

Horizon 2020

Call: H2020-PHC-2014-single-stage

Topic: PHC-15-2014

Type of action: RIA

Proposal number: SEP-210133943

Proposal acronym: APOSEC

Table of contents

Section	Title	Action
1	General information	
2	Participants & contacts	
3	Budget	
4	Ethics	
5	Call-specific questions	

How to fill in the forms

The administrative forms must be filled in for each proposal using the templates available in the submission system. Some data fields in the administrative forms are pre-filled based on the previous steps in the submission wizard.

Proposal ID **643384**Acronym **APOSEC**

1 - General information

Topic **PHC-15-2014**Type of action **RIA**Call identifier **H2020-PHC-2014-single-stage**Acronym **APOSEC**Proposal title* **Clinical Trial testing of APOSEC(TM) as a Regenerative Medicine**

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

Duration in months **54**Fixed keyword 1 **Clinical research****Add**Free keywords **regenerative medicine, paracrine factors, white blood cells, cell secretome, hypoxia induced inflammation, chronic skin wounds, diabetic ulcer, acute myocardial infarction, heart failure**

Abstract

The objective of the APOSEC project is to achieve clinical proof-of-concept by performing clinical Phase I & II studies utilising the patent and trademark protected drug product APOSEC. Authorisation for APOSEC production has already been granted by the relevant Austrian regulatory body (AGES) and complementary studies on toxicology, efficacy and mode of action will ensure full compliance and readiness to swiftly move into Phase III trials at the completion of the proposed H2020 project. The results gained within these trials will provide first-in-man evidence of the ground-breaking potential of APOSEC to provide new therapies to millions of sufferers in modern society worldwide in the fields of wound healing (e.g. for chronic skin wounds and diabetic ulcers) and acute Myocardial Infarction (AMI).

The specific objectives are

- 1. To develop potency and stability assays, to validate its shelf life and to produce sufficient APOSEC for the clinical trials.*
- 2. To conduct toxicology and safety pharmacology studies to assess the safety of the medicinal drug candidate in animal models in order to address preclinical and toxicological matters raised by regulatory authorities.*
- 3. To conduct Phase I topical and systemic clinical trials of APOSEC in healthy subjects.*
- 4. To conduct a Phase II randomised, double blind prospective clinical trial utilising APOSEC as topical treatment for Diabetic foot ulcer.*
- 5. To conduct a Phase I-II clinical trial to determine the safety of a single intravenous application of APOSEC in patients with ST-elevation myocardial infarction (STEMI) as an upstream therapy before primary percutaneous coronary intervention (PCI).*
- 6. To perform parallel, mode of action research for APOSEC to optimise the dosage and the frequency of application for Phase I, II and later on Phase III clinical trials.*
- 7. To promote the APOSEC project and its results widely and effectively to relevant stakeholder groups to fulfil both dissemination and exploitation strategies.*

Remaining characters

0

Has this proposal (or a very similar one) been submitted in the past 2 years in response to a call for proposals under the 7th Framework Programme, Horizon 2020 or any other EU programme(s)?

☐ Yes ☒ No



Proposal ID **643384**

Acronym **APOSEC**

Declarations

1) The coordinator declares to have the explicit consent of all applicants on their participation and on the content of this proposal.	☒
2) The information contained in this proposal is correct and complete.	☒
3) This proposal complies with ethical principles (including the highest standards of research integrity — as set out, for instance, in the European Code of Conduct for Research Integrity — and including, in particular, avoiding fabrication, falsification, plagiarism or other research misconduct).	☒
4) The coordinator confirms:	
- to have carried out the self-check of the financial capacity of the organisation on https://ec.europa.eu/research/participants/portal4/desktop/en/organisations/lfv.html . Where the result was “weak” or “insufficient”, the coordinator confirms being aware of the measures that may be imposed in accordance with the H2020 Grants Manual (Chapter on Financial capacity check); or	☐
- is exempt from the financial capacity check being a public body including international organisations, higher or secondary education establishment or a legal entity, whose viability is guaranteed by a Member State or associated country, as defined in the H2020 Grants Manual (Chapter on Financial capacity check); or	☒
- as sole participant in the proposal is exempt from the financial capacity check.	☐
5) The coordinator hereby declares that each applicant has confirmed:	
- they are fully eligible in accordance with the criteria set out in the specific call for proposals; and	☒
- they have the financial and operational capacity to carry out the proposed action.	☒
The coordinator is only responsible for the correctness of the information relating to his/her own organisation. Each applicant remains responsible for the correctness of the information related to him and declared above. Where the proposal to be retained for EU funding, the coordinator and each beneficiary applicant will be required to present a formal declaration in this respect.	

According to Article 131 of the Financial Regulation of 25 October 2012 on the financial rules applicable to the general budget of the Union (Official Journal L 298 of 26.10.2012, p. 1) and Article 145 of its Rules of Application (Official Journal L 362, 31.12.2012, p.1) applicants found guilty of misrepresentation may be subject to administrative and financial penalties under certain conditions.

Personal data protection

Your reply to the grant application will involve the recording and processing of personal data (such as your name, address and CV), which will be processed pursuant to Regulation (EC) No 45/2001 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data. Unless indicated otherwise, your replies to the questions in this form and any personal data requested are required to assess your grant application in accordance with the specifications of the call for proposals and will be processed solely for that purpose. Details concerning the processing of your personal data are available on the [privacy statement](#). Applicants may lodge a complaint about the processing of their personal data with the European Data Protection Supervisor at any time.

Your personal data may be registered in the Early Warning System (EWS) only or both in the EWS and Central Exclusion Database (CED) by the Accounting Officer of the Commission, should you be in one of the situations mentioned in:

- the Commission Decision 2008/969 of 16.12.2008 on the Early Warning System (for more information see the [Privacy Statement](#)), or
- the Commission Regulation 2008/1302 of 17.12.2008 on the Central Exclusion Database (for more information see the [Privacy Statement](#)).



Proposal ID **643384**

Acronym **APOSEC**

2 - Administrative data of participating organisations

PIC

Legal name

[REDACTED]

[REDACTED]

Short name:

[REDACTED]

Address of the organisation

Street

[REDACTED]

Town WIEN

Postcode

[REDACTED]

Country Austria

Webpage

[REDACTED]

Legal Status of your organisation

Research and Innovation legal statuses

Public body yes

Legal person yes

Non-profit yes

International organisation no

International organisation of European interest no

Secondary or Higher education establishment yes

Research organisation yes

Small and Medium-sized Enterprises (SMEs) no

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name	REDACTED
Street	REDACTED
Town	WIEN
Postcode	REDACTED
Country	Austria

☒ Same as organisation address

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Dr.

Sex

☒ Male ☐ Female

First name

[REDACTED]

Last name

[REDACTED]

E-Mail

[REDACTED]

Position in org.

[REDACTED]

Department

[REDACTED]

Street

[REDACTED]

☒ Same as organisation address

Town

WIEN

Post code

[REDACTED]

Country

Austria

Website

[REDACTED]

Phone

[REDACTED]

Phone 2

+XXX XXXXXXXXX

Fax

[REDACTED]



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

Short name:

Street

Town

Postcode

Country Austria

Webpage

Legal Status of your organisation

Research and Innovation legal statuses

Public body no

Non-profit yes

International organisation no

International organisation of European interest no

Secondary or Higher education establishment no

Research organisation yes

Small and Medium-sized Enterprises (SMEs) no

Legal person yes

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name

Street

☐ Same as organisation
address

Town

Postcode

Country

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Dr.

Sex

☒ Male ☐ Female

First name

[REDACTED]

Last name

[REDACTED]

E-Mail

[REDACTED]

Position in org.

[REDACTED]

Department

-

Street

[REDACTED]

☐ Same as organisation address

Town

[REDACTED]

Post code

[REDACTED]

Country

Austria

Website

[REDACTED]

Phone

[REDACTED]

Phone 2

+xxx xxxxxxxxx

Fax

[REDACTED]

Other contact persons

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



Proposal ID **643384**

Acronym **APOSEC**

PIC

948360368

Legal name

Aposcience AG

Short name: Aposcience AG

Address of the organisation

Street Mariannengasse 14/9

Town Wien

Postcode 1090

Country Austria

Webpage www.aposcience.at

Legal Status of your organisation

Research and Innovation legal statuses

Public body no

Non-profit no

International organisation no

International organisation of European interest no

Secondary or Higher education establishment no

Research organisation no

Small and Medium-sized Enterprises (SMEs) no

Legal person yes

Nace code



Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name

Street

☒ Same as organisation address

Town

Postcode

Country

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Dr.

Sex

☒ Male ☐ Female

First name

[REDACTED]

Last name

[REDACTED]

E-Mail

[REDACTED]

Position in org.

[REDACTED]

Department

-

Street

[REDACTED]

☒ Same as organisation address

Town

Wien

Post code

[REDACTED]

Country

Austria

Website

[REDACTED]

Phone

[REDACTED]

Phone 2

+xxx xxxxxxxxx

Fax

[REDACTED]



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

[REDACTED]

[REDACTED]

Short name: RTDS

Address of the organisation

Street

[REDACTED]

Town Vienna

Postcode

[REDACTED]

Country Austria

Webpage

[REDACTED]

Legal Status of your organisation

Research and Innovation legal statuses

Public body no

Legal person yes

Non-profit no

International organisation no

International organisation of European interest no

Secondary or Higher education establishment no

Research organisation no

Small and Medium-sized Enterprises (SMEs) yes

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name	-
Street	
Town	Vienna
Postcode	
Country	Austria

☒ Same as organisation address

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Dr.

Sex



Male



Female

First name

[REDACTED]

Last name

[REDACTED]

E-Mail

[REDACTED]

Position in org.

CEO

Department

-

Street

[REDACTED]



Same as organisation
address

Town

Vienna

Post code

[REDACTED]

Country

Austria

Website

[REDACTED]

Phone

[REDACTED]

Phone 2

[REDACTED]

Fax

[REDACTED]

Other contact persons

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



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

Short name:

Address of the organisation

Street

Town

Postcode

Country Germany

Webpage

Legal Status of your organisation

Research and Innovation legal statuses

Public body yes

Non-profit yes

International organisation no

International organisation of European interest no

Secondary or Higher education establishment yes

Research organisation yes

Small and Medium-sized Enterprises (SMEs) no

Legal person yes

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name

Street

Town

Postcode

Country

☐ Same as organisation
address

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Sex ☐ Male ☒ Female

First name

Last name

E-Mail

Position in org.

Department

Street

☐ Same as organisation address

Town

Post code

Country

Website

Phone

Phone 2

Fax

Other contact persons

First Name	Last Name	E-mail	Phone



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

Short name: REGIONH

Address of the organisation

Street

Town

Postcode

Country Denmark

Webpage

Legal Status of your organisation

Research and Innovation legal statuses

Public body yes

Non-profit yes

International organisation no

International organisation of European interest no

Secondary or Higher education establishment no

Research organisation no

Small and Medium-sized Enterprises (SMEs) no

Legal person yes

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name	REDACTED	☐ Same as organisation address
Street	REDACTED	
Town	REDACTED	
Postcode	REDACTED	
Country	Denmark	

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Sex ☒ Male ☐ Female

First name

Last name

E-Mail

Position in org.

Department

Street

☐ Same as organisation address

Town

Post code

Country

Website

Phone

Phone 2

Fax



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

[REDACTED]

[REDACTED]

Short name:

[REDACTED]

Address of the organisation

Street

[REDACTED]

Town

[REDACTED]

Postcode

[REDACTED]

Country Spain

Webpage

[REDACTED]

Legal Status of your organisation

Research and Innovation legal statuses

Public body no

Legal person yes

Non-profit no

International organisation no

International organisation of European interest no

Secondary or Higher education establishment no

Research organisation no

Small and Medium-sized Enterprises (SMEs) no

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name

Street

☒ Same as organisation
address

Town

Postcode

Country

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



European Commission - Research - Participants Proposal Submission Forms

Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Mrs

Sex



Male



Female

First name

[REDACTED]

Last name

[REDACTED]

E-Mail

[REDACTED]

Position in org.

Senior Business Development Manager

Department

[REDACTED]

Street

[REDACTED]



Same as organisation
address

Town

[REDACTED]

Post code

[REDACTED]

Country

[REDACTED]

Website

[REDACTED]

Phone

[REDACTED]

Phone 2

+XXX XXXXXXXXXX

Fax

+XXX XXXXXXXXXX



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

[REDACTED]

[REDACTED]

Short name: UMF Tg Mures

Address of the organisation

Street

[REDACTED]

Town

[REDACTED]

Postcode

[REDACTED]

Country Romania

Webpage

[REDACTED]

Legal Status of your organisation

Research and Innovation legal statuses

Public body no

Legal person yes

Non-profit no

International organisation no

International organisation of European interest no

Secondary or Higher education establishment yes

Research organisation no

Small and Medium-sized Enterprises (SMEs) no

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name

Street

☒ Same as organisation
address

Town

Postcode

Country

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Sex ☒ Male ☐ Female

First name

Last name

E-Mail

Position in org.

Department

Street

☒ Same as organisation address

Town

Post code

Country

Website

Phone

Phone 2

Fax



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

Short name: SUM

Address of the organisation

Street

Town

Postcode

Country Poland

Webpage

Legal Status of your organisation

Research and Innovation legal statuses

Public body yes

Non-profit no

International organisation no

International organisation of European interest no

Secondary or Higher education establishment yes

Research organisation no

Small and Medium-sized Enterprises (SMEs) no

Legal person yes

Nace code



Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name

Street

Town

Postcode

Country

☐ Same as organisation address

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Sex ☒ Male ☐ Female

First name

Last name

E-Mail **wojtek.wojakowski@gmail.com**

Position in org.

Department

Street

☐ Same as organisation address

Town

Post code

Country

Website

Phone

Phone 2

Fax

Other contact persons

First Name	Last Name	E-mail	Phone



Proposal ID **643384**

Acronym **APOSEC**

PIC

Legal name

[REDACTED]

[REDACTED]

Short name:

[REDACTED]

Address of the organisation

Street

[REDACTED]

Town

[REDACTED]

Postcode

[REDACTED]

Country Slovakia

Webpage

[REDACTED]

Legal Status of your organisation

Research and Innovation legal statuses

Public body no

Legal person yes

Non-profit no

International organisation no

International organisation of European interest no

Secondary or Higher education establishment no

Research organisation no

Small and Medium-sized Enterprises (SMEs) no

Nace code

Proposal ID **643384**

Acronym **APOSEC**

Department(s) carrying out the proposed work

Department 1

Department name	REDACTED
Street	REDACTED
Town	REDACTED
Postcode	REDACTED
Country	Slovakia

☒ Same as organisation address

Dependencies with other proposal participants

Character of dependence	Participant	
-------------------------	-------------	--



Proposal ID **643384**

Acronym **APOSEC**

Person in charge of the proposal

The name and e-mail of contact persons are read-only in the administrative form, only additional details can be edited here. To give access rights and basic contact details of contact persons, please go back to Step 4 of the submission wizard and save the changes.

Title

Dr.

Sex



Male



Female

First name

[REDACTED]

Last name

[REDACTED]

E-Mail

[REDACTED]

Position in org.

[REDACTED]

Department

[REDACTED]

Street

[REDACTED]

☒ Same as organisation address

Town

[REDACTED]

Post code

[REDACTED]

Country

Slovakia

Website

[REDACTED]

Phone

[REDACTED]

Phone 2

[REDACTED]

Fax

[REDACTED]

Other contact persons

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

Proposal ID 643384

Acronym APOSEC

3 - Budget for the proposal

Participant	Country	(A) Direct personnel costs/€	(B) Other direct costs/€	(C) Direct costs of sub- contracting/€	(D) Direct costs of providing financial support to third parties/€	(E) Costs of inkind contributions not used on the beneficiary's premises/€	(F) Indirect Costs / € (=0.25(A+B-E))	(G) Special unit costs covering direct & indirect costs / €	(H) Total estimated eligible costs / € (=A+B+C+D+F +G)	(I) Reimburse- ment rate (%)	(J) Max. grant / € (=H*I)	(K) Requested grant / €
		?	?	?	?	?	?	?	?	?	?	?
Aposcience AG	AT											

Proposal ID **643384**

Acronym **APOSEC**

Participant	Country	(A) Direct personnel costs/€	(B) Other direct costs/€	(C) Direct costs of sub- contracting/€	(D) Direct costs of providing financial support to third parties/€	(E) Costs of inkind contributions not used on the beneficiary's premises/€	(F) Indirect Costs / € (=0.25(A+B-E))	(G) Special unit costs covering direct & indirect costs / €	(H) Total estimated eligible costs / € (=A+B+C+D+F +G)	(I) Reimburse- ment rate (%)	(J) Max. grant / € (=H*I)	(K) Requested grant / €
		?	?	?	?	?	?	?	?	?	?	?
Total												



Proposal ID **643384**

Acronym **APOSEC**

4 - Ethics issues table

1. HUMAN EMBRYOS/FOETUSES ⁱ		Page
Does your research involve Human Embryonic Stem Cells (hESCs) ?	☐ Yes ☒ No	
Does your research involve the use of human embryos?	☐ Yes ☒ No	
Does your research involve the use of human foetal tissues / cells?	☐ Yes ☒ No	
2. HUMANS		Page
Does your research involve human participants?	☒ Yes ☐ No	14
Are they volunteers for experiments in social or human sciences research?	☒ Yes ☐ No	14
Are they persons unable to give informed consent?	☐ Yes ☒ No	
Are they vulnerable individuals or groups?	☐ Yes ☒ No	
Are they children/minors?	☐ Yes ☒ No	
Are they patients?	☒ Yes ☐ No	15
Are they healthy volunteers for medical studies?	☒ Yes ☐ No	14
Does your research involve physical interventions on the study participants?	☒ Yes ☐ No	14
Does it involve invasive techniques?	☐ Yes ☒ No	
Does it involve collection of biological samples?	☒ Yes ☐ No	13
If your research involves processing of genetic information, please also complete the section "Protection of personal data" [Box 4].		
3. HUMAN CELLS / TISSUES		Page
Does your research involve human cells or tissues? If your research involves human embryos/foetuses, please also complete the section "Human Embryos/Foetuses" [Box 1].	☒ Yes ☐ No	13
Are they available commercially?	☐ Yes ☒ No	
Are they obtained within this project?	☒ Yes ☐ No	13
Are they obtained within another project?	☐ Yes ☒ No	
Are they deposited in a biobank?	☐ Yes ☒ No	



Proposal ID 643384

Acronym APOSEC

4. PROTECTION OF PERSONAL DATA ⁱⁱ		Page
Does your research involve personal data collection and/or processing?	☐ Yes ☒ No	
Does your research involve further processing of previously collected personal data (secondary use)?	☐ Yes ☒ No	
5. ANIMALS ⁱⁱⁱ		Page
Does your research involve animals?	☒ Yes ☐ No	14
Are they vertebrates?	☒ Yes ☐ No	14
Are they non-human primates?	☐ Yes ☒ No	
Are they genetically modified? ^{iv} (directive - regulation)	☐ Yes ☒ No	
Are they cloned farm animals?	☒ Yes ☐ No	14
Are they endangered species?	☐ Yes ☒ No	
Rats and dogs (beagles) for toxicology studies		
6. NON-EU COUNTRIES		Page
Does your research involve non-EU countries?	☐ Yes ☒ No	
Do you plan 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	
Do you plan to import any material - including personal data - from non-EU countries into the EU? If you consider importing data, please also complete the section "Protection of Personal Data" [Box 4].	☐ Yes ☒ No	
Do you plan to export any material - including personal data - from the EU to non-EU countries? If you consider exporting data, please also complete the section "Protection of Personal Data" [Box 4].	☐ Yes ☒ No	
If your research involves low and/or lower middle income countries , are benefits-sharing measures foreseen?	☐ Yes ☒ No	
Could the situation in the country put the individuals taking part in the research at risk?	☐ Yes ☒ No	



Proposal ID **643384**

Acronym **APOSEC**

7. ENVIRONMENT PROTECTION

vi [Directive 2001/18/EC](#) - vii [Directive 2009/41/EC](#) - viii [Regulation EC No 1946/2003](#) - ix [Directive 2008/56/EC](#)
x [Council Directive 92/43/EEC](#) - xi [Council Directive 79/409/EEC](#) - xii [Council Regulation EC No 338/97](#)

Page

Does your research involve the use of elements that may cause harm to the environment, to animals or plants?

☐ Yes ☒ No

Does your research deal with endangered fauna and/or flora and/or protected areas?

☐ Yes ☒ No

Does your research involve the use of elements that may cause harm to humans, including research staff?

☐ Yes ☒ No

8. DUAL USE ^{Xiii}

Page

Does your research have the potential for military applications?

☐ Yes ☒ No

9. MISUSE

Page

Does your research have the potential for malevolent/criminal/terrorist abuse?

☐ Yes ☒ No

10. OTHER ETHICS ISSUES

Page

Are there any other ethics issues that should be taken into consideration? Please specify

☐ Yes ☒ No

I confirm that I have taken into account all ethics issues described above and if any ethics issues apply, I have attached the required documents. ☒

Proposal ID **643384**

Acronym **APOSEC**

5 - Call specific questions

Open Research Data Pilot in Horizon 2020

If selected, all applicants have the possibility to participate in the [Pilot on Open Research Data in Horizon 2020](#)¹, which aims to improve and maximise access to and re-use of research data generated by actions. Participating in the Pilot does not necessarily mean opening up all research data. Actions participating in the Pilot will be invited to formulate a Data Management Plan in which they will determine and explain which of the research data they generate will be made open.

We wish to participate in the [Pilot on Open Research Data in Horizon 2020](#) on a voluntary basis ☐ Yes ☒ No

Participation in this Pilot does not constitute part of the evaluation process. Proposals will not be evaluated favourably because they are part of the Pilot and will not be penalised for not participating.

¹ According to article 43.2 of Regulation (EU) No 1290/2013 of the European Parliament and of the Council, of 11 December 2013, laying down the rules for participation and dissemination in "Horizon 2020 - the Framework Programme for Research and Innovation (2014-2020)" and repealing Regulation (EC) No 1906/2006.

Data management activities

The use of a [Data Management Plan \(DMP\)](#) is required for projects participating in the [Open Research Data Pilot in Horizon 2020](#), in the form of a deliverable in the first 6 months of the project.

All other projects may deliver a DMP on a voluntary basis, if relevant for their research.

Are data management activities relevant for your proposed project? ☐ Yes ☒ No

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

Only applies to topics PHC 8 & 15:

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.	
MARSYAS II - Allogeneic GMP APOSEC topical	
MARSYAS III - Allogeneic GMP APOSEC systemic	
MARSYAS IV - Allogeneic GMP APOSEC Diabetic foot ulcer	
MARSYAS V - Allogeneic GMP APOSEC AMI	

APOSEC – Essential Information on Clinical Trials

MARSYAS II - <i>Allogeneic GMP APOSEC topical</i>	2
MARSYAS III - <i>Allogeneic GMP APOSEC systemic</i>	11
MARSYAS IV - <i>Allogeneic GMP APOSEC Diabetic foot ulcer</i>	19
MARSYAS V - <i>Allogeneic GMP APOSEC AMI</i>	31

1.1 Identifier

MARSYAS II (Allogeneic GMP APOSEC skin)

A Prospective Randomized Double-Blinded Placebo controlled, explorative Phase I trial to investigate the Safety and Tolerability of two different doses of Topically Administered APOSEC in Healthy male subjects with Artificial Dermal Wounds.

EudraCT Nr. Unique Identifier: clinicaltrials.gov will be submitted by [REDACTED]

-

1.2 Study design and endpoints

Study Design:

A prospective randomized, double-blinded, placebo controlled, exploratory Phase I study

This is a prospective, single centre, randomized, double-blinded, placebo-controlled, exploratory Phase I clinical trial in healthy male subjects.

Ten healthy male volunteers will receive two, 4 mm squared punch skin biopsies, one biopsy will receive the study drug, and the second biopsy placebo. The volunteers will consecutively be allocated to 2 different dose groups. 5 volunteers will be allocated to a low dose of APOSEC. Thereafter, 5 additional volunteers will receive a higher dose. Each volunteer will receive both APOSEC and Placebo. The location of application of the investigational medicinal product (IMP) (proximal or distal) will be randomized.

Primary endpoint

- *To investigate safety and tolerability of 2 different doses of APOSEC*

Outcome measures:

1. Assessment of local tolerability or any adverse events will be accomplished by a validated score.
2. Presence of local pain will be assessed by the psychometric “visual analogue scale” (VAS): 0 (no pain) to 5 (severe pain).

Secondary endpoints

- *To investigate the pharmacodynamic effect of APOSEC on wound healing during the treatment phase as well as on wound closure and scar formation after termination of treatment compared to placebo.*
- *To perform a histological comparative evaluation of the baseline biopsy specimen and the re-biopsy specimen taken after termination of treatment with APOSEC, compared to placebo.*
- *To evaluate the physician’s opinion on the use of the gel*

Outcome measures:

1. Assessment of wound healing and condition

- Wound condition in comparison to prior day (stable, improving, impairing)
- Presence or absence of re-epithelialization
- Presence or absence of undermining and tunnelling
- Presence or absence of exudate on the dressing
- Daily photo documentation and planimetric assessment

2. Assessment of wound closure and scar formation

- Assessment of wound closure measured as dichotomic outcome. Complete wound closure yes/no.
- Evaluation of scar formation: The induration of palpable scar tissue will be assessed in length and width [mm]. Moreover, the scar tissue will be evaluated using a calliper.

3. Histological evaluation of biopsy specimen

The baseline biopsy specimen taken at day 1 prior initiation of treatment will be histologically compared with the re-biopsy specimen taken on day 7 after termination of treatment. Baseline and re-biopsy specimens derived from randomized and blinded location of APOSEC application will be compared to placebo. The biopsy specimens will be histologically evaluated with respect to the extent of re-epithelisation and angiogenesis by using CD31 and von Willebrand factor (vWF) as markers.

4. Photographic documentation and planimetric assessment

For evaluation of change in wound size, photographic documentation will be used for planimetric assessment.

1.3 Scientific advice / protocol assistance / communication with regulatory / competent authorities / ethics committees

The GMP production process of APOSEC™ has been developed in the [REDACTED] and is already approved by the Austrian Health Authorities (**Austrian Agency for Health and Food Safety, AGES**) for Phase I trial, for application in healthy volunteers. A phase I trial utilizing topical autologous APOSEC (MARSYAS I; autologous APOSEC) in healthy volunteers will commence in June 2014. A valid ethic board approval by the Medical University is already present.

The present MARSYAS II clinical study will be monitored by an independent CRO and will be designed by a scientific council including peers that are renown in the respective field.

1.4 Subjects/population(s)

Subject population

Ten healthy male volunteers will be included in this study. Staff of the study centre / sponsor or CROs, as well as close relatives of the investigator, are not allowed to participate in this study.

Inclusion criteria

- Healthy male subjects with 18-50 years of age at the day of inclusion
- Written informed consent will be obtained prior to screening examination
- BMI of 19-27 (extremes included)

Exclusion criteria

- Lack of willingness or capacity to co-operate appropriately
- Regular use of medications
- History of malignancies
- History of wound healing abnormalities
- Chronic dermatological disease
- History of chronic autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, diabetes mellitus, Lupus erythematosus

- Tattoos in the region of planned punch biopsy
- Positive HIV serology or evidence of active hepatitis
- Allergy requiring medical treatment within 4 weeks before study initiation
- Active infection of fever $> 38^{\circ}\text{C}$ within 7 days prior randomisation
- Blood donation within 4 weeks before study initiation
- Clinically relevant abnormalities in the laboratory testing, vital signs, ECG or physical examination
- Participation in another clinical trial with an investigational day within 4 weeks before study participation

No subgroup analyses intended.

1.5 Sample size

Sample size considerations

The outlined study is considered to be conducted as an explorative pilot study. Therefore, no statistical hypothesis will be tested or rejected and the study will not be powered accordingly. We assume that 10 subjects will be sufficient to detect major impacts of APOSEC on safety and tolerability in healthy volunteers. No formal sample size calculation can be done in place.

1.6 Statistical methods

Statistical analysis plan

A study-specific statistical analysis plan (SAP) which contains all the detailed information regarding data analysis and software commands used for carrying out the respective calculations will be provided as a separate document. This SAP needs to be completed prior to database lock.

Missing, unused and spurious data

Only subjects for whom data are available will be included in the statistical analysis. Missing values will neither be replaced nor estimated.

Endpoints analysis

All safety and tolerability data will be listed by treatment and subject for the ITT (intention to treat) population. Subjects in the PP (per protocol) population will be identified with an asterisk.

Methods

All variables will be presented using descriptive statistical techniques. Continuous data will be summarized by treatment using the following descriptive statistics: number, mean, standard deviation (SD), minimum, lower quartile (if appropriate), median, upper quartile (if appropriate) and maximum. Categorical data will be summarized by treatment as the number and percentage of treated punch biopsies.

The primary variable will be summarized for subjects in both the ITT and PP population. All other variables will be summarized for subjects in the ITT population only.

Interim analysis

After the low treatment group safety of dosage will be evaluated and decision will be made by the investigator in collaboration with the dermatologists whether or not treatment should continue at a higher dose level.

Criteria for the termination of the trial

All adverse events, serious adverse events and DLT's observed within 15 days following the first administration will be considered and evaluated for safety and can lead to termination of the trial.

For the purposes of this study, a dose limiting toxicity (DLT) will be defined as drug-related serious adverse events or any adverse event that in the opinion of the investigator is thought to be dose-limiting and determined to be related to the investigational medicinal product (IMP). All DLTs will be evaluated and confirmed by the investigator in collaboration with the Department of Dermatology.

1.7 Conduct

I. Study conduct:

Screening of study subjects

- Obtain Informed Consent
 - Demography
 - Medical history
 - Physical examination and vital signs
 - Standard 12-lead ECG
 - Concomitant medication
 - Blood samples for haematology, serum chemistry, virology
 - Urine samples for urine analysis

Inclusion and exclusion criteria are to be verified before run-in phase and randomization.

Randomization and Treatment phase (Day0 to Day 7)

Day 0: test treatment

- On Day 0 a blinded test treatment with APOSEC and Placebo will be performed on intact skin on the inner upper arm, which is opposite to the non-dominant arm where the punch biopsy will be set 24h later to determine possible local tolerability or adverse effects (redness, irritation, inflammation). To exclude any potential adverse events resulting from the use of the wound dressing, Tegaderm Film 10x12 cm is applied to the inner lower arm on the same side.
- Healthy subjects showing any adverse reactions 24h after application of APOSEC, placebo or wound dressing, will be excluded from further treatment phase. If the adverse event is not considered drug related, these subjects will be replaced.
- The AKH Wien pharmacy will perform the blinding procedure, formulation of the IMP as well as the randomization of APOSEC vs. Placebo to proximal or distal area on the intact skin of the inner upper arm. Lyophilized APOSEC will be resuspended in 0.9% Sodium Chloride (NaCl) by the AKH pharmacy. As Placebo, cell free lyophilized medium will be resuspended in 0.9% NaCl and mixed in Gel-Cordes for topical use. Directly at the study site (Department of Clinical Pharmacology), the APOSEC or Placebo will finally be dispersed in Gel Cordes.

Day 1: randomization

- On Day 1, the test treatment on the intact skin will be evaluated and in the absence of any adverse events the subjects will continue with the actual treatment phase. Location of application of APOSEC and Placebo will be randomized to proximal or distal area of the inner upper non-dominant arm.

Day1: treatment day 1

- Blinding and IMP formulation will be performed for the whole treatment period as described for day 0 (test treatment)
- Two punch biopsies (4mm squared) on the inner upper side of the non-dominant arm are set under local anaesthesia. The distance between the biopsies has to be at least 8cm and will be documented as proximal and distal wound. Patients will receive antiseptic treatment on the artificial wound.
- One wound on the upper non-dominant arm (proximal or distal) will be treated with placebo and the second wound will be treated with the allocated concentration of APOSEC. Before application of the randomized assigned treatment, artificial wounds will be cleaned with 0.9 % NaCl. Wound dressing with Adaptic and Mepore Film 10x 12 cm will be done daily and will cover the whole area of IMP application.

Day2 to 7

- From day 2 to 6, punch biopsies will be cleaned with 0.9 % NaCl and then allocated treatment will be administrated. Wound dressing will be done daily and will cover the whole area of application.
- Moreover from day 2 to day 7 the wound will be assessed with respect to its condition in terms of wound healing, local tolerability or any adverse events:

Assessment of local tolerability or any adverse events will be accomplished by a validated score indicated below.

0 = no visible reaction, 1 = faint, minimal erythema, 2 = erythema, 3 = erythema with induration of vesicles, 4 = severe erythema with induration, vesicles or bullae or pustules and/or erosion/ulceration

Any reaction unexpected to be related to the normal wound healing process or the application of the dressing, will be defined as adverse event.

Presence of local pain will be assessed by a psychometric “visual analogue scale” (VAS): 0 (no pain) to 5 (severe pain).

Day 7: End of treatment

- Assessment of wound closure measured as dichotomic outcome. Complete wound closure yes/no.
- Evaluation of scar formation: The induration of palpable scar tissue will be assessed in length and width [mm]. Moreover, the scar tissue will be evaluated using a calliper.
- Photo documentation and planimetric assessment
- After evaluation of wound closure, 2 re-biopsies (6mm) will be taken at the same location of prior punch biopsy, to perform a histological evaluation of re-epithelialization and angiogenesis by using CD31 and von Willebrand factor (vWF) as markers. Artificial wounds will finally be closed with a suture.

Follow-up Day 17: End of study examination

At day 17 or at the final visit day if the subject has discontinued the study, a follow-up visit will be performed including:

- Suture removal
- Physical examination and vital signs
- Change in concomitant medication
- Assessment of adverse events
- Blood samples (haematology and chemistry) for safety issues

II. Randomization and Blinding

Product (APOSEC in 2 doses and placebo) for randomization will be prepared at Blood Transfusion Service for the Red Cross Upper Austria (RED), Krankenhausstraße 7, Linz. The AKH Wien pharmacy will perform the blinding procedure, formulation of the IMP as well as the randomization of APOSEC vs. Placebo to proximal or distal area on the intact skin of the inner upper arm. A predefined randomization list from the web application at www.randomizer.org will be used. Lyophilized APOSEC will be resuspended in 0.9% NaCl by the AKH pharmacy. Subjects' treatment will be randomized to one of the arm sides (proximal, distal) for allocated APOSEC concentration and Placebo. APOSEC treatment will start with the low dose. The sample size per Block will be 5.

III. DOCUMENTATION AND DATA MANAGEMENT

Documentation of study results

A subject screening and enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

Case report form (CRF)

For each subject enrolled, regardless of study drug initiation, an electronic CRF (eCRF) must be completed and approved by the investigator or a designated sub-investigator. This also applies to those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be entered in the eCRF. Case report forms are to be completed on an ongoing basis. eCRF entries and corrections will only be performed by study site staff, authorized by the investigator. After closure of the database, a print-out copy of the completed eCRFs will remain on site [REDACTED].

Original completed eCRF's will remain at the sponsor site and will be archived (in electronic and Paper form) after the study close out. Details about the eCRF will be summarized in a separate Data-Management Plan (DMP).

Data Collection

Data collected at all visits are entered into the electronic individual Case Report Forms (CRF) for later transferal into a database for statistical analysis. The CRFs will be verified against source documents following guidelines established before study onset as detailed in the Monitoring Plan. Maintenance of the study database will be performed by the sponsor.

Identification data to be considered as source data

Photo documentation of wounds will be considered to be source data and therefore be directly entered on the CRF.

Safekeeping

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified (according to ICH-GCP "essential documents"). These documents will be classified into two different categories: investigator's file, and subject clinical source documents.

The investigator's file will contain the protocol/amendments, EudraCT forms, CRFs (eCRF printout), standard operation procedures (SOPs), data clarification and query forms, EC/IRB and Health Authority approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, screening and enrolment logs, and other appropriate documents/correspondence as per ICH/Good Clinical Practice (GCP) and local regulations.

These two categories of documents must be kept on file by the investigator for as long as needed to comply with national and international regulations (in Austria 15 years after end of study). If source documents are not durable as long as needed (e.g. ECG printouts) they must be preserved as a copy.

When source documents are required for the continued care of the subject, appropriate copies should be made for storing outside of the site.

Quality Control and Quality Assurance

Periodic Monitoring

The [REDACTED] Austria will perform the study monitoring. They will contact and visit the investigator regularly and will be allowed to have access to all source documents needed to verify the entries in the eCRFs and other protocol-related documents provided that subject confidentiality is maintained in agreement with local regulations. It will be the monitor's responsibility to inspect the eCRFs at regular intervals according to the monitoring plan throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of the main efficacy, safety, and tolerability endpoints.

The monitor will follow a predefined monitoring plan and will be working according to SOPs and will provide a monitoring report after each visit for the Sponsor. Depending on the quality of the data, additional monitoring visits may be necessary according to the sponsor's discretion. The investigator will resolve discrepancies of data.

Audit and Inspections

Upon request, the investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the sponsor or to competent authority inspectors. The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for assessment of safety and efficacy of the investigational product have appropriately been reported to the sponsor.

Reporting and Publication

Publication of study results

The findings of this study will be published by the sponsor (investigators) in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all co-investigators before submission. Confidentiality of subjects in reports/publications will be guaranteed.

IV. ETHICAL AND LEGAL ASPECTS

Informed consent of subjects

Following comprehensive instruction regarding the nature, significance, impact and risks of this clinical trial, the patient must give written consent to participation in the study. During the instruction the trial participants are to be made aware of the fact that they can withdraw their consent – without giving reasons – at any time without their further medical care being influenced in any way. In addition to the comprehensive instructions given to the trial participants by the investigator, the trial participants also receive a written patient information sheet in comprehensible language, explaining the nature and purpose of the study and its progress. The patients must agree to the possibility of study-related data being passed on to relevant

authorities. The patients must be informed in detail of their obligations in relation to the trial participants insurance in order not to jeopardize insurance cover.

Acknowledgement / approval of the study

The sponsor will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to an Ethics Committee (EC). Approval from the committee must be obtained before starting the study.

The clinical trial shall be performed in full compliance with the legal regulations according to the Drug Law (AMG - Arzneimittelgesetz) of Austria.

An application must also be submitted to the Austrian Competent Authorities (Bundesamt für Sicherheit im Gesundheitswesen (BASG) represented by the Agency for Health and Food Safety (AGES PharmMed) and registered to the European Clinical Trial Database (EudraCT) using the required forms. The timelines for (silent) approval set by national law must be followed before starting the study.

V. CHANGES IN THE CONDUCT OF THE STUDY

Protocol amendments

Proposed amendments must be submitted to the appropriate CA and ECs. Substantial amendments may be implemented only after CA/EC approval has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving CA/EC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Study Termination

If the sponsor or the investigator decides to terminate the study before it is completed, they will notify each other in writing stating the reasons of early termination. In terminating the study, the sponsor and the investigator will ensure the adequate consideration is given to the protection of the subject interests. The investigator, sponsor or (designated CRO on behalf of the sponsor) will notify the relevant CA and EC. Documentation will be filed in the Trial Master and Investigator Files.

Clinical Study Report (CSR)

Within one year after the final completion of the study, a full CSR will be prepared by the sponsor and submitted to the EC and the competent authority.

The Investigator will be asked to review and sign the final study report.

Financial Funding of the Study

The present study is a non-commercial study, whereby the [REDACTED] acts as leader of the study and will be responsible (together with the [REDACTED] for the Scientific Part of the study. The study is planned to be funded by the EU Commission (HORIZON 2020), and the [REDACTED] will be responsible for the Financial Part of the study.

Insurance

During their participation in the clinical trial the patients will be insured as defined by legal requirements. The investigator of the clinical trial will receive a copy of the insurance conditions of the 'patients insurance'.

VI. STUDY CENTRE:

Department of [REDACTED]

The department serves as an international interdisciplinary clinical research and teaching facility, focusing on design, realization, and analysis of clinical studies involving healthy subjects and patients according to highest ethical, scientific and legal standards.

The department is dedicated to the medical care of study participants and has an availability of 12 beds partly with the option of intensive care monitoring.

[REDACTED] ranks among the most renowned departments of the discipline of Clinical Pharmacology in Europe. It closely cooperates with local, national and international clinical departments and research facilities, industry and regulatory authorities to achieve its mission.

[REDACTED] is currently subdivided in following sections:

- Section of Cardiovascular Medicine
- Section of Clinical Pharmacokinetics / Pharmacogenetics and Imaging
- Section of Hematology & Immunology
- Section of Ophthalmology-Pharmacology
- Working Group Drug-Development and Healthcare

The team is furthermore supplemented and assisted by a group of registered nurses with special additional qualification ("study nurses").

MARSYAS II will be performed in the Section of Cardiovascular Medicine.

2.1 Identifier

MARSYAS III (Allogeneic GMP APOSEC Systemic)

A Prospective Randomized Double-Blinded Placebo controlled, explorative Phase I trial to investigate the Safety and Tolerability of four different doses of intravenously administered APOSEC in healthy male and female subjects

EudraCT Nr. Unique Identifier: clinicaltrials.gov will be submitted by [REDACTED]

2.2 Study design and endpoints

Study Design:

A prospective randomized, double-blinded, placebo controlled, exploratory Phase I study

24 eligible volunteers will consecutively be allocated to 4 different doses of APOSEC intravenous infusion. 6 volunteers will serve as controls. APOSEC will be applied to volunteers intravenously in an incremental dosing starting from low to highest dosage. Each volunteer will either receive intravenous APOSEC or placebo.

Primary endpoint

To investigate safety and tolerability of 4 different single doses of APOSEC applied intravenously.

Outcome measures:

Primary measures:

Assessment of systemic tolerability of 4 doses of APOSEC and adverse events

Secondary measures:

Changes in laboratory parameters indicative of inflammation and activation of coagulation

Tertiary measure:

Pharmacokinetics of selected constituents of the infused product

Secondary endpoints

- Laboratory Evaluations: Screening: 24h, 7d
- Infusion duration: 30-45 min
- Blood count including Differential: 0min, 30min, 1hr, 4hr
- Postinfusion 24hrs, 48hrs, 72hrs: CRP, D-dimer, Blood count
- Fade out visit: day 7
- Special evaluations
- ROTEM
- Multiplate
- Nitrates

2.3 Scientific advice / protocol assistance / communication with regulatory / competent authorities / ethics committees

All clinical studies will be monitored by an independent CRO (KKS) and will be designed by a scientific council including peers that are renowned in the respective field. Prior to systemic phase I study, toxicological evaluations have to be performed in GLC laboratories. A valid ethics board approval by the Medical University has to be issued after evaluation of the toxicology report. After these studies are available, MARSYAS III can commence under the supervision of [REDACTED]

The principle investigator has been working as a member of the scientific advice at the European Medicine Agency (EMA) for several years, and is still work in as an expert in centralised scientific advice procedures. Typically, EMA is not concerned about the design of phase I trials because they will generate data, to inform further phase II and phase III trials. For the conduct of this phase I trial, no scientific advice procedure will be necessary, because the investigator has extensive experience in the conduct of trials with biologics and first in man trials. This trial will follow the relevant CHMP guideline, *“Guideline on requirements for first-in-man clinical trials for potential high-risk medicinal products”*

(http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002989.pdf)

2.4 Subjects/population(s)

Subject population

Thirty healthy male or female volunteers will be included in the study. Staff of the study centre / sponsor or CROs, as well as close relatives of the investigator are not allowed to participate in this study.

Inclusion criteria

- Healthy male and female subjects with 18-50 years of age at the day of inclusion
- Written informed consent will be obtained prior to screening examination
- BMI of 19-27 (extremes included)

Exclusion criteria

- Lack of willingness or capacity to co-operate appropriately
- Regular use of medications
- History of malignancies
- History of chronic autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, diabetes mellitus, Lupus erythematosus
- Positive HIV serology or evidence of active hepatitis
- Allergy requiring medical treatment within 4 weeks before study initiation
- Active infection of fever > 38°C within 7 days prior randomization
- Blood donation within 4 weeks before study initiation
- Clinically relevant abnormalities in the laboratory testing, vital signs, ECG or physical examination
- Participation in another clinical trial with an investigational day within 4 weeks before study participation

No subgroup analyses intended

2.5 Sample size

Sample size considerations

The outlined study is considered to be conducted as an explorative pilot study. Therefore, no statistical hypothesis will be tested or rejected and the study will not be powered accordingly. We assume that subjects will be sufficient to detect major impacts of APOSEC on safety and tolerability in healthy volunteers. No formal sample size calculation will be in place. Prof. Jilma expects that the following study design will generate data for safety.

This phase I clinical study will include 30 healthy male and female volunteers. A single intravenous APOSEC infusion will be applied in 24 volunteers. As control, 6 volunteers will receive placebo. APOSEC will be resuspended in physiologic NaCl. The single intravenous infusion will be accompanied by routine laboratory screening 24h post infusion and after 7 days. The APOSEC infusion will be applied within 30-45 minutes. Differential blood count, Routine blood chemistry, and special evaluations will be performed (e.g. ROTEM platelet aggregometry, definition of anti-HLA antibodies).

Dose1: Equivalent: APOSEC derived from 100×10^6 PBMC (n=2 plus Placebo)

Dose2: Equivalent: APOSEC derived from 300×10^6 PBMC (n=2 plus Placebo)

Dose3: Equivalent: APOSEC derived from 1×10^9 PBMC (n=10 plus 2 Placebo)

Dose4: Equivalent: APOSEC derived from 3×10^9 PBMC (n=10 plus 2 Placebo)

The sample size is typical of a first in man phase I trial, and is adequate for the initial assessment of safety and tolerability. Subjects in the placebo group will be pooled for statistical comparisons.

A data safety monitoring board (DSMB) will be established to review safety data throughout the trial.

2.6 Statistical methods

Statistical analysis plan

A study-specific statistical analysis plan (SAP) which contains all the detailed information regarding data analysis and software commands used for carrying out the respective calculations will be provided as a separate document. This SAP needs to be completed prior to database lock.

Missing, unused and spurious data

Only subjects for whom data are available will be included in the statistical analysis. Missing values will neither be replaced nor estimated.

Endpoints analysis

All safety and tolerability data will be listed by treatment and subject for the ITT (intention to treat) population. Subjects in the PP (per protocol) population will be identified with an asterisk.

Methods

All variables will be presented using descriptive statistical techniques. Continuous data will be summarized by treatment using the following descriptive statistics: number, mean, standard deviation (SD), minimum, lower quartile (if appropriate), median, upper quartile (if appropriate) and maximum. Categorical data will be summarized by treatment as the number and percentage of treated punch biopsies.

The primary variable will be summarised for subjects in both the ITT and PP population. All other variables will be summarised for subjects in the ITT population only.

Interim analysis

After including 12 patients and analysing the preliminary results, the Safety Data Monitoring Board will evaluate whether or not treatment should continue at a higher dose level.

Criteria for the termination of the trial

All adverse events, serious adverse events and the dose limiting toxicity (DLT) observed within 15 days following the first administration will be considered and evaluated for safety and can lead to termination of the trial.

2.7 Conduct

I. Study conduct:

Screening of study subjects

- Obtain Informed Consent
 - Demography
 - Medical history
 - Physical examination and vital signs
 - Standard 12-lead ECG
 - Concomitant medication
 - Blood samples for haematology, serum chemistry, virology
 - Urine samples for urine analysis

Inclusion and exclusion criteria are to be verified before run-in phase and randomization.

Run-in phase

Allogeneic GMP APOSEC will be provided in a blinded and labelled fashion at the Blood Transfusion Service for the [REDACTED]. The lyophilisate will be delivered to the Department of Clinical Pharmacology. The product is produced according to the validation process accepted by the Austrian Health Authorities.

Randomization and Treatment phase

Study product will be manufactured and labelled under good manufacturing practice (GMP) as described elsewhere. Four dose levels of the product will be investigated. Doses will be infused intravenously, which correspond to the equivalent of cell supernatant of 0.1, 0.3, 1 and 3 billion cells. The rationale for the doses is based on previous animal trials. The first doses are considered sub-therapeutic and are used primarily for safety reasons. Healthy volunteers will report to the research ward in the morning after an overnight fast and donate blood for baseline determination of parameter. Female subjects will have to undergo urine screening for pregnancy. Heart rate and electrocardiogram of subjects will be continuously monitored on HP Care View systems and blood pressure will be measured repeatedly at 5 minute intervals during the infusion period.

Temperature will be assessed at baseline, hourly during the first 8 hours, then at 12, 16, and 24 hrs. Laboratory safety parameters and adverse events will be followed over 24 hrs, blood chemistry at 0 and 24 hrs, and C-reactive protein as well as D-dimer levels at 0, 8, and 24 hrs. In case of relevant deviations of parameters from baseline, subjects will be followed up for a longer a period of time until, all parameters have

reached baseline again. Subjects will be dismissed after an overnight stay for observation. Blood will be frozen for the determination of cytokines (0-24h) and antibody response at 0 and 4 weeks after APOSEC infusion. Analysis of these parameters will be performed at Dr. Ankersmit's CD Laboratory or subcontracted as appropriate.

II. Randomization and Blinding

Randomization will be performed at the [REDACTED], [REDACTED]. For randomization a predefined randomization list from the web application at www.randomizer.org will be used. The sample size per Block will be 5.

III. DOCUMENTATION AND DATA MANAGEMENT

Documentation of study results

A subject screening and enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

Case report form (CRF)

For each subject enrolled, regardless of study drug initiation, an electronic CRF (eCRF) must be completed and approved by the investigator or a designated sub-investigator. This also applies to those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be entered in the eCRF. Case report forms are to be completed on an ongoing basis. eCRF entries and corrections will only be performed by study site staff, authorized by the investigator. After closure of the database, a print-out copy of the completed eCRFs will remain on site (Department of Clinical Pharmacology).

Original completed eCRF's will remain at the sponsor site and will be archived (in electronic and Paper form) after the study close out. Details about the eCRF will be summarized in a separate Data-Management Plan (DMP).

Data Collection

Data collected at all visits are entered into the electronic individual Case Report Forms (CRF) for later transferal into a database for statistical analysis. The CRFs will be verified against source documents following guidelines established before study onset as detailed in the Monitoring Plan. Maintenance of the study database will be performed by the sponsor.

Safekeeping

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified (according to ICH-GCP "essential documents"). These documents will be classified into two different categories: investigator's file, and subject clinical source documents.

The investigator's file will contain the protocol/amendments, EudraCT forms, CRFs (eCRF printout), standard operation procedures (SOPs), data clarification and query forms, EC/IRB and Health Authority approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, screening and enrollment logs, and other appropriate documents/correspondence as per ICH/Good Clinical Practice (GCP) and local regulations.

These two categories of documents must be kept on file by the investigator for as long as needed to comply with national and international regulations (in Austria 15 years after end of study). If source documents are not durable as long as needed (e.g. ECG printouts) they must be preserved as a copy.

When source documents are required for the continued care of the subject, appropriate copies should be made for storing outside of the site.

Quality Control and Quality Assurance

Periodic Monitoring

The designated monitor will contact and visit the investigator regularly and will be allowed to have access to all source documents needed to verify the entries in the eCRFs and other protocol-related documents provided that subject confidentiality is maintained in agreement with local regulations. It will be the monitor's responsibility to inspect the eCRFs at regular intervals according to the monitoring plan throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of the main efficacy, safety, and tolerability endpoints.

The monitor will follow a predefined monitoring plan and will be working according to SOPs and will provide a monitoring report after each visit for the Sponsor. Depending on the quality of the data, additional monitoring visits may be necessary according to the sponsor's discretion. The investigator will resolve discrepancies of data.

The [REDACTED] will perform monitoring.

Audit and Inspections

Upon request, the investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the sponsor or to competent authority inspectors. The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for assessment of safety and efficacy of the investigational product have appropriately been reported to the sponsor.

Reporting and Publication

Publication of study results

The findings of this study will be published by the sponsor (investigators) in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all co-investigators before submission. Confidentiality of subjects in reports/publications will be guaranteed.

IV. ETHICAL AND LEGAL ASPECTS

Informed consent of subjects

Following comprehensive instruction regarding the nature, significance, impact and risks of this clinical trial, the patient must give written consent to participation in the study. During the instruction the trial participants are to be made aware of the fact that they can withdraw their consent – without giving reasons – at any time without their further medical care being influenced in any way. In addition to the comprehensive instructions

given to the trial participants by the investigator, the trial participants also receive a written patient information sheet in comprehensible language, explaining the nature and purpose of the study and its progress. The patients must agree to the possibility of study-related data being passed on to relevant authorities. The patients must be informed in detail of their obligations in relation to the trial participants insurance in order not to jeopardize insurance cover.

Acknowledgement / approval of the study

The sponsor will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to an Ethics Committee (EC). Approval from the committee must be obtained before starting the study.

The clinical trial shall be performed in full compliance with the legal regulations according to the Drug Law (AMG - Arzneimittelgesetz) of the Republic of Austria.

An application must also be submitted to the Austrian Competent Authorities (Bundesamt für Sicherheit im Gesundheitswesen (BASG) represented by the Agency for Health and Food Safety (AGES PharmMed) and registered to the European Clinical Trial Database (EudraCT) using the required forms. The timelines for (silent) approval set by national law must be followed before starting the study.

V. CHANGES IN THE CONDUCT OF THE STUDY

Protocol amendments

Proposed amendments must be submitted to the appropriate CA and ECs. Substantial amendments may be implemented only after CA/EC approval has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving CA/EC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Study Termination

If the sponsor or the investigator decides to terminate the study before it is completed, they will notify each other in writing stating the reasons of early termination. In terminating the study, the sponsor and the investigator will ensure the adequate consideration is given to the protection of the subject interests. The investigator, sponsor or (designated CRO on behalf of the sponsor) will notify the relevant CA and EC. Documentation will be filed in the Trial Master and Investigator Files.

Clinical Study Report (CSR)

Within one year after the final completion of the study, a full CSR will be prepared by the sponsor and submitted to the EC and the competent authority.

The Investigator will be asked to review and sign the final study report.

The principal investigator of the trial will be [REDACTED] MD, the trial site is the [REDACTED]
[REDACTED]

Data management

Study management, data capture and management, monitoring, biostatistics, pharmacovigilance and reporting will be performed by the [REDACTED] (Director: [REDACTED] MD). Ethical and regulatory approval and agreement from the hospital administration will be in place before initiation of study specific interventions. Subjects are available from a larger database of study participants at the [REDACTED]. All studies will be conducted in agreement with regulatory requirements, ICH-GCP, and the Declaration of Helsinki. All studies will be registered in a publicly available registry.

Financial Funding of the Study

The present study is a non-commercial study, whereby the [REDACTED] is responsible for the Scientific and Clinical part of the study. The study is planned to be funded by the EU Commission (HORIZON 2020).

Insurance

During their participation in the clinical trial the patients will be insured as defined by legal requirements. The investigator of the clinical trial will receive a copy of the insurance conditions of the 'patients insurance'.

VI. STUDY CENTRE:

[REDACTED]

The department serves as an international interdisciplinary clinical research and teaching facility, focusing on design, realization, and analysis of clinical studies involving healthy subjects and patients according to highest ethical, scientific and legal standards.

The department is dedicated to the medical care of study participants and has an availability of 12 beds partly with the option of intensive care monitoring.

[REDACTED] ranks among the most renowned departments of the discipline of Clinical Pharmacology in Europe. It closely cooperates with local, national and international clinical departments and research facilities, industry and regulatory authorities to achieve its mission.

[REDACTED]

[REDACTED] is currently subdivided in following sections:

- Section of Cardiovascular Medicine
- Section of Clinical Pharmacokinetics / Pharmacogenetics and Imaging
- Section of Hematology & Immunology
- Section of Ophthalmo-Pharmacology
- Working Group Drug-Development and Healthcare

The team is furthermore supplemented and assisted by a group of registered nurses with special additional qualification ("study nurses").

MARSYAS III will be performed in the Section of Cardiovascular Medicine.

3.1 Identifier

MARSYAS IV (Allogeneic GMP APOSEC Diabetic foot ulcer)

Randomized, Parallel-Group, Double-Blind, Placebo-Controlled Phase II Clinical Trial to Evaluate the Safety and Effectiveness of APOSEC in Treating Subjects with Diabetic Foot Ulcers

The clinical trial will be registered at EudraCT (European Clinical Trials Database). The study will be registered in www.clinicaltrials.com homepage.

3.2 Study design and endpoints

Study design:

Prospective randomized double-blind multicentre Phase II trial to investigate the efficacy, safety and feasibility of repetitive topical administration of human allogeneic APOSEC in patients with diabetic foot ulcer (DFU) in a 12 week (84-day) trial. APOSEC will be applied topically 2x a week (Monday/Thursday) for 7 weeks. At day 84 the study will be terminated. A screening period of 14 days is included to identify non healing ulcers under best care conditions.

Primary endpoints:

The primary efficacy parameter will be the proportion of Ulcers healed by 12 weeks treatment of APOSEC as defined as 100 % epithelialization without drainage. The healing and complete epithelisation of the diabetic foot ulcer, as well as the progression or regression of grades of the wound will be assessed based on the International Working Group of the Diabetic Foot (IWGDF) classification systems and the area of the wound will be measured and expressed as square centimetre.

Secondary endpoints:

The secondary endpoints will be

1. The proportion of patients in each treatment group reporting adverse events (Designated as safety issue). Adverse events are defined as a) local infective complications during the treatment including osteitis, abscess, osteomyelitis and gangrene, b) necessity of surgical treatment, such as amputation of the leg with the treated wound.
All adverse events (AEs) will be evaluated from the time of informed consent until the last FUP investigation or termination of the study. All SAEs (serious AEs) will be reported within 24h after occurrence to the principal investigator (PI) and data safety monitoring board (DSMB).
2. The rate of re-epithelialization of the Ulcer site during the 12 weeks follow-up, expressed by the overall healing rate of the ulcers, based on the percent of unhealed ulcer area re-epithelialized per week.
3. The time to re-epithelialization of the Ulcer site, expressed as average time to complete re-epithelialization of baseline ulcer area.

3.3 Scientific advice / protocol assistance / communication with regulatory / competent authorities / ethics committees

The study protocol will be submitted to the local Ethical Committee (EC). Patient information and consent will be translated to relevant European language.

Permission for conduction of the study will be requested from the relevant Hospitals and Legal Authorities.

The clinical trial will be registered at EudraCT (European Clinical Trials Database). The study will be registered in www.clinicaltrials.com homepage.

Investigator Responsibility / Performance

Prior to starting enrolment of patients, the investigator must read and understand the Study protocol and must sign and complete the Protocol Signature page. The Investigator Agreement documents agreement to all conditions of the Study protocol and agreement to conduct the study accordingly. This study will be conducted in accordance with the Declaration of Helsinki, and other applicable regulatory requirements, the Study Protocol, or any conditions of approval imposed by the IRB/EC or regulatory authorities. The Investigator will ensure that all work and services will be conducted in accordance with the highest standards of medical and clinical research practice.

Required Documents

The following documents will be submitted to the Sponsor or designee prior to patient enrolment:

- Signed Protocol Signature Page
- Recent signed and dated English Curriculum Vitae (CV) for the Site Principal Investigator and co-investigators. This CV should clearly show the investigator's qualifications and experience.
- Copy of the written confirmation of the EC regarding approval of the protocol including version number and date, patient information sheet and informed consent including version and date and other adjunctive patient material.
- List of EC members, including name, title, occupation and any institutional affiliation of each member. If the EC members list is not available, the General Assurance or EC Recognition number should be provided.
- Signed study agreement.

The investigator's file will contain the protocol/amendments, EudraCT forms, CRFs (eCRF printout), standard operation procedures (SOPs), data clarification and query forms, EC/IRB and Health Authority approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, screening and enrolment logs, and other appropriate documents/correspondence as per ICH/Good Clinical Practice (GCP) and local regulations.

These two categories of documents must be kept on file by the investigator for as long as needed to comply with national and international regulations (in Austria 15 years after end of study). If source documents are not durable as long as needed they must be preserved as a copy.

When source documents are required for the continued care of the subject, appropriate copies will be made for storing outside of the site.

Confidentiality

All data and information collected during this study related to the participating subject will comply with the standards for protection of privacy based on applicable local/ national requirements for subject's confidentiality. All data used in the analysis and summary of this study will be anonymous, and without reference to specific study patient names. Access to study patient files will be limited to authorized personnel of the Sponsor, the Investigator, and research staff. Authorized regulatory personnel have the right to inspect and copy all records pertinent to this study, but all efforts must be made to remove patient's personal data.

Informed consent of subjects

The study patients' written informed consent will use the local an IRB/EC-approved informed consent form. The study will be explained to the study patients in lay language. The Investigator, or representative, will be

available to answer all of the study patient's study-related questions. Study patients will be assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Institutional Review Board/Ethics Committee (IRB/EC) Approval

According to the local regulation the Investigator will have all necessary approvals including written approval from the IRB/EC of the Institution or appropriate other accepted IRB/EC prior to enrolling patients in the study. A copy of the written approval will be provided to Sponsor and will include the following:

- Statement of IRB/EC approval for the proposed study at the institution
- Date the study was approved and the duration of the approval
- Listing of any conditions attached to the approval
- Identification of the approved primary investigator
- Signature of the IRB/EC chairperson
- IRB/EC approval of the informed consent form
- IRB/EC approval of the final protocol

Any substantial amendments to the protocol, as well as associated consent form changes, will be submitted to the IRB/EC and written approval obtained prior to implementation. Minor changes who do not affect the subject safety will be subject of notification.

Serious adverse event reports will be submitted to IRB/EC as requested. Annual and final reports will be provided to IRB/EC as appropriated.

Acknowledgement / approval of the study

The Sponsor will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to the local Ethics Committee (EC). Approval from the committee must be obtained before starting the study.

The clinical trial shall be performed in full compliance with the legal regulations according to the Drug Law of the relevant country.

The study will be registered at the European Clinical Trial Database (EudraCT) using the required forms. The timelines for (silent) approval set by national law must be followed before starting the study.

COMMITTEES

Steering Committee (SC)

The Steering Committee (SC) is formed by a Chairman (Prof. Dr. Hendrik Jan Ankersmit, associate Prof. of Surgery, MUW), representative(s) from the Aposcience AG and a Clinical Research Organization (CRO). The SC is responsible for managing the scientific and operational aspects of the study. The SC has the responsibility to review the daily conduct of the study.

Data Safety Monitoring Board (DSMB)

The Data Safety Monitoring Board (DSMB), being an independent committee, will be composed of at least two well-known dermatologists [REDACTED]

[REDACTED]. For this study the DSMB will be responsible for decision of any potentially significant patient safety-related observations. The DSMB will function in accordance with applicable regulatory guidelines. The DSMB members will not be employees of the sponsor, or serve on the

DSMB or a committee of a competing medical trial. Members will not have any affiliation with the Investigators, CRO or the clinical investigation sites.

The DSMB will be set up prior to the start of the DSMB activities, in which the responsibilities of the DSMB will be described with respect to safety review, formal reporting to the Steering Committee, and if applicable, safety stopping rules.

The DSMB chairperson will notify the Sponsor and / or the Steering Committee (through CRO) by confidential memo of any safety or compliance issues. The committee will also provide confidential recommendations, when necessary, of study termination based on the safety stopping rules, or because a clinically significant result was identified in safety analyses of the data. All DSMB reports will remain strictly confidential, but will be made available to the regulatory body upon request.

Clinical Events Committee (CEC)

The Clinical Events Committee (CEC) is an independent committee comprised of cardiologists who are not participants in the study. The CEC is responsible for the categorization of adverse events in the study, based on the definitions of the protocol.

Prior to any CEC activity, a CEC charter will be developed, which will describe the events to be adjudicated, the minimum amount of data required, and the algorithm followed in order to classify the events.

Periodic Monitoring

The designated monitor will contact and visit the investigator regularly and will be allowed to have access to all source documents needed to verify the entries in the eCRFs and other protocol-related documents provided that subject confidentiality is maintained in agreement with local regulations. It will be the monitor's responsibility to inspect the eCRFs at regular intervals according to the monitoring plan throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of the main efficacy, safety, and tolerability endpoints.

The monitor will follow a predefined monitoring plan and will be working according to SOPs and will provide a monitoring report after each visit for the Sponsor. Depending on the quality of the data, additional monitoring visits may be necessary according to the sponsor's discretion. The investigator will resolve discrepancies of data.

3.4 Subjects/population(s)

According to sample size calculation, 160 study patients will be included with non-healing DFU. Design of Phase II DFU study will be finalized after availability of Phase I study set (MARSYAS II).

Inclusion criteria:

1. Have at least one chronic non-healing Wagner Grade 1 or Grade 2 plantar neuropathic diabetic ulcer between 1.0-6.0 cm² on the mid-foot or forefoot, including the toes but excluding the heel.
2. Type I or Type II diabetes under metabolic control
3. Female subjects must have a negative pregnancy test and be willing to use acceptable method of birth control for the duration of the study

Exclusion criteria:

1. Exposure to any investigational product within the last 30 days
2. Chronic renal insufficiency and/or chronic liver dysfunction
3. Resting blood pressure (at the time of the initial visit of the Screening Period) which exceeds 160 systolic and/or 90 diastolic mmHg on 3 consecutive readings at least 15 minutes apart
4. Malignancy of any kind
5. Receiving dialysis due to terminal renal insufficiency
6. Current history of drug abuse, and/or known to be HIV positive
7. Prior radiation therapy of the foot under study
8. Current use of corticosteroids (within past 8 weeks), immunosuppressants (within past 8 weeks), or use of a biologic agent to include growth factors and skin equivalents (Regranex, Apligraf, or Dermagraft) in the past 90 days
9. Ulcer is deemed clinically infected and requires topical antimicrobials or agents known to affect wound healing, or the subject has been taking systemic antibiotics for more than 7 days for any reason
10. Sickle-cell anaemia, Raynaud's or other peripheral vascular disease
11. Wagner Grade 3 or higher DU, deep abscess or infection of the joint or tendon, or gangrene or osteomyelitis
12. pregnant or breast-feeding
13. uncontrolled anaemia (Hb < 9 g/dL in females and < 10 g/dL in males)
14. hypoalbuminemia (albumin < 3 g/dL)
15. overtly infected target ulcer (as judged by investigator)
16. highly exuding wounds (wounds that require a daily dressing change)
17. osteomyelitis
18. systemic infections
19. acute Charcot foot and severe chronic Charcot deformity
20. suspicion, presence or history of systemic or local cancer or tumour of any kind,
21. ABPI < 0.7 or ankle systolic pressure < 70 mm Hg
22. none of the following findings (only 1 out of 3 tests is required):
 - on Doppler waveform analysis on the dorsalis pedis and posterior tibial arteries a monophasic or biphasic flow (with loss of reverse flow) in either foot artery, or
 - a toe: brachial index < 0.7
 - transcutaneous oxygen pressure (TcPO₂) < 40 mm Hg

Patient withdrawal criteria after inclusion of the patient in the study:

All patients have the right to cancel the study without prejudice at any point during the entire study. The local investigator may withdraw any patient from the study at any time if medically necessary.

If the observations of the patients are discontinued, the reason(s) will be documented in source documents and recorded in the case report form (CRF) and the PI and DSMB will be notified.

Reasons for study discontinuation of an included patient include the followings:

1. Renal insufficiency with a GFR <60 mL/min/1.73 m²
2. Haematologic or other systematic disorders, such as autoimmune disease, anaemia (Hb <10 g/dL), thrombocytopenia (<100.000/mL) or coagulopathy.
3. protocol violation
4. patient written withdrew consent
5. lost to follow-up

Every attempt will be made to collect follow-up information from all patients, independently from the fact that they continue the participation in the study, except in the case of withdrawn of consent.

Consideration of gender aspects

No gender-related differences are predicted in the study, neither in primary nor in the secondary end points. The APOSEC-Skin trial will include patients of both genders as represented in typical populations of patients with diabetic foot ulcer. After finalizing the study, safety issues and treatment effects in men and women will be separately analysed in post-hoc manner.

3.5 Sample size

Supposing a 50% improvement in the re-epithelialization (which is considered effective using a new treatment in diabetic ulcer), and to adequately power the MARSYAS IV trial to detect this improvement (i.e. an 80% chance of correctly detecting a real effect with a 5% chance of incorrectly rejecting a null hypothesis of no effect), 80 patients should be included to each treatment arm. Accordingly, totally 160 patients should be included in the study and randomized to receive either placebo or APOSEC topical application.

As currently no previous study exist using APOSEC in diabetic ulcer, a more exact sample calculation will be possible after completing the MARSYAS I and II trials. After including 30-30 patients in both arm of the MARSYAS IV study, an interim analysis will be performed, and the sample size maybe recalculated. The sample size calculations will be performed in close cooperation with the head of medical statistics [REDACTED] and his team at the [REDACTED]

3.6 Statistical methods

Assessment of the primary and secondary endpoints

All recruited patients will be included in the primary safety analysis independent of any protocol deviations (modified intent-to-treat analysis).

Data management and statistical analysis will be performed by the Department of Medical Statistics at the Medical University of Vienna. The results of the study will be presented utilizing descriptive statistics and making comparisons between treatment groups with respect to demographic, efficacy, and safety parameters. Descriptive statistics by treatment group for continuous variables consist of sample sizes, means, standard deviations, standard errors of least squares means, medians, and minimum and maximum values. Inferential analysis for continuous variables utilizing appropriate analysis of variance models. Frequencies and percentages will be displayed for ordered categorical variables. Results of categorical parameters will be displayed as frequencies and proportions by treatment group and analysed using chi-square, Mantel-Haenszel, or Fisher's exact test procedures, when appropriate.

Summary statistics on demographic parameters will be presented on the following parameters at baseline: age, race, and ethnicity, gender, height, weight, medical history, concomitant medications, vital signs, clinical laboratory parameters, and dimensions of the ulcer. Subject accounting by treatment group will be displayed at each visit, and the proportion of subjects terminating prematurely for any reason, as well as due to adverse events, will be compared between treatment groups. All statistical tests will utilize a two-sided p-value. Differences associated with p-values between 0.005 and 0.10 will be interpreted as reflecting tendencies toward statistical significance. All analysis will be conducted utilizing SAS (SAS Institute, Inc,

Cary, NC). Appropriate analyses of covariance or multiple regressions will be performed in order to identify healing variable independently of jointly affecting healing.

The following three populations will be analysed:

- The Modified Intention-to-Treat (ITT) population: composed of all screened patients.
 - Screening period: the patients included in the study have presented with a non-healing diabetic foot ulcer (DFU) that is present for at least 6 month. A two week screening period will entail standard of care (SOC), including debridement as determined necessary by the investigator. Subjects with ulcers that decreased in area by less than 30% of their baseline surface area during the screening period qualifies as having a non-healing ulcer and will be enrolled in the study and randomized to treatment assignment using an electronic randomization scheme.
 - Subjects with ulcer decreasing in area 30% or more during this period will be considered to be “healing”, and therefore will not be enrolled into the study.
- The Per-Protocol (PP) population: composed of the subset of the ITT population without any major protocol deviations, and received the study drug or placebo and completed the final FUP.
- The Safety population (SP): composed of all included patients who received the study drug or placebo.

Specification of safety parameters

The definition, reporting, adjudication, and recording of safety related events will adhere to the requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting. The local Ethical Committee, the PI and DSMB will be informed about all adverse events within 24h of the events.

3.7 Conduct

Screening of study subjects

Patients with the diagnosis DFU will be screened. Patients eligible for the MARSYAS IV study will be asked to participate in the trial. After obtaining informed consent, a 2 week screening period will be initiated (as describe above). APOSEC or Placebo will be provided by the Sponsor in a blinded fashion and will be labelled patient specific. APOSEC or placebo will be provided in a carrier gel (NUGEL) and will be applied on the DFU at a 2/week term, in seven consecutive weeks. At week 12 the trial will be concluded. Placebo and APOSEC will be applied twice for 7 weeks.

Information of patient and Written Informed Consent

Eligible patients will be informed thoroughly about the trial designs and course, and the background of the proposed study. Risks and benefits of the treatment and study will be explained. If the patient agrees with the participation in the study, he/she has to sign informed consent prior to the first study procedure, in accordance with the Declaration of Helsinki. Failure to obtain signed informed consent declares the patient to be ineligible.

Patient History

Patient history includes demographics (e.g. age, gender), history of diabetes mellitus, hypertension, hypercholesterolemia, current and previous smoking and chronic cardiac, renal and other concomitant illnesses. Lists of regularly taken medicines will be documented.

Laboratory Assessments

Routine laboratory analysis will be taken as determined by study plan. Additional serum will be frozen for later assessment.

Investigational product, dosage and mode of administration

The human allogeneic GMP APOSEC will be resuspended in carrier hydrophilic gel (at an **anticipated** concentration of 15×10^6 and 30×10^6 apoptotic PBMC per ml carrier gel) and will be prepared and managed according to the GMP/GCP/GLP regulations and other applicable general or local regulations. The study drug will be provided by the GMP production laboratory and will be resuspended in a hydrophilic carrier gel (NUGEL) at the production site. The topical application will be applied according to standard operational procedure (SOPs) determined by the SB.

Medical therapy for other medical conditions will be left to the discretion of the treating physicians.

No drug interaction between APOSEC and any standard medication is anticipated. No contraindication of APOSEC is known.

Concomitant medical treatment

Treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blocker lipid-lowering drugs, β -blockers, antidiabetic treatment or other medications may be initiated during the initial hospitalisation and will be continued throughout the study according to current guidelines.

Concomitant medications will be systematically recorded.

Study course

Week 1-2 – Inclusion – Exclusion criteria, best medical treatment of DFU, including debridement as determined necessary by the investigator. The wound is dressed and cleaned twice a week. Subjects with ulcers that decreased in area by less than 30% of their baseline surface area during the screening period qualified as having a non-healing ulcer. Dressing, Photodocumentation, Digital planimetry, Questionnaire will be performed.

Week 1-2 – Inclusion of study patient. Documentation of non-healing DFU with best medical care. After evidencing that best clinical care does not augment DFU healing by >30%, the study patient is included into non-healing DFU group and is included into the ITT DFU trial. The patient will be randomized to treatment APOSEC or Placebo arm, Dressing, Photo, Digital planimetry, Questionnaire.

Week 3 – IMP application Monday and Thursday (MO, THUR), Dressing, Photo, Digital planimetry, Questionnaire, blood sampling

Week 4 – IMP application Monday and Thursday (MO, THUR), Dressing, Photo, Digital planimetry,

Week 5 – IMP application Monday and Thursday (MO, THUR), Dressing, Photo, Digital planimetry, Questionnaire, blood sampling.

Week 6 – IMP application Monday and Thursday (MO, THUR), Dressing, Photo, Digital planimetry

Week 7 – IMP application Monday and Thursday (MO, THUR), Dressing, Photo, Digital planimetry, Questionnaire, blood sampling

Week 8 – IMP application Monday and Thursday (MO, THUR), Dressing, Photo, Digital planimetry

Week 9 – Photo, Digital planimetry, Questionnaire, blood sampling

Week 10 – Dressing, Photo, Digital planimetry, Questionnaire, blood sampling

Week 11- Follow up Nr. 1 Photo, Digital planimetry, Blood drawing, Questionnaire, Blood sampling.

Time table and schedule

Assessment Schedule for clinical phase II trial in diabetic ulcer												
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11
	best care	best care								FU	follow-up	
Incl, Excl. criteria	X											
IMP application			X X*	X X	X X	X X	X X	X X	X X	X X		
Dressing			X X	X X	X X	X X	X X	X X	X X	X X		
Photo			X X	X X	X X	X X	X X	X X	X X	X X	X	X
Digital planimetry			X X	X X	X X	X X	X X	X X	X X	X X	X	X
Blood drawing		X		X		X		X		X	X	X
Questionnaire		X		X		X		X		X	X	X
Safety assessment												
AEs												
SAEs												

*Monday and Thursday

Changes in the Conduct of the Study

Protocol amendments

Proposed amendments must be submitted to the appropriate CA and ECs. Substantial amendments may be implemented only after CA/EC approval has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving CA/EC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Study termination

The Steering Committee will monitor the progress of the study. The DSMB will monitor the safety of the study. If warranted, the study may be suspended or discontinued early if there is an observation or a new development presenting an unreasonable risk to the study population.

In the event of study suspension or termination, the Sponsor will send a report outlining the circumstances to the IRB/EC, Competent Authority and to all Investigators. A suspended or terminated study may not be reinitiated without approval of the reviewing IRB/EC.

Clinical Study Report (CSR)

Within one year after the final completion of the study, a full CSR will be prepared by the sponsor and submitted to the EC and the competent authority. The Investigator will be asked to review and sign the final study report.

Financial Funding of the Study

The present study is a non-commercial study, whereby the Aposcience AG acts as Leader of the study. The study is planned to be funded by the EU Commission (HORIZON 2020).

Our cost calculation for this HORIZON 2020 application for this MARSYAS IV trial was estimated by a German CRO to be around €1.2 million with our envisioned study protocol. This cost estimation is based on already successfully performed phase II studies in Central and Eastern European countries.

Insurance

During their participation in the clinical trial the patients will be insured as defined by legal requirements. The investigator of the clinical trial will receive a copy of the insurance conditions of the 'patients insurance'.

Quality Control Monitoring

A CRO will be sub-contracted by Aposcience AG and will perform the monitoring and organization of the entire study. This will include review of presence of signed informed consent of all patients, review source documentation to verify all data, appropriate inclusion/exclusion criteria. The data management department of CRO will perform automatic checks on the data, and send queries to the sites if applicable.

On site audits

To ensure compliance with regulatory requirements, a member of the consortium (or a designated CRO's) quality assurance unit, may arrange to conduct an audit to assess the performance of the study at the study site and of the study documents originating there. The investigator and/or institution will be informed of the audit time and outcome.

In addition, inspections by regulatory health authority representatives and IEC(s)/IRB(s) are possible. The investigator should notify the sponsor / CRO immediately of any such inspection.

A clinical agreement between the investigators and study sponsor and/or its designee will be signed by both parties. The agreement includes the conduct of the study, audits, financials and other regulatory issues. The clinical study coordinators will provide access to medical records and other relevant documentation to CRO/EC as required. Regulatory authorities worldwide may inspect the investigator during and after the study. The investigator should contact the sponsor / CRO immediately if this occurs, and must cooperate with the regulatory authority inspections as required.

Provision of Information

The CRO, Steering Committee, EC and DSMB should be notified of any Serious Adverse Event within 24 hours or the site's awareness. All appropriate CRFs should be completed as soon as possible and no later than 5 days after enrolment or indicated follow-up. Once the completed forms have been monitored, they shall be sent to CRO.

Documentation and data management

Source Documentation

Investigators must maintain information in the patient's medical records, which corroborate data collected on the case report forms. In order to comply with these regulatory requirements, at minimum, the following is a

list of information that should be maintained and made available as required by monitors and/or regulatory inspectors.

- Medical history/physical condition of the study patient before inclusion in the study sufficient to verify investigational plan entry criteria (if not already present).
- Signed and dated written informed consent form. Dated and signed by the patient and by the physician involved in the study.
- Dated and signed notes on the day of entry into the study, protocol number, Clinical Site, patient number assigned and a statement that informed consent was obtained.
- Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams).
- Serious Adverse events reported and their resolution, including supporting documents such as discharge summaries, lab results, clinical notes.
- Notes regarding investigational plan-required and prescription medications taken during the study (including start and stop dates).
- Study patient's condition upon completion of or withdrawal from the study.

Screening and Enrolment Log

A subject screening and enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

Data Collection

Data collected at all visits are entered into the electronic individual Case Report Forms (CRF) for later transferal into a database for statistical analysis. The CRFs will be verified against source documents following guidelines established before study onset as detailed in the Monitoring Plan. Maintenance of the study database will be performed by the sponsor.

Case report form (CRF)

All required data will be accurately recorded by authorized person on Case Report Forms (CRFs). The training of appropriate clinical site personnel to complete the CRF will be the responsibility of the study monitor.

All CRFs, study records, reports and source documents that support the CRFs must be retained in the Investigator files for a minimum 15 years (Austrian rules) following notification by the Sponsor or designee that all investigations have been completed, and will further be retained in accordance with local and international guidelines as identified in the clinical study agreement. This documentation must be accessible upon request by international regulatory authorities, the Sponsor, DSMB, SC and CEC. The delegates of the sponsor will approve archiving or transfer of the documentation for relocation purpose of premises, in writing, prior to the actual file transfer.

Original completed eCRF's will remain at the sponsor site and will be archived (in electronic and Paper form) after the study close out. Details about the eCRF will be summarized in a separate Data-Management Plan (DMP).

Publication of study results

The findings of this study will be published by the sponsor (investigators) in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all co-investigators before submission. Confidentiality of subjects in reports/publications will be guaranteed.

Study centres:

To be announced. This study is subcontracted to a CRO that has a track record in organizing and performing phase II studies in central and eastern European countries.

4.1 Identifier

MARSYAS V (Allogeneic GMP APOSEC AMI)

Prospective non-randomized First-In-Man multicentre Phase I-II trial to investigate the Safety and Feasibility of single intravenous administration of human allogeneic APOSEC in acute myocardial infarction.

The clinical trial will be registered at EudraCT (European Clinical Trials Database). The study will be registered via the www.clinicaltrials.com homepage.

4.2 Study design and endpoints

Study design:

This is a prospective non-randomized First-In-Man multicentre Phase I-II clinical trial including patients with ST-segment elevation myocardial infarction (STEMI) and receiving single intravenous dose of allogeneic human APOSEC as an upstream therapy of primary percutaneous coronary intervention (PCI).

Primary endpoints:

The primary endpoint of the MARSYAS V is the safety and feasibility of the single intravenous application of allogeneic APOSEC as an upstream therapy in patients with STEMI and primary PCI. The APOSEC treatment will be an additional treatment to the current guidelines. The safety and feasibility will be expressed in NACE (net adverse clinical events, including death, re-infarction, target vessel revascularization of the infarct-related artery, stroke and bleeding).

All adverse events (AEs) will be evaluated from the time of informed consent until the last FUP investigation or termination of the study. All SAEs will be reported within 24h after occurrence to the PI and the independent Data Safety Monitoring Board (DSMB).

Secondary endpoints:

The following secondary endpoints will be evaluated

- microvascular obstruction and myocardial oedema measured by cMRI at day 3 post-AMI
- infarct size and ejection fraction (EF) measured by cMRI with late enhancement (LE) at 6 month FUP,
- Implantation of pacemaker or defibrillator,
- Re-hospitalization due to cardiac events, such as heart failure, angina pectoris, re-AMI.
- Life-threatening ventricular arrhythmias from 24h post primary PCI (ventricular tachycardia, ventricular fibrillation)

Cardiac MRI with LE is currently the gold standard for the assessment of LV volumes, EF and infarct size. All cMRIs will be evaluated by an independent MRI core laboratory, not involved in patient recruitment.

The following cMRI parameter will be evaluated:

- LV end-diastolic volume (LVEDV);
- LV end-systolic volume (LVESV);
- Infarct size (late enhancement);
- Regional systolic and diastolic LV function (peak filling rate, wall thickening);
- Myocardial oedema and fibrosis.

Further parameters to be measured:

- routine laboratory parameters, such as total white blood cell count and peripheral blood leukocyte subsets, granulocytes (basophils, eosinophils and neutrophils). The timing of blood sampling: baseline before infusion, at the end of the infusion, then at 1, 2 and 3rd days post-infusion.
- Cardiac enzymes, such as troponinT and creatine kinase with its MB fraction.
- NT-proBNP levels at baseline, at 3 months and 1-year

Arrangements to minimize biases:

- The primary end point (safety and feasibility) will be evaluated by the PI and a statistician, who is not involved in the recruitment of patients.
- The primary and secondary endpoints will be assessed by the independent DSMB. The members of the DSMB do not participate in the recruitment of patients, and are also independent from the recruiting investigators.
- Statistical data analyses will be performed by an independent statistician, not involved in the treatment or imaging analysis.

4.3 Scientific advice / protocol assistance / communication with regulatory / competent authorities / ethics committees

The study protocol will be submitted to the local Ethical Committees. Patient information and consent will be translated to Romanian, Danish and Polish language.

Permission for conduction of the study will be requested from the relevant Hospitals and Legal Authorities.

The clinical trial will be registered at EudraCT (European Clinical Trials Database). The study will be registered via the www.clinicaltrials.com homepage.

Investigator Responsibility / Performance

Prior to starting enrolment of patients, the investigator must read and understand the Study protocol and must sign and complete the Protocol Signature page. The Investigator Agreement documents agreement to all conditions of the Study protocol and agreement to conduct the study accordingly. This study will be conducted in accordance with the Declaration of Helsinki, and other applicable regulatory requirements, the Study Protocol, or any conditions of approval imposed by the IRB/EC or regulatory authorities. The Investigator will ensure that all work and services will be conducted in accordance with the highest standards of medical and clinical research practice.

Required Documents

The following documents will be submitted to the Sponsor or designee prior to patient enrolment:

- Signed Protocol Signature Page
- Recent signed and dated English Curriculum Vitae (CV) for the Site Principal Investigator and co-investigators. This CV should clearly show the investigator's qualifications and experience.
- Copy of the written confirmation of the EC regarding approval of the protocol including version number and date, patient information sheet and informed consent including version and date and other adjunctive patient material.

- List of EC members, including name, title, occupation and any institutional affiliation of each member. If the EC members list is not available, the General Assurance or EC Recognition number should be provided.
- Signed study agreement.

The investigator's file will contain the protocol/amendments, EudraCT forms, CRFs (eCRF printout), standard operating procedures (SOPs), data clarification and query forms, EC/IRB and Health Authority approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, screening and enrolment logs, and other appropriate documents/correspondence as per ICH/Good Clinical Practice (GCP) and local regulations.

These two categories of documents must be kept on file by the investigator for as long as needed to comply with national and international regulations (in Austria 15 years after end of study). If source documents are not durable as long as needed (e.g. ECG printouts) they must be preserved as a copy.

When source documents are required for the continued care of the subject, appropriate copies will be made for storing outside of the site.

Confidentiality

All data and information collected during this study related to the participating subject will comply with the standards for protection of privacy based on applicable local/ national requirements for subject's confidentiality. All data used in the analysis and summary of this study will be anonymous, and without reference to specific study patient names. Access to study patient files will be limited to authorized personnel of the Sponsor, the Investigator, and research staff. Authorized regulatory personnel have the right to inspect and copy all records pertinent to this study, but all efforts must be made to remove patient's personal data.

Informed consent of subjects

The study patients' written informed consent will use the local IRB/EC-approved informed consent form. The study will be explained to the study patients in lay language. The Investigator, or representative, will be available to answer all of the study patient's study-related questions. Study patients will be assured that they may withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated.

Institutional Review Board/Ethics Committee Approval (IRB/EC)

According to the local regulation the Investigator will have all necessary approvals including written approval from the IRB/EC of the Institution or appropriate other accepted IRB/EC prior to enrolling patients in the study. A copy of the written approval will be provided to the Sponsor and will include the following:

- Statement of IRB/EC approval for the proposed study at the institution
- Date the study was approved and the duration of the approval
- Listing of any conditions attached to the approval
- Identification of the approved primary investigator
- Signature of the IRB/EC chairperson
- IRB/EC approval of the informed consent form
- IRB/EC approval of the final protocol

Any substantial amendments to the protocol, as well as associated consent form changes, will be submitted to the IRB/EC and written approval obtained prior to implementation. Minor changes which do not affect the subject safety will be subject of notification.

Serious adverse event reports will be submitted to IRB/EC as requested. Annual and final reports will be provided to IRB/EC as appropriate.

Acknowledgement / approval of the study

The Sponsor will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to the local Ethics Committee (EC). Approval from the committee must be obtained before starting the study.

The clinical trial shall be performed in full compliance with the legal regulations according to the Drug Law of the relevant country.

The study will be registered at the European Clinical Trial Database (EudraCT) using the required forms. The timelines for (silent) approval set by national law must be followed before starting the study.

COMMITTEES

Steering Committee (SC)

The Steering Committee (SC) is formed by a Chairman ([REDACTED]), representative(s) from the Aposcience AG and the Clinical Research Organisation (CRO) responsible for the monitoring of the study. The SC is responsible for managing the scientific and operational aspects of the study. The SC has the responsibility to review the daily conduct of the study.

Data Safety Monitoring Board (DSMB)

The Data Safety Monitoring Board (DSMB), being an independent committee, will be composed of at least two (interventional) cardiologists [REDACTED] and a biostatistician (TBD). For this study the DSMB will be responsible for decision of any potentially significant patient safety-related observations. The DSMB will function in accordance with applicable regulatory guidelines. The DSMB members will not be employees by the Sponsor, or serve on the DSMB or a committee of a competing medical trial. Members will not have any affiliation with the Investigators, the CRO or the clinical investigation sites.

The DSMB will be set up prior to the start of the DSMB activities, in which the responsibilities of the DSMB will be described with respect to safety review, formal reporting to the Steering Committee, and if applicable, safety stopping rules.

The DSMB chairperson will notify the Sponsor and / or the Steering Committee (through the CRO) by confidential memo of any safety or compliance issues. The committee will also provide confidential recommendations, when necessary, of study termination based on the safety stopping rules, or because a clinically significant result was identified in safety analyses of the data. All DSMB reports will remain strictly confidential, but will be made available to the regulatory body upon request.

Clinical Events Committee (CEC)

The Clinical Events Committee (CEC) is an independent committee comprised of cardiologists who are not participants in the study. The CEC is responsible for the categorization of NACE (Net Adverse Events, including Death, MI, revascularization of the infarct-related artery, CABG, Stent Thrombosis or bleeding complications) in the study, based on the definitions in the protocol.

Prior to any CEC activity, a CEC charter will be developed, which will describe the events to be adjudicated, the minimum amount of data required, and the algorithm followed in order to classify the events.

Periodic Monitoring

The designated monitor will contact and visit the investigator regularly and will be allowed to have access to all source documents needed to verify the entries in the eCRFs and other protocol-related documents provided that subject confidentiality is maintained in agreement with local regulations. It will be the monitor's responsibility to inspect the eCRFs at regular intervals according to the monitoring plan throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of the main efficacy, safety, and tolerability endpoints.

The monitor will follow a predefined monitoring plan and will be working according to SOPs and will provide a monitoring report after each visit for the Sponsor. Depending on the quality of the data, additional monitoring visits may be necessary according to the Sponsor's discretion. The investigator will resolve discrepancies of data.

4.4 Subjects/population(s)

Patients with first ST-segment elevation myocardial infarction will be included. The planned number of recruited patients is 35, with estimated lost to follow-up (FUP) of 5 patients.

Inclusion criteria:

1. Age >18 years and <80 years.
2. Signed written informed consent form.
3. STEMI verified by 12-lead ECG, clinical symptoms, according to guidelines (2013 ACCF/AHA Guideline for the Management of ST-Elevation Myocardial Infarction A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, JACC 2013; Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation, EHJ 2012), intended to perform primary PCI.
4. Time from symptom onset to STEMI diagnosis <6 hours.
5. No previous myocardial infarction assessed by clinical history

Exclusion criteria:

1. Prior myocardial infarction
2. Killip Class 3 and 4 (pulmonary oedema, cardiogenic shock).
3. Cardiac pacemaker or implantable cardioverter defibrillator (ICD), claustrophobia or severe obesity making the cMRI image impossible.
4. Known malignant disease.
5. Known terminal illness.
6. Advanced hepatic disease.
7. Known chronic renal disease: eGFR < 60mL/min/1.73m².
8. Acute or chronic hepatitis, HIV-infection, auto-immune disease.
9. Acute systemic infection or fever.
10. History of bleeding diathesis or coagulopathy.
11. Known hypersensitivity or contraindication to any antiplatelet medication, aspirin, heparin, specified for use in the study (clopidogrel prasugrel, ticagrelor and ticlopidine) or stainless steel.

12. Known medical illness (e.g. cancer, stroke with neurological deficiency) or history of substance abuse (alcohol, drugs, etc.) that may cause non-compliance with the protocol or confound the data interpretation.
13. Pregnancy or unreliable contraception.
14. Patients participating in any investigational drug trial within 30 days of inclusion in the MARSYAS V study.

Patient withdrawal criteria after inclusion of the patient in the study:

All patients have the right to cancel the study without prejudice at any point during the entire study. The local investigator may withdraw any patient from the study at any time if medically necessary.

If the observations of the patients are discontinued, the reason(s) will be documented in source documents and recorded in the case report form (CRF) and the PI and DSMB will be notified.

Reasons for study discontinuation of an included patient include the following:

1. No primary PCI is possible for any reason (indicated acute bypass operation after diagnostic coronary angiography, small vessel disease, no significant coronary lesion etc.)
2. Renal insufficiency with a GFR <60 mL/min/1.73 m²
3. Haematologic or other systematic disorders, such as autoimmune disease, anaemia (Hb <10 g/dL), thrombocytopenia (<100.000 /mL) or coagulopathy.
4. protocol violation
5. patient written withdrew consent
6. lost to follow-up

Every attempt will be made to collect follow-up information from all patients, independently from the fact that they continue the participation in the study, except in the case of withdrawn consent.

Consideration of gender aspects

No gender-related differences are predicted in the study, neither in primary nor in the secondary end points. The APOSEC-AMI trial will include patients of both genders as represented in typical populations of patients with STEMI. After finalizing the study, safety issues and treatment effects in men and women will be separately analysed in post-hoc manner. The ratio men : women in the APOSEC-AMI trial will reflect the incidence of the STEMI in men and women in the participating study sites across Europe. This ratio will not be the object of an a priori stratification. Predefined restrictions and precautions for inclusion of females of childbearing potential will be applied.

4.5 Sample size

Since this is a First-In-Men study with safety and feasibility primary end point, no formal sample size calculation is made. This is a hypothesis generating pilot study, because at present no evidence about the expected magnitude of the effect is available in humans. Data generated from this study will be compared against historical (historical control) and literature controls.

4.6 Statistical methods

Assessment of the primary endpoint

All recruited patients will be included in the primary safety analysis independent of any protocol deviations (modified intent-to-treat analysis).

Descriptive statistics will be provided by the treatment group and for the whole population, which includes the screened (with given informed consent) but not included patients.

The following three populations will be analysed:

- The Modified Intention-to-Treat (ITT) population: composed of all screened patients
- The Per-Protocol (PP) population: composed of the subset of the ITT population without any major protocol deviations, and received the study drug and completed the final FUP.
- The Safety population (SP): composed of all included patients who received the study drug.

Data of all participating centres will be used. Data will be summarized with respect to demographic and baseline characteristics, efficacy observations and measurements, safety observations and measurements.

A single interim analysis will be performed, once 50% of the planned (evaluable) patients have completed the month-6 MRI. This adaptive design interim analysis is to decide on the recommended sample size re-estimation. At the end of this main study part, a complete report of the results will be produced.

Specification of safety parameters

- Clinical visit: vital signs, symptoms
- ECG,
- safety laboratory parameters (electrolytes, blood counts, eGFR, liver function tests, CK/MB and hsCRP),
- documentation of NACE events (major adverse cardiovascular events, such as re-AMI, stroke, target vessel revascularization and bleeding) and any adverse events.
- 24h ECG 1 day after STEMI and drug administration.

Methods and timing for assessing, recording, and analysing safety parameters

- Vital signs will be recorded before and during infusion and before discharge.
- Safety lab (see above) and an ECG will be obtained before and immediately after the end of infusion and before discharge.
- Adverse events will be recorded from time of Informed Consent until last FUP time,
- Serious adverse events will be recorded from time of Informed Consent until 30± 7 days after last FUP.

The definition, reporting, adjudication, and recording of safety related events will adhere to the requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting. The local Ethical Committee, the PI and DSMB will be informed about all adverse events within 24h of the events.

Assessment of secondary endpoints

All statistical tests performed for secondary endpoints are explorative. Due to relatively small number of patients, no subgroup analysis is planned.

- cMRI-LE: LV volumes (EDV, ESV, SV) and EF, infarct size, infarct transmural, segmental wall thickening and diastolic function (peak filling rate), segmental wall contraction velocity, LV mass, LV mass volume, microvascular obstruction, myocardial oedema and fibrosis, cardiac output, cardiac index.
- Transthoracic echocardiography: diameter of the left and right atrium, end-diastolic diameter of the LV, end-systolic diameter of the LV, fractional shortening, degree of mitral insufficiency, degree of tricuspidal insufficiency, pulmonary pressure, diastolic function (E, A wave, E/A ratio, E/E ratio), tissue Doppler image, strain imaging.

4.7 Conduct

Screening of study subjects

All patients with the diagnosis STEMI and planned primary PCI will be screened. Patients eligible for the MARSYAS V study will be asked to participate in the trial. After obtaining informed consent, a blood sample will be taken and the study medication will be administered intravenously under continuous ECG monitoring before primary PCI.

Information of patient and Written Informed Consent

Eligible patients will be informed thoroughly about the trial designs and course, and the background of the proposed study. Risks and benefits of the treatment and study will be explained. If the patient agrees with the participation in the study, he/she has to sign informed consent prior to the first study procedure, in accordance with the Declaration of Helsinki. Failure to obtain signed informed consent declares the patient to be ineligible.

Patient History

Patient history includes demographics (e.g. age, gender), history of myocardial infarction, previous Coronary Artery Bypass Graft Surgery (CABG) and Percutaneous Coronary Intervention (PCI), diabetes mellitus, hypertension, hypercholesterolemia, current and previous smoking and chronic cardiac and other concomitant illnesses. Lists of regularly taken medicines will be documented.

Laboratory Assessments and ECGs

CK and CKMB and cardiac Troponin T will be routinely measured before primary PCI, at day 1, 2, and 3. Before hospital discharge, cardiac proBNP will be measured. Study specified blood sampling will be taken and plasma and serum will be frozen for later enzymatic or other laboratory controls together with the routine blood sampling. At follow-ups 6 week, 6 and 12 months blood sampling will be done and proBNP will be measured. Plasma and Serum will be frozen for later assessment.

Twelve-lead ECG will be performed prior to the primary PCI and within 24h post procedure. Further, a 12-lead ECG will be performed at the 6 weeks, 6 and 12 months visit. Holter 24h ECG will be performed within 24h after drug administration and primary PCI.

Investigational product, dosage and mode of administration

The human allogeneic APOSEC infusion (from 2.5×10^9 apoptotic PBMC) will be prepared and managed according to the GMP/GCP/GLP regulations and other applicable general or local regulations. The study drug will be provided by the GMP production laboratory in a white powder form, and will be diluted into

500ml physiologic solution. The infusion will be given with an infusion pump with 15ml/min in a peripheral (cubital) vein in the emergency room before primary PCI, as an upstream therapy. The infusion duration should be approximately 30 min, and should be finished prior to primary PCI.

Antiplatelet and anticoagulant therapy before and after PCI, before and after discharge from the hospital will be left to the discretion of the treating physicians. Implantation of a bare metal stent or drug-eluting stent will be left to the discretion of the interventional cardiologist.

No drug interaction between APOSEC and any standard medication is anticipated. No contraindication of APOSEC is known.

Concomitant medical treatment

Treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blocker lipid-lowering drugs, b-blockers or other medications may be initiated during the initial hospitalisation and will be continued throughout the study according to current ESC guidelines.

Concomitant medications will be systematically recorded in the CRF during hospital stay, at hospital discharge, at 6 weeks, 6 and 12 months.

Study course

Day-0 Drug (APOSEC) treatment

- STEMI diagnosis, planned primary PCI
- Patient screening, eligibility criteria
- Written informed consent
- Blood sampling
- Administration of intravenous infusion of APOSEC
- Primary PCI

Day-1 Clinical treatment

- Blood sampling
- Transthoracic echocardiography to evaluate left ventricular function
- Holter 24h ECG recording

Day 2 Clinical treatment

- Blood sampling

Day 3 Cardiac LE-MRI

- Blood sampling
- Cardiac LE-MRI with 1.5 T equipment

Week 6 Clinical Follow-up

- Blood sampling
- Clinical follow-up
- ECG

Month 6 Clinical follow-up

- Blood sampling
- Clinical follow-up
- ECG
- Cardiac LE-MRI

Month 12 Clinical final follow-up

- Blood sampling
- Clinical follow-up
- ECG

Time table and schedule

Sequences of Procedures	Day 0/1.	Day 0/2.	Day 0/3.	Day 1	Day 2	Day 3	Week 6	Month 6	Month 12
STEMI Symptom onset	X								
Infusion		X							
PCI/Stent			X						
Informed consent	X								
cMRI-LE						X		X	
Clinical Visit	X						X	X	X
Echocardiography				X					X
ECG	X					X	X	X	X
Blood sampling	X		X	X	X	X	X	X	X
24h ECG				X					
Safety assessment									
AEs									
SAEs									

Changes in the Conduct of the Study

Protocol amendments

Proposed amendments must be submitted to the appropriate CA and ECs. Substantial amendments may be implemented only after CA/EC approval has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving CA/EC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Study termination

The Steering Committee will monitor the progress of the study. The DSMB will monitor the safety of the study. If warranted, the study may be suspended or discontinued early if there is an observation or a new development presenting an unreasonable risk to the study population.

In the event of study suspension or termination, the Sponsor will send a report outlining the circumstances to the IRB/EC, Competent Authority and to all Investigators. A suspended or terminated study may not be reinitiated without approval of the reviewing IRB/EC.

Clinical Study Report (CSR)

Within one year after the final completion of the study, a full CSR will be prepared by the sponsor and submitted to the EC and the competent authority. The Investigator will be asked to review and sign the final study report.

Financial Funding of the Study

The present study is a non-commercial study, whereby the [REDACTED] acts as the Sponsor. The study will be completely funded by HORIZON 2020.

Insurance

During their participation in the clinical trial the patients will be insured as defined by legal requirements. The investigator of the clinical trial will receive a copy of the insurance conditions of the 'patients insurance'.

Quality Control Monitoring

A CRO will be appointed to perform the monitoring. This will include review of presence of signed informed consent of all patients, review source documentation to verify all data, appropriate inclusion/exclusion criteria. The data management department of the CRO will perform automatic checks on the data, and send queries to the sites if applicable.

On site audits

To ensure compliance with regulatory requirements, a member of the consortium (or a designated CRO's) quality assurance unit, may arrange to conduct an audit to assess the performance of the study at the study site and of the study documents originating there. The investigator and/or institution will be informed of the audit time and outcome.

In addition, inspections by regulatory health authority representatives and IEC(s)/IRB(s) are possible. The investigator should notify the sponsor / CRO immediately of any such inspection.

A clinical agreement between the investigators and study sponsor and/or its designee will be signed by both parties. The agreement includes the conduct of the study, audits, financials and other regulatory issues. The clinical study coordinators will provide access to medical records and other relevant documentation to CRO/EC as required. Regulatory authorities worldwide may inspect the investigator during and after the

study. The investigator should contact the Sponsor / CRO immediately if this occurs, and must cooperate with the regulatory authority inspections as required.

Provision of Information

The CRO responsible for monitoring, Steering Committee, EC and DSMB should be notified of any Serious Adverse Event within 24 hours or the site's awareness. All appropriate CRFs should be completed as soon as possible and no later than 5 days after enrolment or indicated follow-up. Once the completed forms have been monitored, they shall be sent to the CRO. All echocardiographic, LE-MRI and 24h Holter ECG recordings will be forwarded as soon as possible to the Core Lab (defined later) and not later than two weeks.

Documentation and data management

Source Documentation

Investigators must maintain information in the patient's medical records, which corroborate data collected on the case report forms. In order to comply with these regulatory requirements, at minimum, the following is a list of information that should be maintained and made available as required by monitors and/or regulatory inspectors.

- Medical history/physical condition of the study patient before inclusion in the study sufficient to verify investigational plan entry criteria (if not already present).
- Signed and dated written informed consent form. Dated and signed by the patient and by the physician involved in the study.
- Dated and signed notes on the day of entry into the study, protocol number, Clinical Site, patient number assigned and a statement that informed consent was obtained.
- Dated and signed notes from each study patient visit with reference to the CRFs for further information, if appropriate (for specific results of procedures and exams).
- Serious Adverse events reported and their resolution, including supporting documents such as discharge summaries, cath lab reports, ECGs, lab results, clinical notes.
- Notes regarding investigational plan-required and prescription medications taken during the study (including start and stop dates).
- Study patient's condition upon completion of or withdrawal from the study.

Screening and Enrolment Log

A subject screening and enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

Data Collection

Data collected at all visits are entered into the electronic individual Case Report Forms (CRF) for later transferal into a database for statistical analysis. The CRFs will be verified against source documents following guidelines established before study onset as detailed in the Monitoring Plan. Maintenance of the study database will be performed by the sponsor.

Case report form (CRF)

All required data will be accurately recorded by an authorized person on Case Report Forms (CRFs). The training of appropriate clinical site personnel to complete the CRF will be the responsibility of the study monitor.

All CRFs, study records, reports and source documents that support the CRFs must be retained in the Investigator files for a minimum 15 years (Austrian rules) following notification by the Sponsor or designee that all investigations have been completed, and will further be retained in accordance with local and international guidelines as identified in the clinical study agreement. This documentation must be accessible upon request by international regulatory authorities, the Sponsor, DSMB, SC and CEC. The delegates of the sponsor will approve archiving or transfer of the documentation for relocation purpose of premises, in writing, prior to the actual file transfer.

Original completed eCRF's will remain at the sponsor site and will be archived (in electronic and Paper form) after the study close out. Details about the eCRF will be summarized in a separate Data-Management Plan (DMP).

Publication of study results

The findings of this study will be published by the sponsor (investigators) in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all co-investigators before submission. Confidentiality of subjects in reports/publications will be guaranteed.

Study centres:

1. [REDACTED]
2. [REDACTED]
3. [REDACTED]

Clinical Trial testing of APOSEC™ as a Regenerative Medicine

Short name: APOSEC

List of participants

Participant No.		Participant organisation name	Country
1 (Coord.)			Austria
2			Austria
3	APO	Aposcience AG	Austria
4			Austria
5			Germany
6			Denmark
7			Spain
8			Romania
9			Poland
10			Slovakia

Table of Contents

1	EXCELLENCE.....	2
1.1	OBJECTIVES	2
1.2	RELATION TO THE WORK PROGRAMME	3
1.3	CONCEPT AND APPROACH	5
1.4	AMBITION	17
2	IMPACT.....	24
2.1	EXPECTED IMPACTS	24
2.2	MEASURES TO MAXIMISE IMPACT	32
3	IMPLEMENTATION.....	39
3.1	WORK PLAN — WORK PACKAGES, DELIVERABLES AND MILESTONES.....	39
3.2	MANAGEMENT STRUCTURE AND PROCEDURES	59
3.3	CONSORTIUM AS A WHOLE	64
3.4	RESOURCES TO BE COMMITTED	65

1 Excellence

1.1 Objectives

The strategic objective of the APOSEC project is to achieve clinical proof-of-concept by performing clinical Phase I & II studies utilising the patent and trademark protected drug product APOSEC™. Authorisation for APOSEC™ production has already been granted by the relevant Austrian regulatory body (AGES) and complementary studies on toxicology, efficacy and mode of action will ensure full compliance and readiness to swiftly move into Phase III trials at the completion of the proposed H2020 project. The results gained within these trials will provide first-in-man evidence of the ground-breaking potential of APOSEC™ to provide new therapies to millions of sufferers in modern society worldwide in the fields of:

- Wound healing (e.g. for chronic skin wounds and diabetic ulcers)
- Acute Myocardial Infarction (AMI)

The specific objectives of the APOSEC project are:

1. To develop and apply potency and stability assays, to validate its shelf life and to produce sufficient APOSEC™ for the clinical trials.
2. To conduct toxicology and safety pharmacology studies to assess the safety of the medicinal drug candidate in animal models in order to address preclinical and toxicological matters raised by regulatory authorities.
3. To conduct Phase I topical and systemic clinical trials of APOSEC™ in healthy subjects.
4. To conduct a Phase II randomised, double blind prospective clinical trial utilising APOSEC™ as topical treatment for Diabetic foot ulcer.
5. To conduct a Phase I-II clinical trial to determine the safety of a single intravenous application of APOSEC™ in patients with ST-elevation myocardial infarction (STEMI) as an upstream therapy before primary percutaneous coronary intervention (PCI).
6. To perform parallel, mode of action research for APOSEC™ to optimise the concentration and the frequency of application for Phase I, II and later on Phase III clinical trials.
7. To promote the APOSEC project and its results widely and effectively to relevant stakeholder groups to fulfil both dissemination and exploitation strategies.

The fundamental need for the clinical approval of APOSEC™ is to provide new and innovative supplementary treatments in the fields of wound healing and acute myocardial infarction. These health issues are associated with the modern society epidemic of diabetes in connection with the trend towards an aging society. Diabetes leads to chronic skin wounds (diabetic ulcer) and atherosclerosis which causes acute myocardial infarction. In Europe, 56.3 million people suffered from diabetes in 2013 and by 2035, this number will increase by 22% to 68.9 million corresponding to about 10% of the population over 25 years of age.

Compelling research results and subsequent publications as well as APOSEC™ worldwide IP protection and innovation potential are described in Section 1. The requested H2020 funding will leverage these significant investments enabling APOSEC™ to revolutionise standard therapies as a comparatively cost-effective, easy-to-use product that will be available as an “off-the-shelf” supplementary therapy to improve overall treatment effectiveness and an accelerated recovery time.

APOSEC™ will be uniquely the most sustainable drug of its type on the market being produced from the white blood cell pellet, an abundant blood transfusion waste product.

1.2 Relation to the work programme

The proposal relates to topic PHC 15 – 2014/15: “Clinical research on regenerative medicine” in the call “Innovative treatments and technologies” of the work programme 2014 – 2015 “8. Health, demographic change and wellbeing”.

1.2.1 Clinical research

We are proposing the performance of four clinical trials in the phases I and/or II utilizing the patented therapeutic APOSEC™ in the indications:

- Phase I first-in-man in topical application
- Phase I first-in-man in systemic application
- Phase II diabetic foot ulcer (topical application) and
- Phase I-II first-in-man acute myocardial infarction (AMI)

APOSEC™ is a new powerful drug compound that is therapeutically effective in a number of different disease entities involving hypoxia and inflammatory processes leading to healing deficiencies. The APOSEC project, comprising clinical phase I trials (safety studies) and clinical phase II trials (establishing the efficacy of the drug) is designed to bring forward the ‘proof of concept’ of the biological APOSEC™, that is, to decide, whether this new therapy can be taken forward to the next level of testing or can be discarded.

1.2.2 Regenerative medicine

The drug compound APOSEC™ is a novel development in regenerative medicine answering to a recent shift of paradigm in the field of stem cell research. It is recognized that the secreted factors of the transplanted cells play a major role in the tissue regeneration having 1) own regenerative capacity; 2) chemotactic effect for recruiting and homing of autologous reparative cells; 3) immune-modulating character contributing to allograft survival and microenvironment regulation and 4) influence on the secretory profile of the transplanted and host tissue cells. As a result, cell-free regenerative therapy came into the forefront in recent years with an increasing body of evidence of angiogenesis, neuroprotection, immunomodulation, and maintenance of a homeostatic environment by the secretome of the cells. The regenerative power of APOSEC™ is due to the stimulation of regenerative processes, manipulation of the adaptive immune system, platelet activation, induction of cytoprotection, effects on microvascular obstruction, and induction of neoangiogenesis. Within the APOSEC project it shall be shown that APOSEC™ supports human tissue in recovering from damage caused by hypoxia induced inflammation.

1.2.3 The innovative potential of APOSEC™

The drug compound APOSEC™ is such that it takes the current standard therapies to a new level of innovation. APOSEC™ is a therapy for widespread, non-communicable disease entities like diabetic foot ulcer (DFU), which afflicts up to 25% of patients with diabetes. As the diabetes epidemic continues to grow, this is a significant public health issue with 56.3 million sufferers in Europe in 2013. Acute myocardial infarction (AMI) and the prevention of myocardial remodelling, the process responsible for heart insufficiency also represents a potential market for the product. APOSEC™ can revolutionize standard therapies due to its character of being a comparatively cost-effective, easy-to-use product that will be available “off-the-shelf” as a supplementary therapy to the currently recommended treatment guidelines, enhancing overall treatment effectiveness and speeding recovery time.

The therapeutic power of APOSEC™ could also be extended towards other diseases involving hypoxia and inflammatory processes, such as myocardial ischemia/injury, infarction, stroke, myocarditis and spinal cord injury. Based on existing research results, we expect that further orphan indications can be treated by intravenous and topical applications of APOSEC™. **The aim of the APOSEC project is to establish all mandatory regulatory and safety prerequisites for broad clinical testing and ultimately regulatory approval.**

1.2.4 Improve the ability to treat and manage diseases

Cell products derived from autologous and allogeneic sources are already in phase II-III studies in multiple indications. The handling of these cell products in the therapeutic setting is difficult because of storing capacity, issues of cell transport to mandated clinics, the need of trained personal and quality control of final preparation of the cell product at the clinic. All these obstacles need to be overcome before the final drug compound can be delivered to patients. In addition, only physicians trained in the art of cellular therapy can apply the advanced cellular therapy. It can be concluded that current cell products are in the honest sense, not “off-the-shelf” products since preparatory work has to be initiated before clinical application can occur.

The medicinal product APOSEC™ represents a true “off-the-shelf” product. The lyophilized powder can either be resuspended “on site” with physiologic saline for conditions involving internal inflammatory conditions (e.g. AMI) or can be applied topically in a sterile GMP carrier cream. In June 2014, the worldwide first secretome based clinical phase I trial utilizing autologous GMP APOSEC in healthy male subjects will commence (MARSYAS¹ I), having already ethical approval of the MUW ethics committee. This clinical trial is funded by the Christian-Doppler Society and is independent from the APOSEC project.

1.2.5 Benefits for an ageing population

The therapeutic effectiveness of APOSEC includes treatment of diseases typically associated with old age, such as metabolic diseases (e.g. diabetes mellitus) and cardiovascular diseases. Hence, the elderly will especially benefit from APOSEC, in more than one way: (a) improved wound healing and prevention of cardiac remodelling and (b) preserve the patients in a better state of health after suffering from chronic wound healing impairments and AMI due to a decreased likeliness to develop complications or to suffer from sequelae. This in turn means shorter hospital stays as well as less sickness days.

1.2.6 Stimulation of growth and competitiveness of European regenerative medicine

Aposcience AG (APO) is a spin-off company from the Medical University of Vienna (MUW) founded by a MUW surgeon in association with angel investors and has been granted patents and trademark registration for APOSEC™ in all main markets worldwide as described in Section 2.2.1. In a private public partnership, Aposcience AG has funded the Christian Doppler Laboratory for Diagnosis and Regeneration in Cardiac and Thoracic Diseases located at the University Clinics of Surgery. The development of APOSEC™ and its promising pre-clinical large animal experiments in AMI and wound healing led us to establish a production site for APOSEC™ at the Blood Transfusion Service for Upper Austria. This production site was inspected and audited by the Austrian Agency for Health and Food Safety (AGES) in February 2014 and **regulatory authorisation for the APOSEC production was issued**. With increasing demand for APOSEC, the production can be licensed out to other European transfusion units with GMP facilities.

The APOSEC project partners consider the H2020 PHC 15 call as very timely with regards to their efforts to accelerate the development of regenerative medicine products and to leverage the significant investments already made both in APOSEC™ R&D as well as its extensive IP protection.

The aim of the APOSEC project is to bring allogeneic GMP APOSEC™ into the realm of clinical application. In case of the success of paracrine factors derived from peripheral white blood cells (PBMC), Europe will (re)gain a leading role in the field of regenerative medicine, as Aposcience AG will make a serious contribution to establishing a new and exciting aspect to the European regenerative medicine sector. This project aims to drug a drug designed in the EU to all patients in Europe and beyond. As the indications treated in the planned trials are of worldwide concern, the export potential of APOSEC is evident. In addition the revenue generated will allow for further research on APOSEC as a potential treatment for orphan indications.

¹ MARSYAS = Unique identifier for all clinical trials using APOSEC; name taken from Greek mythology.

1.3 Concept and Approach

1.3.1 Background

APOSEC™ is an innovative medicinal drug candidate comprising a lyophilized mixture of active components secreted from cultured apoptotic peripheral white blood cells (PBMC) for the treatment of hypoxia induced inflammatory conditions, such as chronic wounds and acute myocardial infarction (AMI).

Section 1.3.1 contains a summarized description of (a) the scientific path from preclinical stem cell research leading to the alternative development of APOSEC; (b) the performed preclinical investigations utilizing APOSEC in vitro and vivo; (c) the planned clinical trials as well as preparatory investigations (toxicology etc) as required by the regulatory authorities within this APOSEC Horizon 2020 application (Figure 1).

From 2006-2009, the development of APOSEC was performed by the research group “Applied Immunology” (www.applied-immunology.at) of Dr Hendrik Ankersmit and from 2009 – today by the Christian Doppler Laboratory for Diagnosis and Regeneration of Cardiac & Thoracic Diseases, both based at the [REDACTED]. The entire technique regarding production process of APOSEC and its indications for treatment were patented for the indication skin healing and treatment of internal inflammatory conditions (e.g. stroke, spinal cord injury, AMI and others). The EPO has granted a European Patent (No. 2379085) for APOSEC as pharmaceutical preparation for treating skin indications and wound healing. The treatment of internal hypoxia induced inflammation; WO 2010/070105A1 “Pharmaceutical preparation comprising supernatant of blood mononuclear cells culture” is still pending. Both patents were filed on Dec. 18, 2008.

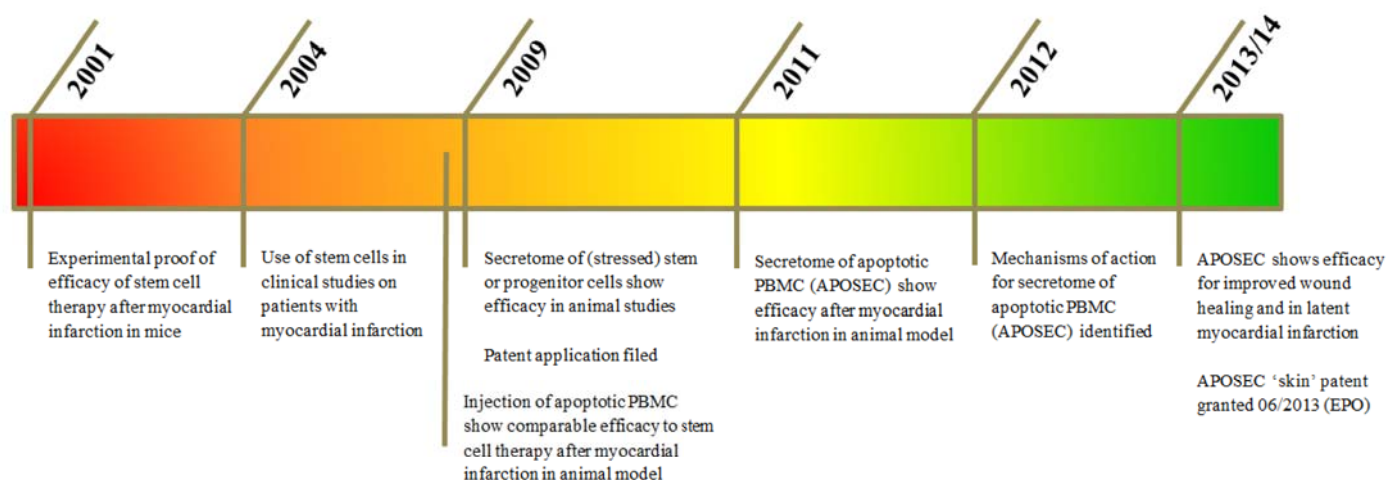


Figure 1: Overview of developments leading to the APOSEC Project

1.3.1.1 From stem cells to paracrine factors derived from PBMC

In 2001, two reports in *Nature* and *Nature Medicine* provided evidence that bone marrow derived mononuclear cells (stem cells) attenuate myocardial infarction and remodelling in a rodent model of acute myocardial infarction (Orlic et al. 2001; Kocher et al. 2001).

In consecutive years, multiple academic centres initiated clinical trials in humans utilizing autologous stem cells in treating patients after myocardial infarction (Strauer et al. 2001, Wollert et al. 2004). In 2010, Wollert et al. published a review article that summarized all published clinical experience with stem cell based therapies after acute myocardial infarction. Wollert concluded that the available summarized clinical data were inconsistent and that uncertainty persisted regarding the mechanism of action and prospect of cell therapy for patients with heart disease. It was found that stem cells which were injected intramyocardially or

infused via intracoronary route did not remain in the hypoxic tissue, but migrated to other organs such as liver, spleen, lung and bone marrow. Wollert et al. further speculated that, based on all published and available data, paracrine factors (soluble proteins secreted by stem cells) might be responsible for all effects seen in preclinical stem cell experiments. This hypothesis is currently generally accepted given the limited success of stem cell therapies. Experimental data supporting this speculation were published as early as 2005 by Gneccchi et al.

1.3.1.2 From stem cells to the secretome of PBMC

As shown by Ankersmit et al. (2009) and Lichtenauer et al. (2011), apoptotic PBMC suspensions prevented ventricular remodelling after experimental rodent AMI and attenuated post infarct hemodynamic impairment. This was the first evidence that apoptotic PBMC and their secretory products are able to mimic stem cell effects published in the literature.

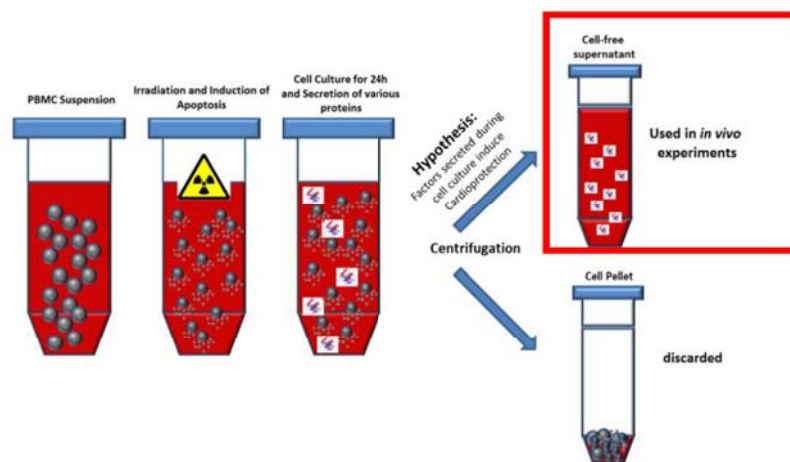


Figure 2: Having already shown that infusion of apoptotic PBMC suspension in an acute rodent AMI model prevented ventricular remodelling, it was further shown that the administration of “paracrine factors only” of stressed PBMC (= **APOSEC** – this acronym stands for **APO**ptotic **SEC**retome) evidenced similar properties and caused long term hemodynamic cardioprotection in an acute rodent AMI model (Lichtenauer et al. 2011).

1.3.1.3 Clinically relevant animal model for the testing of APOSEC in hypoxia induced inflammation

Since large animal models are more representative for the clinical reality in the human setting, a porcine closed chest reperfused AMI model was used for the evaluation of APOSEC as a therapeutic agent. APOSEC was generated in a species-specific manner. The cultured medium was lyophilized in order to generate a potential “off-the-shelf product” for the treatment of acute AMI.

A single intravenous infusion of APOSEC caused a significant short and long-term reduction of myocardial damage as determined by cardiac magnetic resonance imaging (MRI). A dose dependency in the effect of APOSEC was found. In the high dose group, APOSEC infusion reduced the extent of myocardial infarction by approximately 50%, from 18.17% to 8.66% after 3 days ($p=0.0019$) and from 12.6% to 6.92% after 30 days ($p=0.015$), respectively. Conversely, Left Ventricular Ejection Fraction (LVEF), cardiac output (CO) and cardiac index (CI) were improved significantly compared to controls and low-dose treatment (Figure 3; Lichtenauer et al. 2011).

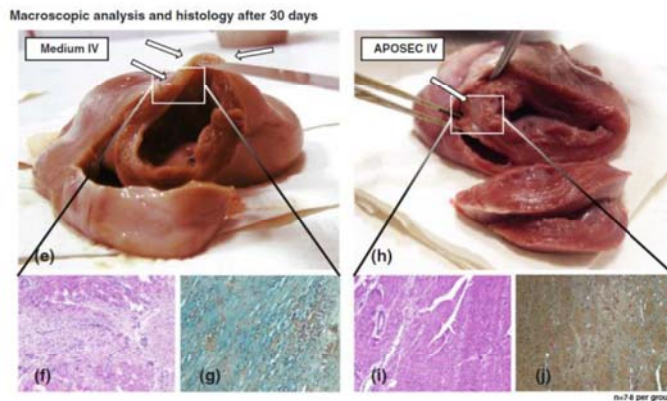


Figure 3: Results of the porcine closed chest reperfused AMI model. e,h show representative images of hearts explanted 30 days after AMI. Hearts of APOSEC-injected pigs evidence only marginal formation of scar tissue in the myocardium compared to control animals where large infarcts were common. H&E-stained (f,i) and Movat's pentachrome-stained (g,j) specimens of the infarcted myocardium shown in lower part of Figure 3 indicate fewer signs of collagen deposition and more viable cardiomyocytes within the scar tissue (I,j) compared to control animals (f,g). (adapted from Lichtenauer et al. 2011)

1.3.1.4 Identified mechanisms of action of APOSEC in vitro and vivo

In order to understand why conditioned medium derived from stressed PBMC prevents myocardial infarction, multiple in vitro and vivo investigations were performed in order to define biological mechanisms causative for the experimental results in AMI. By utilisation of membrane array and ELISA technique, it was shown that APOSEC contains IL8, GRO- α , ENA-78, RANTES, sICAM-1, MIF, VEGF, IL-1RA and IL-16. Little if any secretion was detected for MCP1, IL-10, IGF-1, HGF, FGF-2, TGF- β , SDF-1, G-CSF and GM-CSF. All of the above mentioned proteins are associated with a specific role in immune response after AMI.

It was shown in large animal AMI studies that APOSEC significantly reduced microvascular obstruction (MVO) in a porcine AMI model in comparison to medium treated animals (Hoetzenecker et al. 2012). MVO after AMI determines the process of ventricular remodelling and development of congestive heart failure (Larose et al. 2010).

It was demonstrated that one intraperitoneal application of APOSEC in autoimmune myocarditis significantly reduced inflammation and caused Caspase8-dependent apoptosis of highly purified CD4-positive cells. (Hoetzenecker et al. 2013) All these results were preceded by the finding that APOSEC causes cytoprotection in primary cultured human cardiomyocytes (Lichtenauer et al. 2011). The data of Pavo et al. (2014) showed that APOSEC increased significantly ejection fraction (EF) and cardiac index. This suggests that APOSEC enhances the accumulation of endogenous cardiac stem cells and augments angiogenesis in latent AMI model as compared to medium treated animals (Pavo et al. 2014).

In summary, seven mechanisms of action were identified:

APOSEC induces:

- (a) cytoprotection,
- (b) reduction of microvascular obstruction (MVO),
- (c) exerts an anti-inflammatory effect,
- (d) induces keratinocyte and fibroblast migration,
- (e) induces pro-angiogenesis (new vessel growth),
- (f) inhibits platelet aggregation, and
- (g) causes vasodilation, therefore protecting hypoxic areas from tissue damage and causes wound healing.

1.3.1.5 APOSEC and skin regeneration and wound healing – in vitro and vivo results

Holzinger et al. has shown that topical application of in vitro activated autologous PBMC effectively improves granulation and epithelialisation of diabetic skin ulcers (Holzinger et al. 1994). Concomitantly, reports showed that topical application of stem cells accelerates wound re-epithelialisation and

neovascularisation in wound models. More recently it was shown that paracrine factors of stem cells augment cutaneous wound healing (Jayaraman P et al. 2013).

Figure 4 shows the beneficial effects of APOSEC in skin wounds.

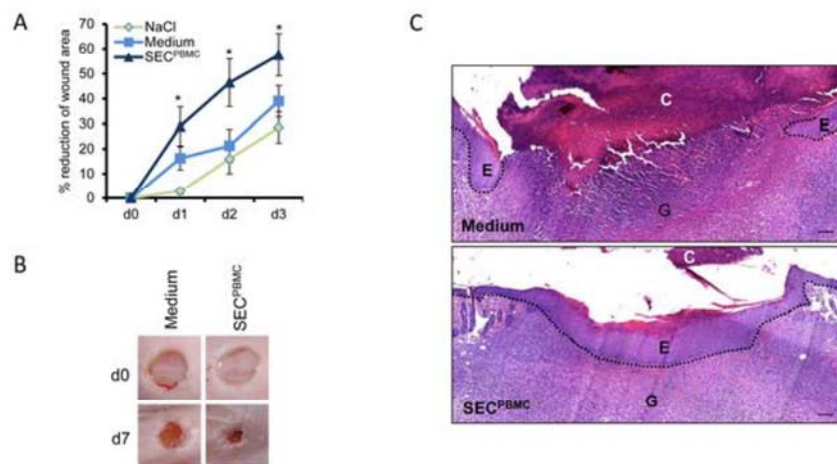


Figure 4: SEC^{PBM} (Secretome of PBMC) leads to enhanced wound closure and re-epithelialization. (A) Wound areas were measured during the first 3 days after wounding. Treatment with SEC^{PBM} significantly enhanced wound closure. Error bars represent standard deviation calculated from 15 animals for 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 of SEC^{PBM} 7 days after wounding are shown. While medium treated wounds still show a thick crust and little re-epithelialization, SEC^{PBM} treated wounds are fully re-epithelialized. C=crust; E=newly formed epidermis; G= granulation tissue. Scale bars: 100µg. One representative animal of 15 is shown. (adapted from Mildner et al. 2013)

1.3.1.6 Migratory capacity of dermal fibroblasts and epidermal keratinocytes

We have shown that paracrine factors of PBMC significantly induced cell migration in both, dermal fibroblasts (FB) and epidermal keratinocytes (KC). No significant changes in cell cycle progression after exposing FB and KC to cultured medium were revealed by cell cycle analysis, indicating that paracrine factors only induce migration. This was consistent with the in vivo observation, where no significant increase in proliferating cells in the regenerated epidermis in the skin wounds treated with PBMC paracrine factors containing emulsions could be detected (Mildner et al. 2013).

1.3.1.7 Neoangiogenesis in and tube formation in PBMC secretome treated murine wound models

Since a crucial event during wound healing is the sprouting of newly formed blood vessels into the wounded tissue, the efficacy of PBMC paracrine factors to induce neoangiogenesis in vivo was examined. Skin wounds harvested after wounding were stained for CD31, a specific marker for blood vessels. A significant increase in CD31 positive cells in the wounds treated with PBMC paracrine factors was found, indicating enhanced new formation of vessels (neoangiogenesis) (Mildner et al. 2013).

1.3.1.8 APOSEC enhances wound healing in large animal model

Since the validity of mouse models for wound healing or any inflammatory condition was shown to have limited correlation with what is observed in humans (Seok et al. 2013), the research was extended to a large animal model. An animal model that is most comparable to human skin tissue, namely a porcine burn and split-thickness skin grafting model (Branski et al. 2008). Figure 5 shows increased epidermal thickness in APOSEC treated skin wounds in pigs as compared to controls.

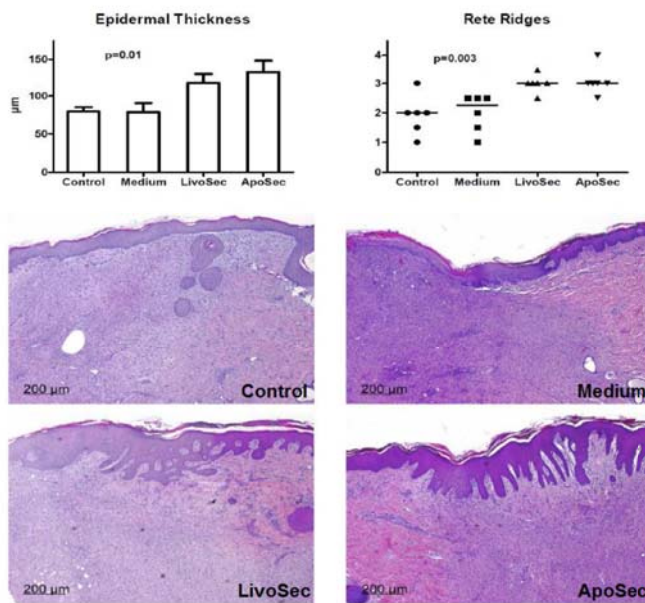


Figure 5: Epidermal thickness and quality of rete ridges at the epidermal/dermal border zone were improved significantly after repeated application of non-irradiated PBMC Secretome (LIVOSEC) or APOSEC (above) at the last day of examination (day 10). Example of the wound edge after application of NaCl- or medium control or LIVOSEC or APOSEC. Note: LIVOSEC is non irradiated PBMC secretome. (adapted from Hacker et al. 2013, manuscript in submission – Abstract: Hacker S et al.: Regenerative Effects Of Secreted Factors Derived From Peripheral Blood Mononuclear Cells in Cutaneous Wound Healing After Full Thickness Skin Defects, Burn and Skin Grafting: Results Of Animal Studies. Interact Cardiovasc Thorac Surg. 2013 Feb;16(2):229.)

In addition it was investigated if neo-angiogenesis is also present in the porcine split skin regeneration model treated with topical APOSEC. Figure 6 shows strongly induced angiogenesis after five days after split-thickness skin autografting.

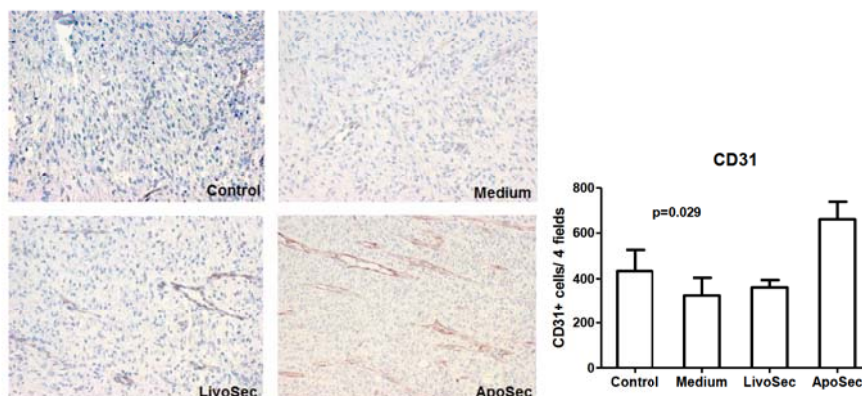


Figure 6: Human APOSEC induces neoangiogenesis in split skin grafted pigs in a significant manner ($p=0.029$); Histological evaluation of wound tissue after application of NaCl- or medium control (below, upper left and right) or LIVOSEC or APOSEC (below, lower left and right). Note: LIVOSEC is non irradiated PBMC secretome. (adapted from Hacker et al. 2013, manuscript in submission)

We have shown that paracrine factors derived from stressed PBMC (APOSEC) increases wound healing in animal models relevant for humans. Mechanistically, we have identified **several modes of action of APOSEC relevant to wound healing:**

- (a) induction of endothelial cell sprouting,
- (b) induction of fibroblast and keratinocyte migration and
- (c) neo-angiogenesis.

1.3.1.9 APOSEC from pre-clinical experiments to human clinical application

In order to move towards clinical trials in humans, the [REDACTED] part of the regional association of the [REDACTED], has been chosen as a partner to produce APOSEC under GMP conditions. **In February 2014 and regulatory authorisation for the GMP production of APOSEC has been granted.** This national licence is the prerequisite for communications with the European Medicines Agency (EMA) as well as toxicological and clinical investigations proposed in this grant application.

Autologous and allogeneic GMP APOSEC

“Autologous APOSEC” is a medicinal product originating from PBMC derived from one blood donor for the treatment of the same blood donor. Thus “Autologous APOSEC” can be considered as a prototype of a “personalized” medicinal product. However, due to multiple testing procedures, the “Cost of Good” (COG) is extremely high.

“Allogeneic viral cleared GMP APOSEC” is a medicinal product originating from PBMC derived from multiple blood donors. The Cost of Good of allogeneic GMP APOSEC will decrease drastically (e.g. 20 treatments for skin ulcer at █████ per treatment; █████ per treatment in the indication AMI).

Of utmost importance is the fact that APOSEC is produced from a current waste product at all transfusion units worldwide.

1.3.1.10 Viral clearance of allogeneic APOSEC – Background

When utilizing pooled secretomes from multiple donors, e.g. allogeneic APOSEC, the risk of transmission of blood-borne viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) have to be tackled. Viral safety derives from three complementary approaches during manufacturing, i.e. donor selection, testing of donations and plasma pools, and the introduction of viral inactivation and removal procedures in the course of manufacture, each of which requires strict adherence to good manufacturing practices (GMPs).

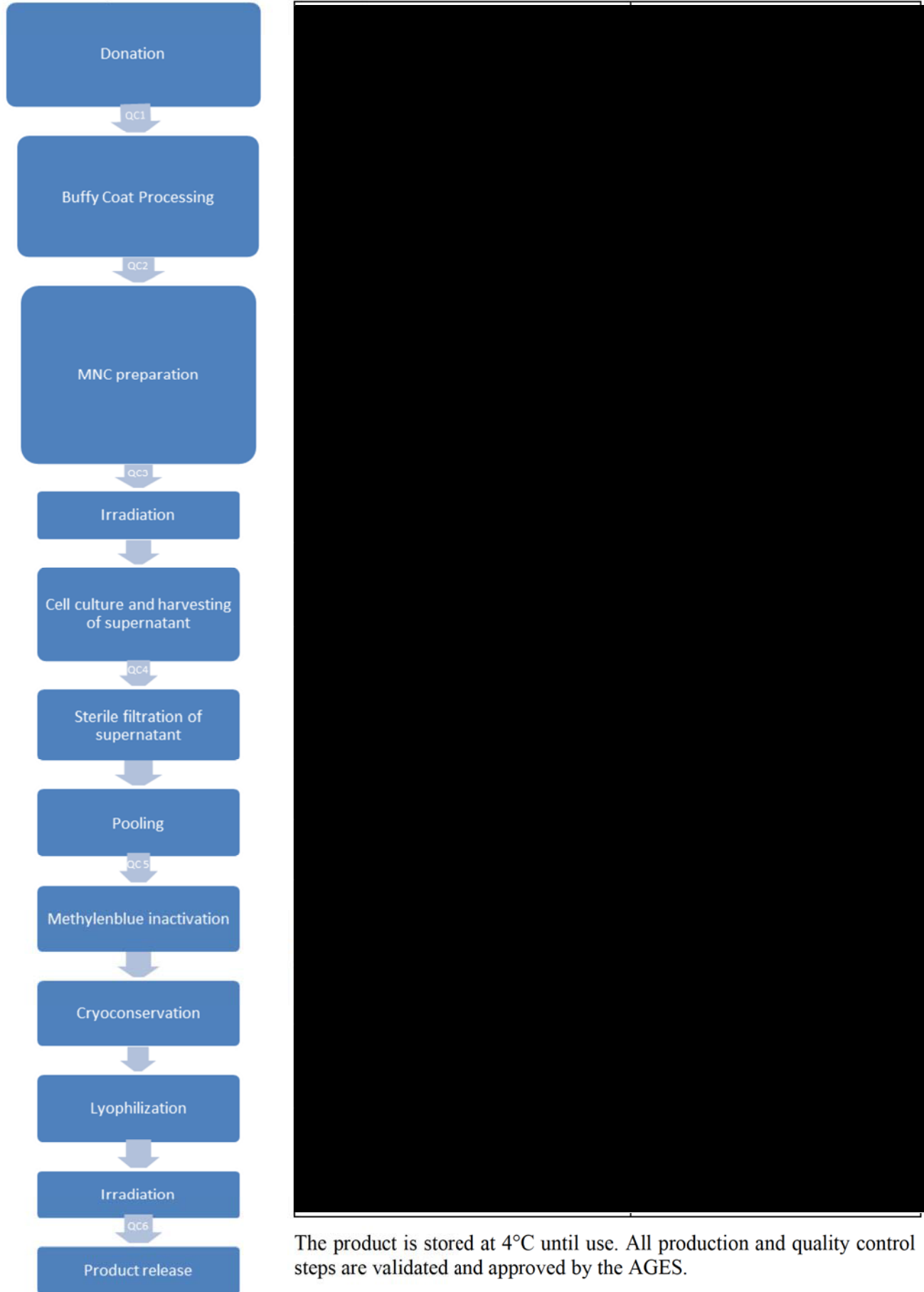
For the product to be safe, the production process must inactivate and/or remove possible virus contamination. By considering the nature and characteristics of APOSEC, it was decided to implement two different, but complementing, well validated and universally used methods of viral clearance into the manufacturing process: **Methylene Blue Technology** and **Gamma irradiation**. Table 1 provides an overview of the APOSEC production process including the two viral clearance steps.

1.3.1.11 Proof of effectiveness of viral cleared APOSEC in a large animal AMI model

Since a viral clearance process is mandatory for the allogeneic production of APOSEC, it had to be investigated whether the anticipated medicinal product has kept its potency in a porcine closed chest reperfused infarction model. The above described AMI model was repeated by utilizing APOSEC from a cell equivalent of 1.5 billion PBMC that had undergone viral clearance (methylene blue and 25 KGy gamma irradiation). **It could be shown that viral clearance did not compromise the efficacy of the product.**

Table 1 shows the protocol of allogeneic APOSEC production validated by the Austrian Health Agency (AGES).

Table 1: Final production and testing scheme of allogeneic APOSEC



1.3.1.12 Overview of national research & innovation activities linked to the APOSEC project

The Medical University of Vienna (MUW) supports the **“Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration”** which is financed by Aposcience AG and the Ministry of Science, Research and Economy for 7 years. The total amount of funding is [REDACTED] and is coordinated by Dr. Hendrik Jan Ankersmit (11/2009 - 10/2016).

Use of synergies Between the CD-Laboratory and the “APOSEC HORIZON 2020” project

According to the definitions of the Christian Doppler Society, the research performed in CD-Laboratories led to the pre-clinical available data presented in this grant application including mechanistic explanations of effects seen. Due to the nature of the Christian Doppler Society, only basic science pertaining to the lead products can be supported. This included also the GMP facility at [REDACTED] and the commencement of the phase I trial. The further development of the mandated “allogeneic APOSEC” is far beyond reach of the CD Lab and fits perfectly to the current Horizon 2020 call.

Summary of toxicology studies utilizing “human non-GMP APOSEC” and “rat non-GMP APOSEC” in Seibersdorf Laboratories

In order to investigate whether APOSEC has any toxicological effects, two incremental toxicology studies were initiated. Both reports concluded that no evidence for toxicity of rat as well as human APOSEC was determined at any dosage examined (Report SL-LT-349/2010, Report SL-LT-350/2010). Prior to clinical trials, extensive toxicology examinations of GMP APOSEC have to be performed in a [REDACTED] approved facility (see WP 2).

Summary of pathological reports of large animals exposed to APOSEC by an accredited veterinary pathologist at the Veterinary University of Vienna

In order to investigate whether a single high dose of allogeneic APOSEC (Pavo et al, 2014) causes pathological findings in pigs, multiple organs were sampled and delivered to Prof. Weissenböck, Head of Veterinary Pathology at the Veterinary University of Vienna for histological evaluation. No pathological changes were detected in any organs studied (Official Report No. W/5060-73/12, 7 March 2012).

Summary of regulatory strategy report on allogeneic APOSEC

In order to receive external advice for the development of APOSEC, a former EMA expert, [REDACTED] was consulted in early 2012. [REDACTED] provided a regulatory strategy report indicating that APOSEC has to be produced under GMP conditions, needs viral clearance and has to be tested for batch-to-batch comparability. Furthermore, it was emphasized that an authorisation of a GMP production site for allogeneic APOSEC is a prerequisite for further clinical trials. Pertaining to our grant application it is noted that the Austrian National Health Authority (AGES) has licensed the APOSEC production.

Summary of background leading to design of the proposed clinical trials

- ✓ Proof of therapeutic effect of APOSEC in small animal AMI model;
- ✓ Proof of therapeutic effect of APOSEC in large animal AMI model
- ✓ Proof of therapeutic effect of APOSEC in small animal skin wound model
- ✓ Proof of therapeutic effect of APOSEC in large animal skin wound model
- ✓ Identification of mechanisms of action of APOSEC
- ✓ Validation and Authorisation of the GMP production facility for APOSEC by the Austrian National Health Authority (AGES)

1.3.2 Approach and methodology

1.3.2.1 Achieving the proof-of-concept through first-in-man clinical trials

The aim of the APOSEC project is to achieve the proof-of-concept for the medicinal product APOSEC i.e. the proof of therapeutic effectiveness of APOSEC in human patients (see Figure 7). This will be achieved by:

- GMP production of APOSEC
- Potency assays and stability assays;
- Toxicology studies;
- Topic and Systemic Phase I clinical trials;
- Topical clinical phase II trial in the indication diabetic foot ulcer (skin wound healing);
- Clinical phase I-II clinical trial for the indication AMI;
- Additional basic research on mode of action of APOSEC

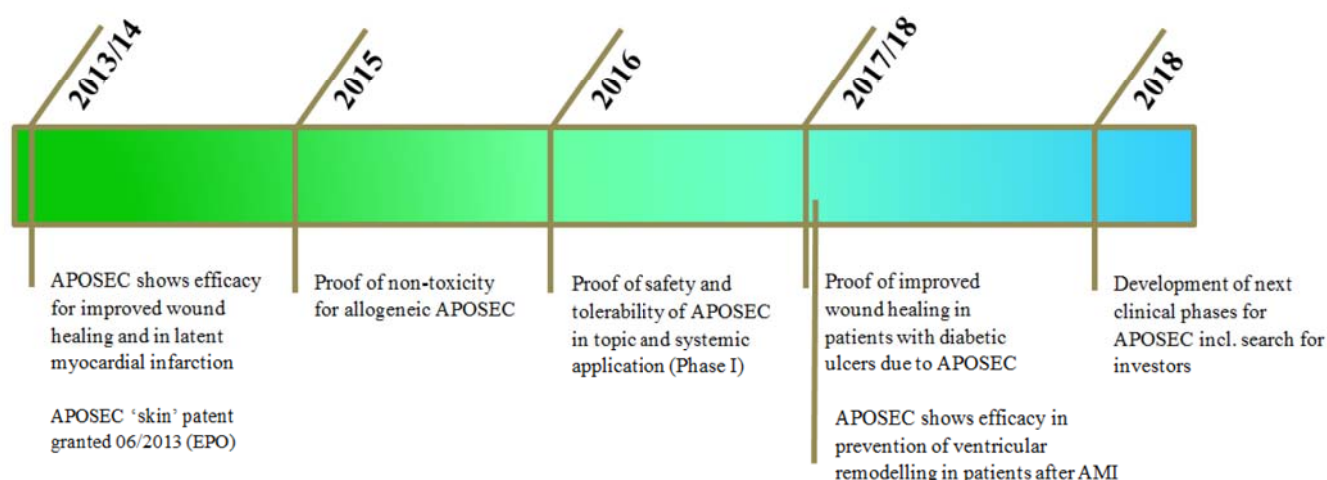


Figure 7: Overview of key achievements anticipated in the APOSEC Project

Below is an overview of the main steps of work planned for the APOSEC project.

Details of work packages and a Gantt- chart are provided in Section 3 and the Clinical Trial Annexes.

1.3.2.2 GMP production of APOSEC (WP1)

The mandated APOSEC production site at RED will produce the required quantities of the medicinal product APOSEC according to currently planned study designs. The following numbers represent the cell equivalent of stressed irradiated PBMC needed for each part of the project:

1. Toxicology studies	200 x 10 ⁹ APOSEC GMP
2. Stability Assays	30 x 10 ⁹ APOSEC GMP
3. Potency assays	30 x 10 ⁹ APOSEC GMP
4. Clinical Phase I trial Topic	1.125 x 10 ⁹ APOSEC GMP
5. Clinical Phase I trial Systemic	40.8 x 10 ⁹ APOSEC GMP
6. Clinical phase II study Diabetic Foot Ulcer	38 x 10 ⁹ APOSEC GMP
7. Clinical phase I-II trial AMI	100 x 10 ⁹ APOSEC GMP
8. Biotests UKW	30 x 10 ⁹ APOSEC GMP

1.3.2.3 Potency assays and stability assays (WP1)

Potency assays for medicinal products require adherence to the Pharmacopoeia, guideline ICH Q5C. Development of stability assays has to adhere to ICH Q1A (R2). RED has a Good Laboratory Practice (GLP) laboratory capable of developing such potency assays that will be utilized in consecutive years for testing the quality of the produced APOSEC. Furthermore, a setup of stability studies, including relevant analysis, will be performed.

1.3.2.4 Toxicology Studies (WP2)

After GMP APOSEC is available, toxicology studies are mandatory according to the guidelines ICH M3(R2) and S6 + Addendum, Guideline 2001/83/EG. Toxicology studies, stability studies and potency assays have to be finalised before clinical studies can be initiated. Toxicology studies need to be performed in order to provide evidence of non-toxicity of APOSEC. According to these results, dosage calculation in conjunction with available preclinical data can be calculated. Close cooperation with members of the Dept. of Clinical Pharmacology (MUW) are mandatory to design clinical phase I trials utilizing APOSEC.

1.3.2.5 Regulatory affairs (WP2)

Aposcience AG (APO) owns the IP-rights of APOSEC and is qualified as SME by the EC and the European Medical Association (EMA, EMA SME number of Aposcience AG: EMA/SME/248/13). APOSEC as a complex product, comprising a variety of active factors, raises special issues in the drug approval process by the European regulatory authorities. Within the framework of this project, we want to meet this challenge by pro-actively communicating with the European Medicines Agency, by receiving regulatory advice and by providing additional scientific data required by the authority.

1.3.2.6 Clinical Trial Phase I – Topic (skin) – MARSYAS II (WP3)

The clinical phase I trial in topical application of allogeneic APOSEC is designed to investigate if APOSEC is safe and tolerable in ten male subjects. Female subjects are excluded because of a possible negative effect on appearance. For this reason two different dosages will be applied on six consecutive days (once a day) to an artificial skin wound (4 mm punctures) set on the day of study initiation. Daily photographic and clinical evaluations will be performed, on day 7 the healthy proband will be re-biopsied (6 mm puncture) and the final clinical evaluation will take place on day 17. This phase I trial will be performed at the [REDACTED] according to the study protocol, authored by [REDACTED]. Further details on exclusion criteria, inclusion criteria and study conduct of MARSYAS II can be found in the Annex ‘APOSEC – Essential Information on Clinical Trials’.

For information: a similar trial (MARSYAS I) will commence in June 2014, utilizing autologous APOSEC in the same setting. This study has already been approved by the Ethics Committee of the [REDACTED] for autologous APOSEC (MARSYAS I, EudraCT number: 2013-000756-17) and represents the first secretome based clinical trial in wound healing (see Section 5, MUW Ethics Committee approval for MARSYAS I).

1.3.2.7 Clinical Trial Phase I – Systemic (single intravenous infusion) – MARSYAS III (WP3)

It has been shown in large animal models that one single infusion of APOSEC does attenuate myocardial damage caused by hypoxia. Moreover, multiple small animal models in other indications (experimental myocarditis, experimental stroke, and experimental spinal cord injury) were performed, evidencing that APOSEC has therapeutic effects in preventing hypoxia induced inflammation in multiple experimental settings. Since intravenous infusion of APOSEC has to be tested in humans, a clinical phase I trial has to be performed. This phase I trial will be performed at the [REDACTED]. According to the study protocol, authored by [REDACTED] the following study protocol is envisioned: a single intravenous administration of APOSEC is administered in four different doses (low dose, middle dose, therapeutic dose, high dose) to 24 healthy male and female probands. Heart rate and electrocardiogram of subjects will be continuously monitored, temperature will be

assessed, laboratory safety parameters and adverse events will be followed. Differential blood counts, blood chemistry, and C reactive protein as well as D-dimer levels will be assessed. Cytokines and antibody responses will be determined by analyses of the serum of the probands.

Further details on exclusion criteria, inclusion criteria and study conduct of MARSYAS III can be found in the Annex 'APOSEC – Essential Information on Clinical Trials'.

1.3.2.8 Clinical Trial Phase II – Diabetic Foot Ulcer (DFU) – MARSYAS IV (WP4)

It has been shown that topical application of human allogeneic APOSEC augmented wound healing in a murine model as well as enhances neo-angiogenesis and re-epithelialisation in a porcine full thickness burn model (xenogeneic topical application of human APOSEC in murine and porcine animal experiments). In order to provide further supporting evidence, a phase II clinical trial in diabetic foot ulcer patients is planned within the APOSEC project by the MUW. The trial conduct and monitoring will be subcontracted to a Clinical Research Organization (CRO) that has a track record in conducting trials on dermatological indications in central and Eastern Europe. The study design will be orchestrated by an international steering committee headed by [REDACTED] and with the involvement of [REDACTED].

The anticipated trial design is an interventional randomized, parallel-group, double blind, placebo-controlled prospective study investigating safety/efficacy in treating DFU in a 12-week trial, administering APOSEC twice a week over seven weeks. Dose specification will take place after phase I data is available. The primary endpoint will be the proportion of ulcers healed, defined as 100% epithelialization without drainage. Secondary endpoints will include the proportion of patients reporting adverse effects, the rate of re-epithelialisation of the ulcer site and the time to re-epithelialisation of the ulcer site. A detailed description of the trial design and conduct can be found in annex 'MARSYAS IV – Essential Information CT'.

This trial will evaluate the safety and feasibility of APOSEC in the dermatological indication, conducting a Phase II trial. The indication diabetic foot ulcer was chosen for this trial in order to recruit a patient group which is homogenous and large enough to allow for statistically representative results.

Further details on exclusion criteria, inclusion criteria and study conduct of MARSYAS IV can be found in the Annex 'APOSEC – Essential Information on Clinical Trials'.

1.3.2.9 Clinical Trial Phase I-II – Acute Myocardial Infarction – MARSYAS V (WP5)

The scientific and technological objective of the APOSEC-AMI trial is to achieve a robust regeneration of the ischemic injured myocardium by increasing the anti-inflammatory and angiogenic mechanisms in the very early phase of AMI and prevent ventricular remodelling, life-threatening arrhythmias and heart failure, by intravenous administration of the myocardial protective protein-mix gained by the irradiated apoptotic (PBMC) cytokine factory.

Specifically, the objectives are:

- 1) To develop a new and innovative myocardial regenerative therapy addressing patients with infarcted myocardium and to achieve a robust regeneration of the myocardium after AMI in patients, injecting intravenously APOSEC in the up-streaming phase of primary percutaneous coronary angioplasty, beside the standard medical and interventional therapy of AMI; in order to **a)** prevent left ventricular remodelling and post-AMI congestive heart failure, and **b)** to reduce the morbidity and mortality associated with ischemic heart disease.
- 2) To evaluate the safety and feasibility of the APOSEC in the clinic, conducting Phase I-II clinical trial (first in man).

Further details on exclusion criteria, inclusion criteria and study conduct of MARSYAS V can be found in the Annex 'APOSEC – Essential Information on Clinical Trials'.

1.3.2.10 Additional basic research in defining modes of action of APOSEC (WP6)

APOSEC has shown to influence regeneration and healing in models of hypoxia induced inflammation. Besides of already defined mechanisms, further pre-clinical evaluations have to be performed in order to satisfy regulatory affairs. This work package is designed to further understand the a) mode of action, b) optimization of dosage, and c) frequency of APOSEC application in planned phase I, II and III clinical trials.

The following tissue culture models will be utilized: human full skin equivalent simulating the topical application in the planned clinical trials, healing model based on a vascularized human full skin equivalent, model of human myocardial equivalent simulating the application in the planned clinical trials and most importantly human vascularized tumour model simulating the intravenous application of APOSEC. All these planned assays will suffice to answer obligatory questions by the regulatory affairs. Besides mechanistic approaches, generation of novel intellectual properties can be expected.

In vitro tissue models

One of the first epidermal skin models reconstructed in vitro first described by Cannon et al. 1994. The model was composed of human epidermal keratinocytes cultivated on inert polycarbonate membranes, which developed a fully differentiated epidermal tissue comprising a basement membrane, a zone of proliferative keratinocytes as well as a stratum corneum after exposure to an air-liquid interphase. This model was introduced to the commercial market (EpiDerm™) by MatTek Corporation (Ashland, MA, USA) and was mainly used by several industries in product development, safety assessment and drug discovery.

UKW (WP6 leader) developed more complex human skin equivalents based on primary endothelial cells, fibroblasts and keratinocytes. These models have been shown to be useful tools for functional in vitro tests, including toxicological screenings and drug development. Such complex 3D skin models are used routinely in the industry as well as in research laboratories worldwide. In general they are capable to test the efficacy or study mode of action of new drugs but also allow distinguishing significantly agents of reversible from those of irreversible toxicity without validation in repetitive animal experiments. These tissue models also provide accurate and measurable information that can be utilized to modify the particular agent in order to reduce its toxicity without loss of efficacy and to identify the metabolites being responsible for the biological activity of the drug in the human body.

UKW has established different complex human skin models, including a technology to injure automatically the skin in a defined manner on a high-standardized level (wound healing model). Immune competent cells like macrophages or dendritic cell can be co-cultured in the complex 3D human skin models as well.

Additionally have a vascularized complex human skin models (SkinVaSc) was developed, including a feeding artery, a capillary network and a vein. These in vitro angiogenesis models are suitable models to study the effect of pro-angiogenic factors, anti-angiogenic substances, matrix metalloproteinases (MMPs), cell adhesion molecules and fibrolysis. These models mimic the in vivo situation more closely and enable the interaction of the ECs with the ECM and the surrounding cells. As a result, ECs formed capillary-like structures in the dermis.

Tumorigenicity studies

Cancer is one of the leading causes of death worldwide. Current therapeutic strategies are predominantly developed in 2D culture systems, which inadequately reflect physiological conditions in vivo. Biological 3D matrices provide cells an environment in which cells can self-organize, allowing the study of tissue organization and cell differentiation. Such scaffolds can be seeded with a mixture of different cell types to study direct 3D cell-cell-interactions. To mimic the 3D complexity of cancer tumours, the research group at UKW has developed a 3D vascularized in vitro tumour test system (OncoVaSc). These human vascularised tumour tissues will be incubated with APOSEC through the blood vessel system with different doses of APOSEC over time. The invasion and EMT capability will be compared with the standardized induction of

invasion and EMT by TGF- β . Transformed tumour/healthy cells will be isolated by laser capture technology to evaluate the APOSEC induced signalling networks. A promising new approach is to study cellular signalling networks and their changes through targeted agents in tumour models that have specific mutations. Through this network analysis, modes of drug action can be understood

1.3.2.11 Gender relevant issues

APOSEC is expected to be equally therapeutically effective in both men and women. This hypothesis is to be tested in the course of the planned clinical studies in this project. In general, the therapeutic effectiveness of APOSEC comprises diseases typically associated with old age, irrespective of the gender, such as cardiovascular diseases and metabolic diseases (diabetes mellitus). However, due to the higher life expectancy of women, women are more exposed to age-related diseases and could therefore profit more from APOSEC. Nevertheless a treatment with APOSEC is expected to prevent prolonged suffering in the elderly population due to diabetic foot ulcers and consequences of myocardial infarctions.

The Phase I clinical trial – topic application (MARSYAS II) includes only healthy male volunteers. Female volunteers are excluded because of a possible negative effect on appearance.

In the in vitro tissue model, male and female donors will be used to isolate the cells and to build up the skin, myocardial and tumour models. Using Tissue engineering as a tool to generate multiple test tissue, gender and age related effect of APOSEC can be determined in a very early clinical phase. Especially for the regeneration capacity of the myocardium this complex human tissue models are relevant new tools.

The ratio men : women in the APOSEC-AMI (MARSYAS V) trial will reflect the incidence of the STEMI in men and women in the participating study sites across Europe. This ratio will not be the object of an a priori stratification. Predefined restrictions and precautions for inclusion of females of childbearing potential will be applied. Gender (& Ethical) issues will be addressed at Executive Board level led by Prof. Heike Walles.

1.4 Ambition

1.4.1 Comparison - State of the art and beyond

To date, the main focus in regenerative medicine is still on the research and application of autologous and allogeneic stem cells. The original concept stated that pluripotent stem cells can transform into a variety of cells and through that replace e.g. damaged heart tissue. In the meantime it has been recognized that: stem cells do not usually remain permanently in the organ in which they were introduced; in case they transform into differentiated tissue cells, they grow in the wrong place at times. The concept has therefore been largely discarded. Recent findings suggest that the observed effect of stem cell applications is mostly due to their secreted paracrine factors.

APOSEC consists of paracrine factors and thus, can be regarded as a “second generation” regenerative medicinal product. Stem cells are obtained in a complex process e.g. through painful bone marrow biopsy and subsequent costly stem cell separation and purification. The innovative concept of APOSEC is to provide a drug, which, in concentrated form, has the proven therapeutic efficacy of stem cells and at the same time is simple and inexpensive in the making. APOSEC can be stored without loss of quality and it is expected to be used "off -the-shelf".

1.4.2 Development beyond state-of-the-art achieved by the APOSEC clinical phase II diabetic foot ulcer trial

“Chronic wounds” are seen seldom in healthy humans. Most patients suffering from impaired wound healing have the diagnosis of diabetes, obesity or pressure ulcer development after neurological impairment. Chronic

wound is defined as one that is unresponsive to initial therapy or persistent in the face of appropriate care. The most common types of chronic wounds of the lower extremity are described by their etiology: 1) vascular (e.g. arterial, venous, or mixed ulcers), 2) pressure ulcers, and 3) neuropathic (e.g. diabetic ulcers). Chronic wounds are not defined by size, complexity or failure to heal within a limited timeframe. (ASPS, 2007)

Wound therapy can be divided in a **traditional (dry)** and **moist (advanced) wound care**. The traditional approach is the treatment with gauze-based dressings such as woven and non-woven sponges, conforming bandages, non-adherent bandages, and aid pads. This treatment still accounts for over 50% of the total wound care dressings market. In the 1960's, advanced wound care was demonstrated to add quality and increased wound closure by applying moist wound healing products to the wound area. These ointments are provided with and without bio-active additives. Such products can contain bovine collagen, peptides of porcine collagen, or growth factors. Also biological skin or skin replacement therapies as well as antimicrobial dressings and negative-pressure wound therapy (VAC) are available.

Figure 8 provides an overview of the of the global wound care market divided by segments.

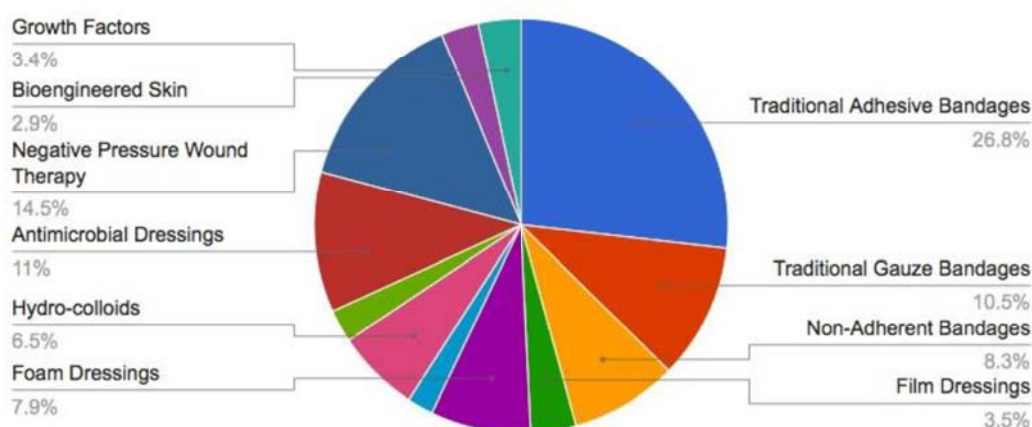


Figure 8 Global wound care market, by segments. Medilignce 2013.

Visiongain, a business information provider based in London, UK predicts that the world **market for advanced wound care** will reach \$8.7bn in 2015. Four companies, KCI, Smith & Nephew, Molnlycke and ConvaTec, together constituted 55.7% of the total advanced wound care market in 2012 (Visiongain, 2013).

1.4.2.1 Potential advantage of APOSEC

APOSEC has proven efficacy in augmenting wound healing in a clinical relevant large animal model via massive induction of angiogenesis and epidermal thickening. APOSEC treatment is envisioned by our study team to be included in the advanced wound care market, since the drug product will be applied in a moist hydrophilic carrier (e.g. NuGel). We believe that, after successful trials, cost effectiveness will be the strongest argument for penetration into the market. Further breakthroughs by APOSEC are shown in Table 2.

APOSEC dosages of 25-50 million cell equivalents / 3ml carrier gel (e.g. NUGEL) are anticipated in humans. This dosage is delineated from our large animal data. In this large animal experiment we have shown effectiveness at a dosage of 50 M resuspended 3 ml NUGEL equalling to a final dosage of 16.6 million/ml NUGEL; the COG for APOSEC derived from 1×10^9 PBMC is around €760. Therefore: if a dosage of 25/50 million/3ml NUGEL is anticipated in the clinical arena, one treatment would cost €19 or €38 respectively. We therefore are convinced that our secretome derived therapy with APOSEC will, when proven successful in phase II trials, offer the society a cost effective drug substance for the treatment of diabetic ulcer.

Table 2: Breakthroughs by APOSEC in wound treatment compared to cell based therapies

Current status in cell based therapies	Breakthroughs by APOSEC
Different types of cells (fibroblasts, keratinocytes) and/or stem cells used for the production either need to be of autologous origin or require additional processing in order to avoid cell-related immunity response on behalf of the recipient of the therapy (e.g. extensive expanding of the cells in vitro for the production of OrCel)	Secretome of PBMC is used; since APOSEC is cell-free, autologous cells are not needed
In the case of some products, cells are acquired from the patient in a painful process and/or causing a new wound (ReCell requires a puncture of the size of a stamp; EpiDex requires plucking out about 30-70 hairs from the head of the patient)	APOSEC is produced from a current waste product of blood transfusion units (PBMC – white blood cells)
Production process is complex and costly. (E.g. extraction of cells from neonatal foreskin (APLIGRAF) or mesenchymal stem cells (Grafix), cell cultivation on some kind of matrix etc.)	Simple and cost-effective GMP-production process
The acquisition of the components of the products – living cells – in general is complicated.	APOSEC is produced from a current waste product of blood transfusion units (PBMC – white blood cells)
Limited number of cells available for skin repair	No limitation of the amount of cell equivalent (dosage) of the used protein-mix
In some products, the cells are used in an autologous manner and need some time (six weeks in the case of EpiDex) to be cultivated and prepared for use.	APOSEC, once produced and packed, is available anytime.
For storage cell products usually have to be frozen, and afterwards thawed for use. This process may infringe the vitality of the cells (e.g. in Dermagraft 60% of the cells regain vitality)	As APOSEC is a cell-free product, the full therapeutic power of its components is conserved.
Shipment and preparation for use of these products as well as patient management require detailed attention due to very limited product preservability	APOSEC is a robust and easy to store product because the end product is a lyophilized powder.
Clinical utilization is often complicated and time consuming (e.g. if a wet and limp layer of living cells has to be cut to fit the extensions of the wound before being spread out onto the wound utilizing surgical instruments)	Utilization of APOSEC in topical application is easy, for APOSEC can be resuspended in a cream, or may even be commercialized for topical use in the form of a cream.
Time consuming treatments (living cells have to be prepared and applied to the wound with great care)	After debridement of the wound, the application of APOSEC is that of a simple cream
Highly cost consuming treatments	Cost-effective treatments with a cost of good of about 38€/treatment
Not approved for commercialisation in the EU	As APOSEC is a cell-free product, no repulsive reactions of the immune system of recipient of the treatment are elicited; this might facilitate approval of the medicinal drug candidate for commercialisation in the EU

1.4.2.2 Current situation on the market - Advanced wound care products

Various advanced wound care products based on cells and in combination with scaffolds are currently available on the market in the USA, only one product, ReCell[®], is currently available in the European Union. In the area of biologics (wound care products utilising compounds such as growth factors) most products are still in the development stage and are undergoing clinical studies. Table 3 and Table 4 are showing a selection of competitive products of APOSEC.

Table 3 Some competitive products of APOSEC for the treatment of skin wounds - Cell based products (on scaffolds)

Product	Company	Application	Status / Price
Dermagraft [®]	Shire/Advanced BioHealing	Diabetic Foot Ulcers	On the market in USA, CAN
Apligraf [®]	Organogenesis	Venous Leg Ulcers Diabetic Foot Ulcers	On the market in USA
Grafix [®]	Osiris	Acute and chronic wounds	On the market in USA
EpiDex [®]	Euroderm	Chronic wounds	Close to market
ReCell [®]	Avita Medical	Acute injuries, scar remodelling, pigmentation issues	On the market in EU, CAN, AUS Pending in USA
OrCel [®]	Forticell Bioscience	acute surgical excisions	On the market in USA
AlloDerm [®]	LifeCell	Soft-tissue repair	On the market in USA

There are no “official” publically available prices for the products mentioned in Table 3 although they are already on the market for some years (<http://celltrials.info/2013/08/17/grafix/>). However, they are estimated to be rather expensive: “Cell-derived wound care products in addition to standard care generate very high costs” (Langer A, Rogowski W, 2009). Their comparative advantages are usually justified by the reduction of treatment time and concomitant reduction of hospitalisation costs (<http://www.smith-nephew.com/about-us/what-we-do/advanced-wound-management/economic-cost-of-wounds/>).



Cost efficacy studies can be found in the literature, however they are often consolidated from various sources and therefore do clearly not reveal individual product prices (Langer A, Rogowski W, 2009). Some calculate costs for one additional successfully treated patient (Hankin et al. 2012) without mentioning the cost of good of the therapeutic products. Hankin et al. mention \$4,992 per treatment for Apligraf, \$630 per treatment for Oasis, and \$800 per treatment for Tallymed. Hanft et al. (2003) mention the prices of Apligraf (\$1,000 - \$1,200 per use) and OrCel \$995 per unit (<http://www.podiatrytoday.com/article/1771>). The costs per wound healed range from US \$10,323 - \$16,936 for Apligraf, Australian \$12,500 for Dermagraft and US \$11,708 for Regranex (Langer A, Rogowski W, 2009).

Table 4 Competitive products of APOSEC for the treatment of skin wounds - Biologics

Product	Company	Application	Status
Regranex [®]	Johnson & Johnson	Diabetic Foot Ulcers	On the market in USA; Warning on increased rate of mortality secondary to malignancy
BioChaperone [®] PDGF-BB	ADOCIA	Diabetic Foot Ulcers	Phase II – III
DSC-127	Derma Sciences	Diabetic Foot Ulcers	Phase III
TMBP-3	Tissuemed Biosciences	Diabetic Foot Ulcers	Pre-clinical studies + Phase I

1.4.3 Developments beyond state-of-the-art achieved by the clinical phase I-II AMI study

Acute myocardial infarction (AMI) is a leading cause of death worldwide. Advances in treatment for patients after AMI have led to a decrease in early mortality, but as a result there is a higher incidence of heart failure (HF) among survivors (Velagaleti et al. 2008). Current therapies do not address the central problem associated with the aftermath of AMI—the massive loss of cardiomyocytes, vascular cells, and interstitial cells—so these patients continue to experience frequent hospitalizations and premature death. Regeneration of the infarcted heart is a daunting task considering that the cardiomyocyte deficit could be in the order of 1 billion cells, that the environmental cues required to guide transplanted cells into multicellular, three-dimensional (3D) heart structures might be absent from damaged myocardium. Advanced patient age, cardiovascular risk factors (in particular diabetes), and heart failure appear to have a negative impact on the functional activity of the bone marrow (BM)-origin and blood-derived progenitor cells used for cardiac repair in clinical trials (Hill et al. 2003). Furthermore, human cardiac stem cell regenerative therapy is currently limited by low rates of cell engraftment and poor survival of the injected cells after direct surgical or catheter-based delivery. Owing to the limited availability of differentiated cardiomyocytes and cardiac resident stem cells, or the number of deliverable pluripotent stem cells however, it is unrealistic to use these cells for large-scale clinical applications (Wollert et al. 2010).

While bone marrow and blood-derived cells seem to have a favourable impact on the function of the infarcted heart, they cannot replenish lost cardiomyocytes and vascular cells to a meaningful extent. A large body of evidence indicates that the beneficial effects of cell therapy are related to the secretion of soluble factors acting in a paracrine manner. Human peripheral blood-derived EPC, nucleated BMC, and skeletal myoblasts secrete large arrays (>100) of bioactive molecules. Potential effects of paracrine factors include myocardial protection, neovascularization, modulation of inflammatory and fibrogenic processes, cardiac metabolism and contractility, increase in cardiomyocyte proliferation, and activation of resident stem and progenitor cells. Endothelial progenitor cell conditioned medium (EPC-CM) enhanced the formation of capillary outgrowth in a rat aortic ring model and enhanced survival of serum-starved human umbilical cord endothelial cells (HUVEC) (Di Santo S et al. 2009). EPC-CM contains massive amounts of cytoprotective proteins causing anti-apoptotic and growth factor mediated effects. Most importantly, neutralization of combined or selective cytokines such as VEGF, HGF, IL-8 and MMP9 did not significantly reverse the cytoprotective effect of EPC-CM. It seems that EPC secrete factors, which cause a broad synergistic effect and exert strong cytoprotective properties on differentiated endothelium through modulation of intracellular antioxidant and defensive mechanisms and pro-survival genes (Yang et al. 2010). Apoptotic cell ingestion by macrophages induces further expression of anti-inflammatory cytokines, such as interleukin (IL)-10 and transforming growth factor (TGF)-beta. The increased expression of anti-inflammatory cytokines by macrophages phagocytosing apoptotic cells suppress synthesis of pro-inflammatory mediators (e.g. tumour necrosis factor [TNF]-alpha, IL-1-beta and IL-6) in the infarcted heart, resulting in accelerated resolution of the inflammatory response. Apoptotic cells are also recognized by immature dendritic cells, which are present in all tissues, including heart (Kocher et al. 2006, 2007, Wollert et al. 2010).

The mechanism of BMC-SN and EPC-CM is similar to that which we described concerning PBMC, which documents the first evidence that culture medium from apoptotic PBMC (APOSEC) induces cytoprotective mechanisms in co-cultured cardiomyocytes in vitro. However, unlike the BMC-SN process, which requires BM stem cell acquisition and preparation, PBMC can be obtained from venous whole blood, which can be much more easily obtained via simple venal puncture. Our obtained short and long term magnet resonance imaging (MRI) results evidenced that one high dose APOSEC infusion led to a highly significant improvement of cardiac function and attenuation of AMI, indicating a dose-dependent effect. Thus APOSEC appears to represent an “endogenous biological effective substance” in concentrated high dose, which prevents experimental myocardial infarction by causing “peri-infarct conditioning” and stimulation of

regenerative effects in the acutely hypoxic myocardium. An overview of developments beyond state-of-the-art in stem cell research through APOSEC is given in Table 5.

Table 5: Breakthroughs by APOSEC in treatment of AMI compared to stem cell research

Current status in stem cell research	Breakthroughs by APOSEC
Different type of stem cells are used for cardiac repair, need of autologous origin	Secretome of PBMC are used; since no cell-related immunity, autologous cells are not needed
Harvesting of bone marrow or peripheral blood from acutely severely ill patients	No need of bone marrow or blood sampling in patients with AMI
Limited number of cells available for cardiac repair	No limitation of the amount of cell equivalent (dosage) of the used protein-mix
Cardiac regeneration therapy starts with delay	Cardiac regeneration therapy starts during the very acute phase of infarction, carrying the possibility for early repair mechanism
Difficulties of intracoronary or intramyocardial or transvenous (via sinus coronarius) delivery	Simple iv. infusion
Risk of side effects, such as no reflow after intracoronary delivery, perforation after intramyocardial delivery	No side effect expected
No or minimal efficacy after iv. cell delivery	Effective after iv. delivery
Cost-consuming interventions, especially the intramyocardial delivery	Cost-effective, with minimal additional costs for the patient
Numerous exclusion criteria of the human stem cell therapy, such as open-infarct-related artery, wall thickness >8 mm for intramyocardial delivery, etc.	Every patient can be treated
Additional intervention is necessary, such as coronary angiography	No additional intervention is necessary
Time-consuming therapeutic procedure	Easy to use, no additional time-consuming procedure is needed
Effect is dependent of the viability and function of the stem cells; for example decreased in old or diabetic patients	Effect is independent from the function of the stem cells or the morbidity of the patient
Current medical therapy did not address the myocardial regeneration	Medical therapy, addressing the regeneration of the myocardium via protection from ischemia

1.4.3.1 Current treatment options following AMI

Currently, the initial therapy for AMI focusses on limiting the injury to the cardiac tissue and is therefore directed towards restoration of perfusion as soon as possible. This may be accomplished through mechanical means, such as percutaneous coronary intervention (PCI) or Coronary Artery Bypass Grafting (CABG), or the use of substances like oxygen, nitrates, analgesic medications, antiplatelet agents, fibrinolytics, β -blockers, and angiotensin-converting enzyme (ACE) inhibitors.

Prevention of myocardial remodelling and advanced treatment options

Despite a decrease in post-MI mortality and morbidity, all-cause mortality over a five year period is still at approx. 20%, with a 12% mortality rate over the first two years after the index infarction. At least 10% of patients surviving an infarction will develop congestive heart failure, which severely impairs their well-being and carries a five-year mortality of 50% (Yeh et al., 2010, Fox et al., 2003, Spencer et al., 2004, Roger et al.,

2004, Levy et al., 2002). Therefore advanced therapies to prevent myocardial remodelling or improve cardiac regeneration are highly sought after. A number of potential drug compounds are currently under scrutiny in the various clinical phases and must be seen as competitive products of APOSEC (Table 6, Table 7)

The APOSEC project will try to demonstrate in clinical studies that an additional treatment with APOSEC significantly improves left ventricular function in a sustained manner, and thereby reduces the incidence of post-MI heart failure. This will provide a very important advance in the care for AMI patients and in addition, the very significant cost burden to health systems caused by the large number of heart failure patients can be significantly alleviated.

Table 6 Competitive products of APOSEC for prevention of ischemia-reperfusion injury

Product	Company	Application	Status
KAI-9803	KAI Pharmaceuticals	Acute myocardial infarction	Phase IIb
IK-6001	Ikaria	Stabilisation of endothelial integrity	Phase II - III
Danegaptide	Zealand Pharma A/S	ST-segment elevation myocardial infarction	Phase II

Table 7 Competitive products of APOSEC regarding cell therapy for cardiac regeneration

Product	Company	Application	Status
Prochymal®	Osiris	acute graft versus host disease (GvHD) Crohn's disease	Phase III
Mesenchymal Precursor Cells	Mesoblast	Myocardial Infarction	Phase IIa/IIb
Bone Marrow Cells	T2Cure	Acute myocardial infarction	Phase II
MyoCell	Bioheart	severe heart damage	Phase II-III

“Unique selling proposition of APOSEC versus Stem cells”

- ✓ APOSEC is a cost-effective, easily obtainable medicinal product derived from stressed peripheral blood mononuclear cells, currently a waste product in all transfusion units worldwide. The process of APOSEC production and utilisation in multiple indications are covered by two patent families.
- ✓ A GMP Production line for APOSEC was established in Austria that has undergone validation and authorisation by the Austrian Agency for Health and Food Safety (AGES).
- ✓ Extensive pre-clinical data was obtained in clinically relevant large animal models by the lead applicant as a preparatory step to the clinical trials proposed in the project.
- ✓ In contrast to allogeneic and autologous stem cell therapies, APOSEC represents a drug substance that can be easily stored in lyophilized form or can be distributed in a carrier gel in conditions of chronic wounding or physiologic solution of sodium chloride for internal inflammatory diseases such as AMI.
- ✓ The aim of this project is to bring allogeneic GMP APOSEC into the realm of clinical application. Upon successful clinical trials, APOSEC will contribute to establishing a whole new industrial sub-sector within European regenerative medicine.

2 Impact

2.1 Expected impacts

The core aspects of the APOSEC regenerative medicine project are the **phase II clinical trial on diabetic ulcers** and the **phase I-II clinical trial on AMI**. The results gained within these trials will provide first-in-man evidence of the ground-breaking potential of APOSEC™ to provide new therapies to millions of sufferers in modern society worldwide in the fields of:

- Wound healing (e.g. for chronic skin wounds and diabetic ulcers)
- Acute Myocardial Infarction (AMI)

Particular to the APOSEC project is that it begins with a thoroughly researched, **patented therapeutic drug candidate as background**, owned by the Aposcience AG (APO) for the most important target markets worldwide. These patents will be utilised within the project and further exploitation options will be sought including attracting post project financial support via investors for subsequent phase III clinical trials as well as searching for potential licensees. **Regulatory authorisation** has already been **granted for the APOSEC™ GMP production** facility at the [REDACTED]

APOSEC™ is produced from a blood transfusion waste product, making it unique as the most sustainable drug of its type on the market. The proposed project is the next vital step in bringing APOSEC™ a step closer to benefiting the public i.e. Phases I & II clinical (in-patient) trials which are prerequisite to the Phase III trials and the subsequent licensing to pharmaceutical companies for industrial scale production. The proposed H2020 funding provides required leverage to the significant investments already invested in APOSEC fundamental research and the associated worldwide IPR protection, pre-requisite to stimulate growth and competitiveness of the science-drive SMEs, both directly for Aposcience AG as patent owner and for the European regenerative medicine sector.

2.1.1 Diabetes as cause for both cardiovascular diseases and chronic wounds

The main fundamental need for the clinical approval of APOSEC™ is to combat the global diabetes ‘epidemic’. According to the International Diabetes Federation, 56.3 million people in Europe suffered from diabetes in 2013 and by 2035, this number will increase by 22% to 68.9 million (Figure 9). This corresponds to about 10% of the population over 25 years of age currently living with the disease while also straining national economies and public health systems.

IDF REGION	2013			2035			Increase in the number of people with diabetes %
	Population MILLIONS	Number of people with diabetes MILLIONS	Comparative diabetes prevalence %	Population MILLIONS	Number of people with diabetes MILLIONS	Comparative diabetes prevalence %	
AFR	407.9	19.8	5.7	775.5	41.5	6.0	109.6
EUR	658.7	56.3	6.8	668.7	68.9	7.1	22.4
MENA	374.5	34.6	10.9	583.7	67.9	11.3	96.2
NAC	334.9	36.8	9.6	404.5	50.4	9.9	37.3
SACA	300.5	24.1	8.2	394.2	38.5	8.2	59.8
SEA	883.2	72.1	8.7	1,216.9	123.0	9.4	70.6
WP	1,613.2	138.2	8.1	1,818.2	201.8	8.4	46.0
World	4,572.9	381.8	8.3	5,861.8	591.9	8.8	55.0

Figure 9: Regional estimate for diabetes (20 – 79 years), 2013 and 2035 (AFR=Africa, EUR= Europe, MENA=Middle East and North Africa, NAC=North America and the Caribbean, SACA=South and Central America, SEA=Southeast Asia, WP=Western Pacific) (IDF, 2013)

Despite numerous preventive European and national health policy efforts as well as by developments in the food industry, this prevalence is accelerated by a demographic shift to an aging society. APOSEC offers a regenerative medicine breakthrough in the treatment of diabetes related diseases because:

- Diabetes causes chronic wounds (diabetic ulcer) in the lower extremities (→Main APOSEC patient group: wound healing and skin reconstruction)
- Diabetes leads to atherosclerosis which, in consequence, leads to acute myocardial infarction (AMI) (→Main APOSEC patient group – AMI)

In both cases, the diseases are caused by hypoxia (insufficient sustenance of the tissue with oxygen) and insufficient sustenance of the tissue with nutrients due to the impaired state of the body's blood vessel system. Up to 25% of all diabetes sufferers develop a diabetic ulcer once in their lifetime. The current therapy costs are about 7,500 and 20,000 USD per hospital stay (Singh et al. 2005; Rogers et al. 2008). Furthermore, 20% of all hospital stays related to diabetic ulcer are caused by the diabetic foot and 12% of all patients suffering from a diabetic foot lose their foot to amputation (ACFAS, 2006).

2.1.2 Main APOSEC patient group – Wound healing and skin reconstruction

The wound healing market is very broad in terms of in disease, surgical incision and surface damage from burns, cuts and especially as a result of diabetes. Important disease entities besides diabetic limb ulcers are venous ulcers caused by hypoxia in heavy smokers or as a consequence of cardiac insufficiency and pressure ulcers in immobilized patients i.e. as a result of poor circulation.

Chronic skin wounds

Chronic skin wounds represent a significant burden to patients, health care professionals and public health systems overall. Alone in the US, 5.7 million patients are affected costing the health care system an estimated \$20 billion annually. The worldwide wound-care market, which can be broadly divided into wound-closure products, anti-infectives, basic wound care, advanced wound care, active wound care, and miscellaneous therapies, is expected to continue to grow at a compound annual growth rate (CAGR) of 6% with the direct cost of wound-care products to the U.S. healthcare system being over \$7 billion in 2007 (Timmers et al. 2008). It is estimated that almost 1-1.5% of the population in the industrialized world will have a problem wound at any one time. In Europe, the average cost per episode is €6,650 for leg ulcers and €10,000 for foot ulcers, which accounts for 2-4% of health-care budgets. This figure can be expected to rise with an increasingly elder and diabetic population (Gottrup et al. 2010).

Diabetic skin ulcer

Diabetic skin ulcers represent a major, high impact sub-group of the chronic skin wound patient group. Every year, 5% of diabetics develop foot ulcers and 1% requires amputation with lower limb amputations (Sen et al. 2009) - at least ten times more common in people with diabetes than in non-diabetic individuals in developed countries with more than half of all non-traumatic lower limb amputations being due to diabetes (Icks et al. 2009). People with diabetes require at least two to three times the health-care resources compared to people who do not have diabetes, and diabetes care may account for up to 15% of national health care budgets (Zhang et al. 2010). The health economics of topical treatments for skin wounds and in particular diabetic skin ulcers is not easy to evaluate because of the variations in therapeutic approaches i.e. type and cost of products used, frequencies of dressing change and the relatively long treatment periods. Arguably, the highest, and often underestimated cost to the health care system, is the time spent by medical staff i.e. the frequency of dressing change and whole healing time. Another study by (Schaper, 2012) confirmed the average (direct & indirect) cost of treating one foot ulcer at €10,000 with the majority of these costs being related to hospital admissions. This corresponded to an overall annual cost of €10 billion for 2005 in Europe. If the APOSEC clinical trials are positive, and it could achieve a conservative 5% reduction in the overall cost, this would immediately save the European public health systems €50 million in one year.

In the United States, the total direct cost for diabetic foot care was estimated by Barshes et al. (2013) to be \$17 billion, comparable to the annual costs of breast cancer and colorectal cancer (Figure 10).

In Europe, in a study comprising 821 patients in 14 European diabetic foot centres, the costs have been estimated to be about €10,000 per treatment (Prompers et al. 2008).

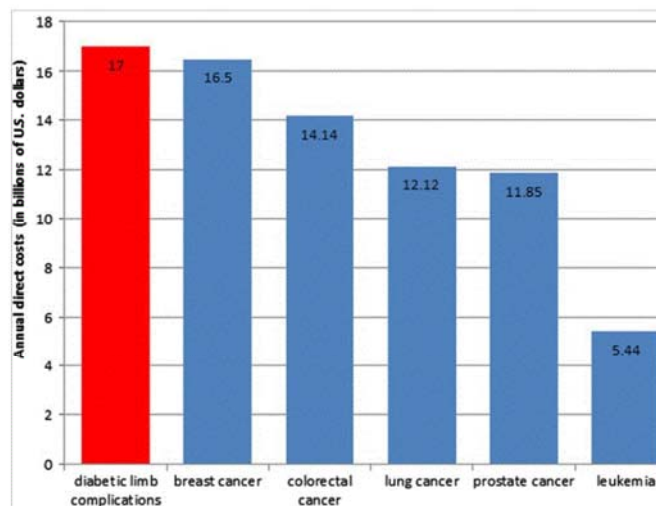
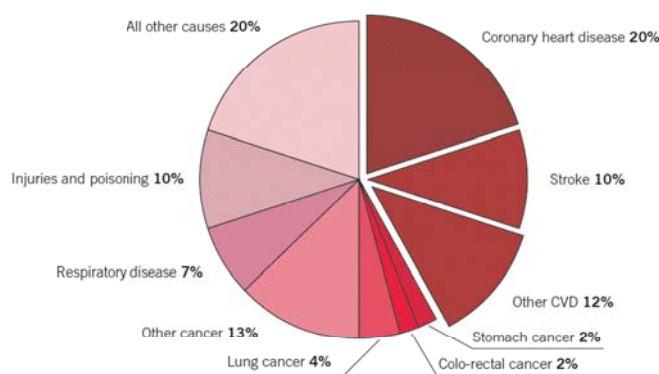


Figure 10: Annual direct costs to the US Health System by diseases (Barshes et al. 2013)

2.1.3 Main patient group - Acute Myocardial Infarction (AMI)

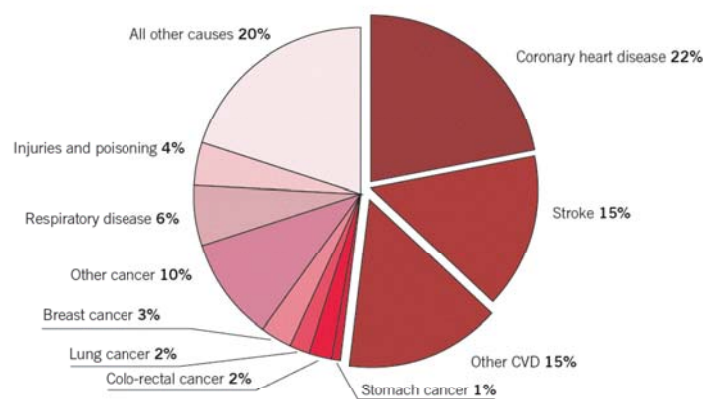
Cardiovascular diseases (CVD) cause annually more than four million deaths in Europe (47%). Although CVD mortality saw large increases until early 21st century, it is falling in most European countries. Absolute numbers are, however, offset by the demographic trend towards an ageing society. The main forms of CVD are coronary heart disease (CHD) and stroke (Figure 11).

Figure 11: Cardiovascular diseases (CVD) mortality in men, last available year, Europe



Coronary heart disease (CHD) is the single most common cause of death in Europe, accounting for 1.8 million deaths each year; stroke is responsible for about 1.1 million deaths per year in Europe. Over one in five women (22%) and one in five men (20%) die from CHD as the single most common cause of death in the EU, accounting for over 681000 deaths each year: 15% of deaths among men, and 13% of deaths among women (Figure 12, Nichols et al. 2012).

Figure 12: Cardiovascular diseases (CVD) mortality in women, last available year, Europe



AMI leads to chronic heart failure (CHF)

In standard treatment, after diagnosis of acute myocardial infarction (AMI), the patient is admitted as quickly as possible to the nearest hospital where reperfusion of the infarct-related artery takes place immediately. Unfortunately, myocardial necrosis starts early after coronary occlusion, usually before reperfusion can be achieved. The resulting loss of viable myocardium initiates an inflammatory process that leads to a replacement of the infarcted area with scar tissue, and sets the stage for systolic dysfunction and progressive ventricular remodelling in many patients. This process results in a weakened heart and potentially subsequent heart failure (HF) or coronary heart disease (CHF) (Korf-Klingebiel et al. 2008).

Heart failure (HF) affects 2–3% of the population in industrialised countries with a marked rise in those aged above 65. It has been estimated that about 15 (of 900) million Europeans and 5.8 (of 300) million US Americans suffer from HF. The prevalence of HF has been increasing over the past decades and is expected to further increase in the future, due to the increasing proportion of elderly in the population and the improved survival of patients with cardiac and non-cardiac conditions such as acute myocardial infarction, hypertension, diabetes, renal failure, and metabolic syndrome that may trigger the development. In the USA, the annual cost of HF in 2010 is estimated to be \$39.2 billion or about 2% of the total US health-care budget. Evaluations from different European countries indicate a similar share of HF-related costs in relation to overall health-care expenditure. As a consequence of the demographic trends, the estimated annual cost for HF in the USA rose from \$24.3 billion in 2003 to 39.2 billion in 2010 (Braunschweig et al. 2011).

Major advances in treatment over the last 4 decades have translated into a considerable decline in mortality rates after myocardial infarction (MI). However, HF is a common complication of MI with the estimated incidence varying from 10%-40%. Post-MI HF is associated with a markedly elevated risk of death, with an estimated median survival of about 4 years (Velagaleti et al. 2008).

The standard treatment of AMI (angioplasty (= surgical reopening of the occluded artery followed by reperfusion of the hypoxic area)) unfortunately also leads to reperfusion. If instituted in a timely manner, reperfusion can prevent a potential transmural AMI and limit the extent of necrosis greatly. However, some injured cardiomyocytes at the edge of the wave front become irreversibly injured during the reperfusion phenomenon. This effect is due to the peroxidation of lipid membranes caused by oxygen-derived free radicals which results in necrosis and cell death. The earlier the reperfusion, the less necrosis occurs; conversely reperfusion can be rendered less effective by microvascular damage and obstruction that develop during the ischemic phase (Dominguez-Rodriguez et al. 2014). Ischemic Heart disease is the number one cause of death worldwide, killing 7 million people annually WHO, 2013) and APOSECTM is expected to alleviate reperfusion injury through its immunomodulatory and cytoprotective effects.

In the rule, prevention is better than cure, but current and trending statistics show that treatments will need to become more effective and affordable to have a widespread effect in rendering the impact CVD and HF is having on society and public health care systems. Current treatments usually combine use of drugs such as aspirin, beta blocker, diuretic and statin and can lead to reductions in MI and HF amongst high risk groups. APOSECTM has a main benefit as a treatment to prevent for cardiac remodelling and for prevention of recurrences and complications in those with established disease.

2.1.4 Stimulate growth and competitiveness of European regenerative medicine

Due to substantial budget cuts in recent years and a conservative investor market in Europe, researchers, and therefore the EU is struggling to establish leadership in cell based regenerative medicine. As a result and the fact of being closer to a larger, more stable market, investors currently take more interest in medical and pharmaceutical research undertaken in the US than in the EU. Furthermore, in European value systems commercialisation and commoditisation of the human body and its parts is generally not very well accepted. In the area of cell-based regenerative medicine, ethical concerns and doubts still prevail in the general public.

Economic globalisation has changed the world's economic order, bringing with it new challenges and opportunities. Europe cannot compete in this new environment unless it becomes more innovative and responds more effectively to practitioners' and patients' needs and preferences. APOSEC™ is a cell free compound derived from a waste product obtained from conventional blood donations. This novel methodology is unique in the world and sets the basis for new approaches towards the treatment of various indications e.g. skin wounds and myocardial infarction, clinically investigated in the project, but also has high potential for therapeutic treatment for stroke or spinal cord injuries.

Setting the basis through the APOSEC project would allow for the development of core competencies on cell free regenerative medicine in Europe. This will help in gaining more momentum in order to lever further (private) investments needed to perform necessary basic research as well as additional phase III and IV clinical trials plus clinical trials for other indications than skin indications i.e. AMI. This innovative research and clinical trial project and its results could be a stepping stone towards Europe achieving a leading role in cell free regenerative therapy.

European small and medium sized enterprises (SMEs) and industry working in the field of regenerative medicine and production of pharmaceuticals could in addition valorise the APOSEC project results in order to:

- develop new therapies using APOSEC™ for the treatment of other indications than diabetic ulcers and AMI and;
- produce ready-made pharmaceutical products containing APOSEC™.

As the indications targeted in the APOSEC project are of major concern in all industrialised countries and patented protection has been granted for most major countries, the potential for exporting and international business development placing Europe at the forefront in this field is a strategic impact goal of this project.

2.1.5 Levering existing investments in fundamental research in regenerative medicine and improving innovation capacity

In the Seventh Framework Programme (2007-2013), €6 billion were spent on health related topics. For Horizon 2020, the budget was increased and amounts to €7.4 billion for tackling the societal challenge on 'Health, demographic change and wellbeing'. These investments offer an unparalleled resource and the APOSEC project will provide innovative treatment options in the indications diabetic ulcer and AMI, using a novel approach with cell free regenerative therapies. The market for advanced therapeutics for skin wound healing, especially in the case of diabetic ulcers (as described in Section 2.1.1) is expected to be the initial indication for APOSEC exploitation. However, it must not be forgotten that the extensive research and ground-breaking results leading up to this shows clear signs that APOSEC™ has much more potential. To reduce risk, it makes scientific and economic sense to leverage the existing investment in fundamental research by running a parallel investigation using APOSEC™ to treat AMI. With the compelling results gained to date, it was possible to motivate some of Europe's leading cardiologists to join the consortium to leverage this valuable research with scientific data from human trials. In addition, further developments of therapies for other indications (e.g. stroke, spinal cord injury) are expected.

2.1.6 APOSEC in the context of European Regenerative Medicine Research

Several ongoing European initiatives relevant to the APOSEC project, to which various linkages are assured, include:

EUROSKINGRAFT - A clinical trial project aiming to develop novel generation of skin substitutes to clinically treat a broad spectrum of severe skin defects. Contact will be taken up via the D&E work package to explore collaboration and exchange possibilities.

BAMI - The effect of intracoronary reinfusion of bone marrow-derived mononuclear cells (BM-MNC) on all-cause mortality in acute myocardial infarction. The project will develop a standardised method of bone marrow cell collection, optimising reparative potential of bone marrow derived cells and preparation procedure and bone marrow cell delivery post AMI and conduct the first large scale all-cause mortality clinical trial. Two APOSEC partners [REDACTED] are BAMI project partners who will ensure synergies and knowledge sharing.

In addition, close contact will be maintained with **ECRIN**. The European Clinical Research Infrastructures Network is dedicated to improving the health of patients and citizens across the world through clinical research and in particular, the resulting ECRIN-AI project in which [REDACTED] is a WP Leader. ECRIN-AI organises a Clinical Trial Day around 20th of May, a transnational communication event on multinational clinical trials, with the objective of facilitating better clinical research relevant to the needs of patients and highlighting the important role of patients in clinical research. APOSEC will be pursuing an active role within the Clinical Trial Day programme.

2.1.7 APOSEC and new approaches to currently untreatable diseases

The novel drug candidate APOSEC is expected to provide an essential improvement of the currently unsatisfactory treatment of chronic wounds. Furthermore, APOSEC is capable of preventing the therapeutically untreatable state of heart failure (HF) when used as additional therapy to the conventional therapy of acute myocardial infarction (AMI).

Chronic wounds, such as e.g. diabetic ulcers, are characterised by damages of tissue and the vascular system caused by the underlying disease. This damage is associated with an impairment of the supply of the wound with oxygen and other nutrients leading to prolonged inflammation and impaired neovascularization. Chronic wounds are painful, they take months or even years to heal, often relapse and in a considerable number of cases amputation of the affected limb is the last resort. The current treatment of chronic wounds requires the utilisation of significant resources in terms of treatment materials, nursing time and hospitalisation days while eliciting only very limited treatment success.

Heart failure (HF) describes the state of a damaged heart consecutive to acute myocardial infarction (AMI). It is characterised by left ventricular remodeling and scarring of the myocardial tissue. The muscle of the heart is weakened, and heart function has become insufficient. The only two therapy options for heart failure are pharmacological therapy and cardiac transplantation. Pharmacological agents are not effective in severe (class IV) cases of heart failure, and the therapeutic success of heart transplantation is limited by complications of long-term immunosuppression.

The therapeutic power of APOSEC lies in the fact that it is not aimed at just one biological target. It exerts a series of immunomodulatory effects (see: section 1.3.1.4, “Identified mechanisms of action of APOSEC”) attenuating inflammatory responses, preventing microvascular obstruction and improving the supply of skin or myocardial tissue affected states of hypoxia and inflammation.

2.1.8 Relevant EU policies: APOSEC contributes to EU health policy implementation

EU policies set out to help citizens to live not only longer but also healthier lives by fostering good health in an ageing European population, supporting dynamic health systems and new technologies and tackling health inequalities. APOSEC contributes to the following EU health policy objectives laid down in the EU Health Strategy “Together for Health” and hence supports the overall Europe 2020 strategy:

I. Fostering good health in an ageing Europe

Projections show that the proportion of people over 65 in Europe will increase from 17% (2010) to almost 30% by 2060 (EC, 2014). The therapeutic effectiveness of APOSEC™ comprises diseases typically associated with old age, such as metabolic diseases (e.g. diabetes mellitus) and cardiovascular diseases. The APOSEC project will not only provide for more economic treatment options, it is also expected that wound closure of diabetic ulcers in patients treated with APOSEC™ is achieved sooner and with less scarring when compared to the current standard therapy. Also, patients treated with APOSEC™ after an AMI are less likely to develop complications or to suffer from sequelae, such as cardiac remodelling or chronic heart failure. This in turn means shorter hospital stays as well as less sickness days and reduced costs associated with co-morbidity.

II. Supporting dynamic health systems and new technologies

Health systems in the EU are under increasing pressure due to emerging epidemiological and economic challenges e.g. increasing prevalence of chronic diseases. New technologies can contribute to the efficiency and sustainability of health systems. Therapies developed within the APOSEC project are based on the new concept of cell free regenerative medicine, valorising a waste product of conventional blood donations. The developed therapies are expected to improve effectiveness and reduce recovery time of current ‘standard’ treatments as well as helping to prevent heart failure and thus reduce overall treatment costs and subsequently lowering the financial burden on the European health systems.

III. Tackling health inequalities

In 2010, the gap in life expectancy at birth between the highest and lowest values for EU-27 Member States was 11.6 years for males and 7.9 years for females (EC, 2013). This can be due to e.g. barriers in the access to healthcare for disadvantaged groups or persons in vulnerable situations ^(Devaux et al. 2012) or higher levels of health-damaging behaviours. For the EC, one solution is to provide for broad access to affordable, high-quality health services (EC, 2012). The APOSEC project contributes to this through the development of an easy-to-use, off-the-shelf product with advanced storage properties as well as more economic therapy options for diabetic ulcers and AMI.

2.1.9 Barriers or obstacles to the impact achieved in the APOSEC project

Challenges of drug development

Small businesses often have difficulties financing growth, innovations and, especially in the field of regenerative medicine, clinical trials. The significant successes in basic research as described in section 1 now needs the H2020 financing to leverage the previous investments to bring APOSEC™ through the Phase I and II clinical trials en route to Phase III trials and eventual commercialisation. If successful, APOSEC will serve as a flagship project to the European life science SME community and will help stimulate the investor interest in this sector.

The development of a new medicinal drug can exceed \$1 billion (or even Euros) so that for small, scientifically driven companies, which are seen as the driver of innovation and the economy, the idea of developing a new medicinal drug appears as an impossible hurdle. However, what SMEs, such as Aposcience AG, can do is link up with leading researchers and medical professionals to motivate them to collaborate on the hope of contributing to improving individual citizen’s health and the society at large. Although the chance of receiving economical payback within ten years is low, without their commitment

there would be no progress in this branch. The daunting reality is: a drug development timeline of about 15 years (Figure 13) requiring astronomical investment levels, that without direct backing of pharmaceutical industry, is well beyond the capacities of any SME, and more so in Europe than in the US, where the capital markets are more accessible.

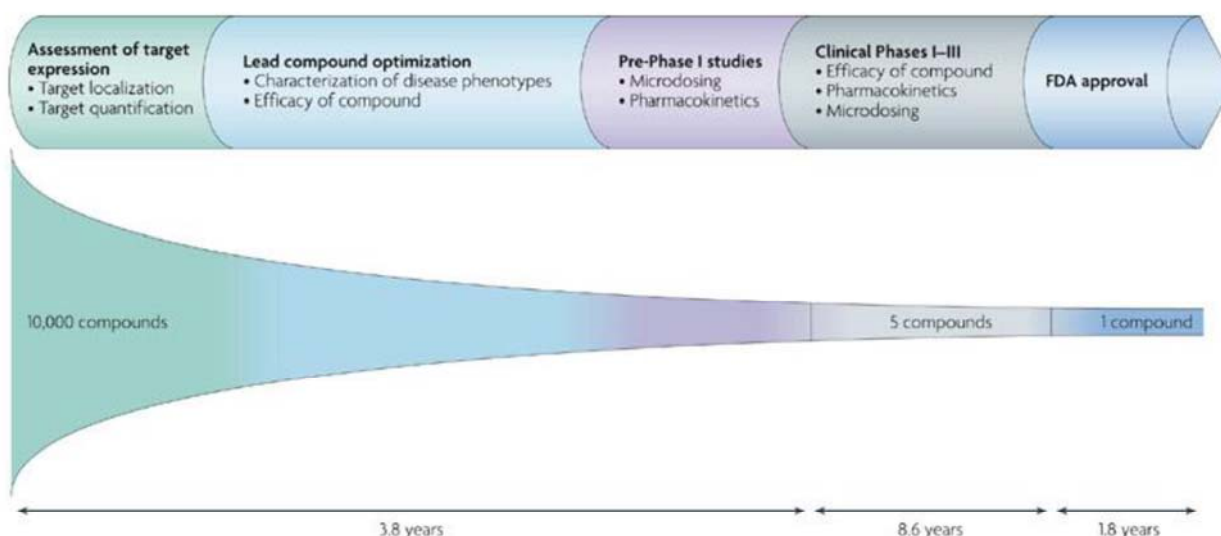


Figure 13: Drug development timeline according to Willemann et al. 2008.

This means that the path to commercialisation is much longer than the economic cycles of an SME, which is mostly due to regulatory requirements. The APOSEC project intends to overcome a number of regulatory hurdles through potency and toxicology assays, further research to ascertain product stability and finally with clinical studies. The route to commercialisation requires the generation of reliable scientific data in humans in order to find partners for phase III clinical trials.

2.2 Measures to maximise impact

a) Dissemination and Exploitation Strategy

The main aim of the APOSEC D&E Strategy is to pave the way for successful exploitation of the patented Aposcience AG therapeutics which can be broken down into specific D&E objectives:

1. To communicate and where necessary, establish a dialogue with the relevant stakeholders to ensure that the clinical trials can be implemented without obstacles created through either regulatory, gender or ethical opposition as well as to overcome any public or political scepticism.
2. To establish relationships with investors and pharmaceutical companies to convince them of the quality of the science and clinical trials so as to ensure timely access to resources to finance Phase III clinical trials of APOSEC™ ‘Skin’ therapeutics.
3. To pro-actively manage IP, seek its appropriate protection and then disseminate research results relating to the APOSEC™ ‘AMI’ therapeutics.

To implement the D&E objectives as described above, a range of dissemination tools and channels will be used. IPR will be managed as a dedicated task and will accumulate in development of business plans according to the various product pipelines to be followed up post project. An outline dissemination and exploitation (D&E) plan is provisionally designed in the proposal phase, which will be elaborated in the first four months of the project leading to the first D&E Deliverable (D7.1).

The D&E strategy balances interests of various stakeholder groups associated with the APOSEC project (see Section 2.2.2) which includes sensitive ethical issues surrounding human clinical trials, a stringent regulatory environment, but also aimed at attracting investors so as to secure post project financing of Phase III clinical trials. Particular to the APOSEC project is that it begins with patented therapeutics as background so that the planned accompanying research work is focused on ensuring safety and control of any negative side effects in parallel to the clinical trials but with potential commercial value relevance.

The exploitation efforts will focus on securing post project financial support via investors, which would be accompanied by a portfolio of dissemination activities and tools. The project will be supported by a state of the art website with appropriate use of social media, making use of professional network platforms and discussion groups (beginning with LinkedIn) and presenting at relevant scientific and industry conferences. As an innovative dissemination tool, animated whiteboard videos will be produced to cost-effectively convey scientific APOSEC concepts to non-scientific audiences i.e. “The Invisible World of APOSEC Regenerative Medicine”. These videos will be distributable via the internet and can be used at any public event.

2.2.1 APOSEC Background and Further Development Plans

Aposcience AG, the main exploitation partner has invested solidly in IP protection of APOSEC™ across most of the important markets worldwide (Table 8). Furthermore, they have registered the name APOSEC as a word mark in Nice classes 5 and 9.

Table 8: International patents APOSEC granted and owned by Aposcience AG (state 03/2014)

Subject Matter	PCT number	Priority	International Protection
Pharmaceutical preparation for treatment of condition associated with ischemia (‘AMI’)	WO2010070105	18/12/2008	AU, BR, CA, CN, EP, JP, NZ, RU, US, ZA
Pharmaceutical preparation comprising supernatant of blood mononuclear cell culture (‘Skin’)	WO2010079086	18/12/2008	AU, BR, CA, CN, EP, JP, NZ, RU, US, ZA, AT, CH, DE, ES, FR, GB, HU, IT, NL, PL, SE, TR

The APOSEC project is the key financial instrument required to exploit the patents for which is seen as the potential revenue generator to grow the SME Aposcience AG, both in terms of personnel as well as to fund further research and patenting activities. An overview of APOSEC background and the planned development are shown in Figure 14.

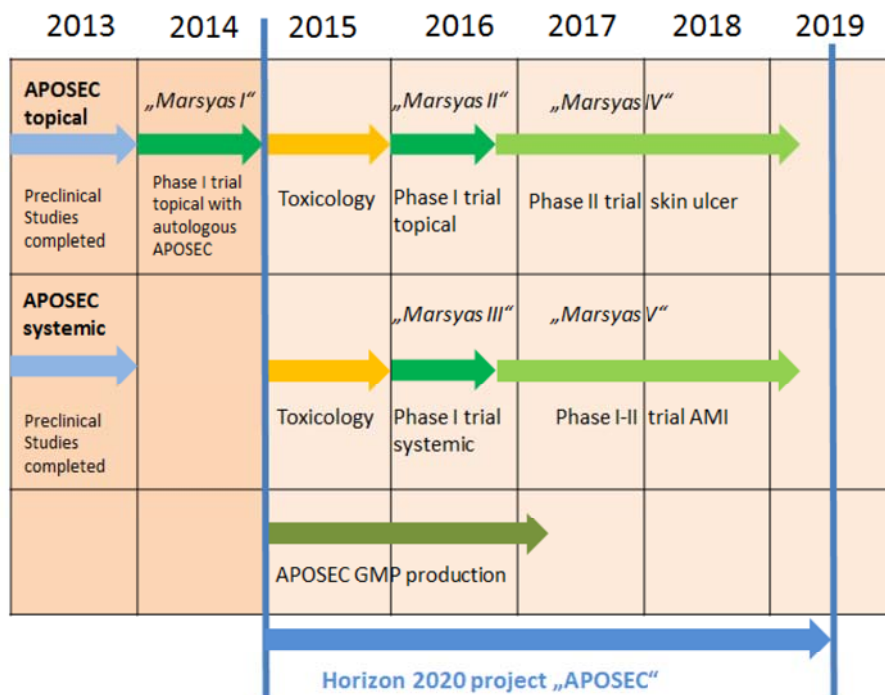


Figure 14: APOSEC Background and planned development within H2020

2.2.2 Research data management

Types of data the project will generate/collect and standards to be used

The APOSEC project will generate and collect data from clinical trials. This includes personal data of the patients enrolled in the clinical trials, thus including several aspects of their identity. As a matter of course, data protection will be of great importance in this project. ICH- good clinical practice (GCP) guidelines will be followed in all steps of data collection and reporting and the APOSEC project will comply with all local/national regulations. In the clinical trials, sensitive, personal information will be de-identified (anonymised) to ensure performance of double-blinded trials. In our clinical trials the risk of re-identification of patients is greatly reduced because treatment will be offered for diabetic skin ulcer and AMI, high incidence diseases in Europe, and not rare diseases. This makes re-identification by some individual subject characteristics very unlikely.

Data dissemination and accessibility for verification and re-use

The main means of the APOSEC project data dissemination will be publication in scientific, peer reviewed journals. Wherever possible, open access to these publications will be ensured by the “golden model”, allowing immediate access to the publication. A description of the trial (protocol) provided by APOSEC will be stored by the national competent authority in the EudraCT database of the EMA, together with the authorisation and opinion from the ethics committee. The protocol can be accessed via the EU Clinical Trials Register (www.clinicaltrialsregister.eu/). Result related information is currently not included automatically in the database. A summary of the original data will be posted also in the EudraCT database to make it accessible, complying with the EudraCT result related information guidelines. This will foster the proactive approach of EMA regarding the disclosure of documents.

To allow for public scrutiny, access to all data will be based on the EMA policy on “Publication and access to clinical-trial data” (Policy 70). As this policy is still in its’ draft version, the final details of which data will

be shared to which extent will be decided upon in the near future. To what extent APOSEC will make this raw data on a patient level available will also depend on policy 70 by EMA.

Data curation and preservation

Data uploaded to the EudraCT database will be continuously updated on any changes to the clinical trials unless there are substantial modifications and have to be reported as such. Paperwork including sensitive patient data will be stored in a dedicated, locked room with restricted access. Data archiving will be in agreement with current legislation (medical files, essential documents, study report). The full clinical trial data set and locked database will also be archived electronically with read-only access at the MUW central storage facilities.

2.2.3 Knowledge & IP management and protection and Open Access Dissemination

All knowledge (*results*) obtained will be reviewed for its' exploitation potential by the Executive Board as described in the Management section. If *results* are classified as being not potentially commercial i.e. requiring some form of IP protection, the material is deemed free to disseminate is the range of means to various stakeholder groups as described in section b) Communication (Figure 15).

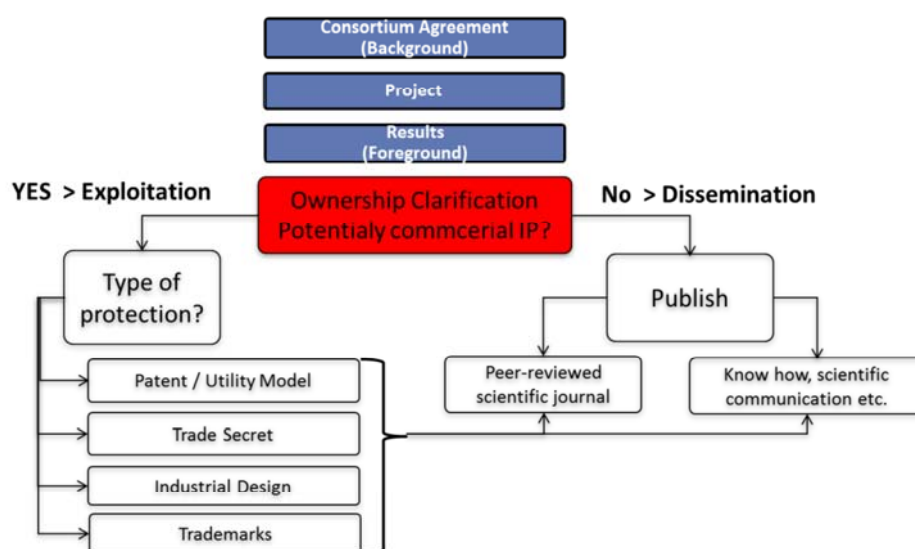


Figure 15 Overview of the Knowledge and IP Management

The general dissemination and exploitation “rules” for H2020 collaborative (multi-beneficiary) projects are defined in Section 3 “Rights and Obligations related to Background and Results” within the Multi beneficiary Grant Agreement as well as being elaborated on in the DESCA 3.0 Consortium Agreement which will be used for the APOSEC project. The Task 7.5 IPR Management is jointly implemented by RTDS and APO and essentially aims:

1. To facilitate a transparent process for the documentation of the production of *results*.
2. To clarify ownership of *results* (which can be often co-ownership).
3. To initially identify whether the *results* are capable of industrial or commercial application or can be readily disseminated as scientific results.
4. To prepare IP documentation for evaluation by the Executive Board, acting as Exploitation Committee with the Task 7.5 IPR & Innovation Management as described in the Management section and WP7.

Open Access Dissemination

To ensure open access to all APOSEC scientific publications the so called ‘golden’ open access model is clearly preferable in a fast-evolving field. Despite its’ additional cost (author processing charges) in case of a hybrid open access journal, it guarantees immediate access to the publications by the publisher. This ensures a high visual quality and an easy accessibility, directly on the publishers’ website. The golden open access model allows authors to retain the copyright for their publications, as encouraged by the EC, and grants licences only to the publishers. The most commonly used licence is the CC-BY (Creative Commons Attribution), which is the most open of the Creative Commons licences.

In addition, most journals allow for specific underlying data to be stored as “supplemental information” along with the publication, making it easy to find and providing access to data necessary to validate the results presented in the publication. In case the underlying data is so vast as to exceed the possibility of including it as supplemental information, its’ repository will be mentioned in the main text of the publication. If the Open Access Infrastructure for Research in Europe (OpenAIRE) allows for it in the future, APOSEC partners will use it to link underlying data with publications.

Depending on who is the lead author, all scientific publications, also publications making use of the golden open access model, will either be deposited via OpenAIRE in their institutions repository, or, if such a repository does not exist, in the OpenAIRE Orphan repository. When depositing via OpenAIRE, APOSEC beneficiaries will also check that the metadata of the publication is adequate for EU funded projects. An additional advantage of the golden open access approach is that the publications will be available for other APOSEC dissemination activities. Publications are provided by publishers as pdfs in a visually attractive design and this format makes them easy to use in other dissemination areas and to spread them to a wider audience. Up to now all publications and preclinical results utilizing APOSEC™ in small and large animal models have been made publicly accessible after IP protection was filed.

D&E Documentation

An often underestimated factor in the success of the project is a clear system of documentation, both for internal (incl. exploitation) and dissemination, which means any information, digital or print entering the public domain i.e. outside the realm of confidentiality defined by the consortium as shown in Figure 16.

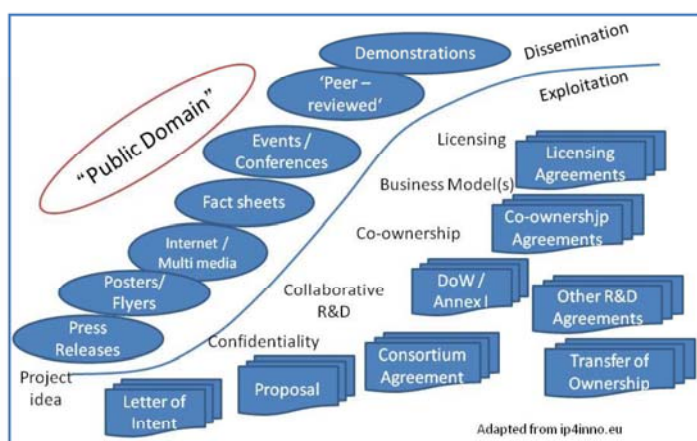


Figure 16: Documents relevant for D&E

Each beneficiary is responsible for documenting their research progress and collaborations through e.g. laboratory books with sufficient detail to identify significant contribution to creation of results i.e. to internationally recognized standards and good practice.

RTDS is an SME specialised in managing dissemination and exploitation in collaborative projects and will be leading the D&E work package. This begins with implementing D&E guidelines and procedures and includes supporting the consortium in defining IP

strategies and appropriate business models and planning as well as managing stakeholder communications and relations.

b) Communication activities

Before communication activities can begin, it is necessary to identify and classify the stakeholder groups i.e. those with an interest, be it negative or positive (excluding the participants themselves) in the project. There is a relatively wide range of stakeholder groups to consider (Table 9) and these are prioritised into three classifications relating to extent of interaction (Table 10).

Table 9: Identifying and categorising stakeholder groups

GOVERNMENT Hospitals and Social (health) insurance Health policy makers (EU / national) Regulatory authorities (EU / national) European Commission (DG Health & Consumers), EU Agencies (e.g. European Medicines Agency)	MEDIA Scientific journals, popular science magazines TV, Radio, Press – newspapers Internet & social media
FINANCIAL Entrepreneurs (business angels) investors and the banking sector, stock market	CUSTOMERS Healthcare, private and state-provided European Commission (funding agency)
GENERAL PUBLIC Patients (various groups) and representative patient decision makers	LEGAL Ethics governance and gender groups
ACADEMIA / RESEARCH COMMUNITY Medical universities, researchers and other EU projects Scientific organizations e.g. Austrian Surgical Society, European Assoc. of Cardiothoracic Surgery (Regeneration Unit), European Society of Dermatological Research and European Society of Cardiology	TRADE/ INDUSTRY/ SMES Healthcare professionals, specifically specialist physicians Pharmaceutical and biotech industries Private clinics, & (health) insurance companies Pharmaceutical industry associations
“NEGATIVE-INTEREST” GROUPS Stem cell advocates and lobbyists Animal rights groups	“POSITIVE-INTEREST” GROUPS Patients’ interests/ disease support groups (IAPO/EPPOSI) EU/National health campaigns Innovative Medicines Initiative (IMI) Alliance for Advanced Therapies Religious groups (those having ethical problems with embryonic stem cells) Blood transfusion units (blood banks)

Table 10: Prioritising stakeholder interactions

KEEP SATISFIED AND MONITOR (MINIMUM EFFORT) Religious groups (those having ethical problems with embryonic stem cells) Stem cell advocates and lobbyists Animal rights groups European Commission (DG Health & Consumers), Stem cell research & development groups	MANAGE CLOSELY Healthcare professionals, specifically specialist physicians Medical universities, researchers and other EU projects Scientific organisations Patients’ interests/ disease support groups EU/ National health campaigns European Commission (funding agency) Ethics governance and gender groups EU Agencies (e.g. European Medicines Agency) Entrepreneurs (business angels) investors and the banking sector Blood transfusion units (blood banks)
	KEEP INFORMED Regulatory authorities (EU / national) Health policy makers (EU / national) Patients’ interests/ disease support groups Healthcare, private and state-provided General public (via media/ online) Trade journals, magazines, Press – newspapers Internet & social media

The project addresses two patient groups (diabetic ulcers and AMI) which have some overlapping stakeholders, but their own specific markets. It is clearly recognised that the APOSEC™ ‘skin’ (= wound) market is ready for new products and the potential is immediately obvious from the statistics. It is planned that Aposcience AG will itself lead exploitation of the ‘skin’ product by partnering with interested pharmaceutical companies and investors to move into Phase III clinical trials and will look for potential licensees for AMI-related patents, backed with solid scientific data and small scale clinical trial results performed within the APOSEC project. Dissemination has several parallel, partially synergetic aims:

1. Gaining credibility within the scientific community;
2. Informing potential customers and investors;
3. Addressing and keeping satisfied a range of positive and negative interest groups;
4. Communicating compliance with regulations and policy objectives.

An overview of the dissemination tools for the main stakeholder groups is shown in Table 11.

Table 11: Overview of Dissemination tools and Stakeholder groups for APOSEC

APOSEC Dissemination Tools (Materials / Activities)	Stakeholder Groups addressed			
	Science/ Other industry/ academia	Customers/ Financial/	Media/ Positive and Negative-interest groups/ General Public	Government/ Legal
Logo/ Brand identity	X	X	X	X
Website/ Social media	X	X	X	(x)
Intranet	(x)	--	--	(x)
Print (flyers, posters, press releases, newsletters, etc.)	X	X	X	(x)
Presentations	X	(x)	(x)	X
Animated whiteboard videos	(x)	X	X	(x)
Scientific Publications	X	(x)	--	(x)
Events / Training / Symposia	X	X	(x)	(x)

X = main target group (x) secondary target group

APOSEC identifies and will utilise key stakeholder representation groups one of which is the Alliance for Advanced Therapies (AAT), whose objectives include exchanging information and coordinating input to European regulatory and reimbursement authorities to facilitate greater understanding and improvement of regulatory requirements as well as increasing awareness creation among stakeholders, especially investors to promote both public and private funding. Being the European arm of the US-based alliance, there will be efforts to strengthen and share scientific knowledge and know-how also involving manufacturing and commercialisation challenges surrounding cell & gene therapy and tissue engineering, being most relevant to APOSEC. Other such multiplier organisations will be sought to improve dissemination efficiency and effectiveness.

Innovative Dissemination Aspect – Animated Whiteboard Video

A challenge with scientific communication is to relay complex processes and interactions which cannot even be observed with the naked eye to non-scientific audiences. Particularly in the medical field, where patients are sceptic of new treatments, but also as a tool to convince business and/or policy decision makers, an innovative approach is needed. The APOSEC project will produce 2 short animated videos to ‘make visual’ the processes happening at microscopic level to cleverly combine information and entertainment (infotainment). The working title: “The Invisible World of APOSEC Regenerative Medicine” has already been coined. These short videos will be able to be used on website, as well as ‘eye catchers’ at conferences and exhibitions. The SME partner [REDACTED] has its own in-house resources to produce the whiteboard animated videos having already gained good experience and feedback from FP7 projects such as the example from the PERFORMANCE project (<http://www.performance-fp7.eu/>) with a still shot in Figure 17.

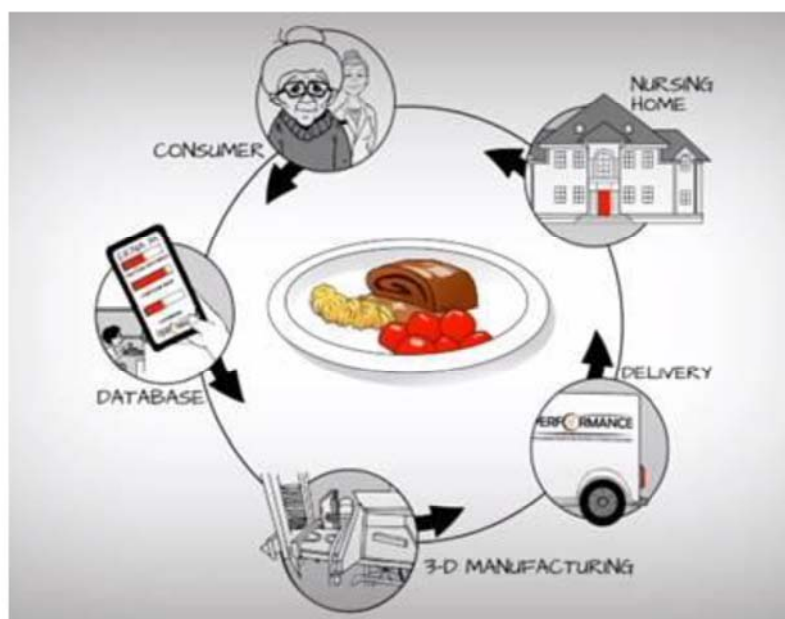


Figure 17: Still shot from the PERFORMANCE whiteboard animation video

Dissemination Events & Materials – Periodic Planning and Implementation

There are a range of potentially relevant dissemination events each year, each having their particular pros and cons, but all of them incurring significant costs. The D&E planning will include planning events (amongst other D&E activities and materials on a periodic basis according to the project progress and ‘newsworthy’ items which will be disseminated ad hoc throughout the year. Events can range from public/political (Long Night of Research, ESOF, EU Open Days) through to high level scientific (Advanced Therapies Summit, EACTS, TERMIS) or business, industry & brokerage events (BIO-Europe Spring, BioEquity Europe). A relatively cost effective, rather informal type of dissemination events includes lunchtime symposia at annual meetings of relevant scientific societies such as those mentioned stakeholder groups table above. Dissemination materials, both in print and digital, will be efficiently planned and produced on a periodic basis to suitably implement the dissemination and exploitation strategy. RTDS, having more than 10 years D&E experience, is currently leading D&E in six FP7 projects and will ensure that the APOSEC project will achieve its impact goals both in dissemination and exploitation.

3 Implementation

3.1 Work plan — Work packages, deliverables and milestones

At the core of the APOSEC project (Figure 18) are the two work packages:

- WP4 - Phase II clinical trial on diabetic foot ulcer (MARSYAS IV) and;
- WP5 - Phase I-II clinical trial on AMI (MARSYAS V).

The results of WP3 - Phase I clinical trials topical application (MARSYAS II) and systemic application (MARSYAS III) will feed into WP4 and WP5 respectively to determine the general safety in human application and the optimum dosage to be used in Phase II clinical trials. Preceding this, WP2 - Safety Assessment & Regulatory Affairs will ensure that APOSEC™ complies with all regulatory requirements before entering into clinical trials.

WP6 will run in parallel to the clinical trials and the safety assessment to determine the modes of action of APOSEC™ which will use tissue models under laboratory conditions to simulate volunteers'/patients' treatments in the clinical trials. The results of WPs 4 and 5 will feed into WP6 to allow for in vitro and in vivo correlations in wound healing and AMI. In addition, tumour invasion studies will be performed in order to rule out tumorigenicity which is fundamental for further development and exploitation of APOSEC (WP7).

WP1 will produce allogeneic GMP APOSEC™ necessary for WPs 2, 3, 4, 5 and 6. In addition to that, WP1 involves development of potency and stability assays to ensure a stable product with consistent characteristics and determine shelf life.

Dissemination & Exploitation (WP7) will run the full duration, with a clear focus on dissemination of clinical trial results and exploitation of the same (incl. securing further investments) to immediately move forward with Phase III clinical trials after project completion. WP8 is related to project management and co-ordination.

For the last quarter year of the project, only WPs 7 & 8 activities are foreseen, but in addition the last three months can be seen as buffer time for the clinical trials in case organisation issues (e.g. patient recruitment) cause a slight delay.

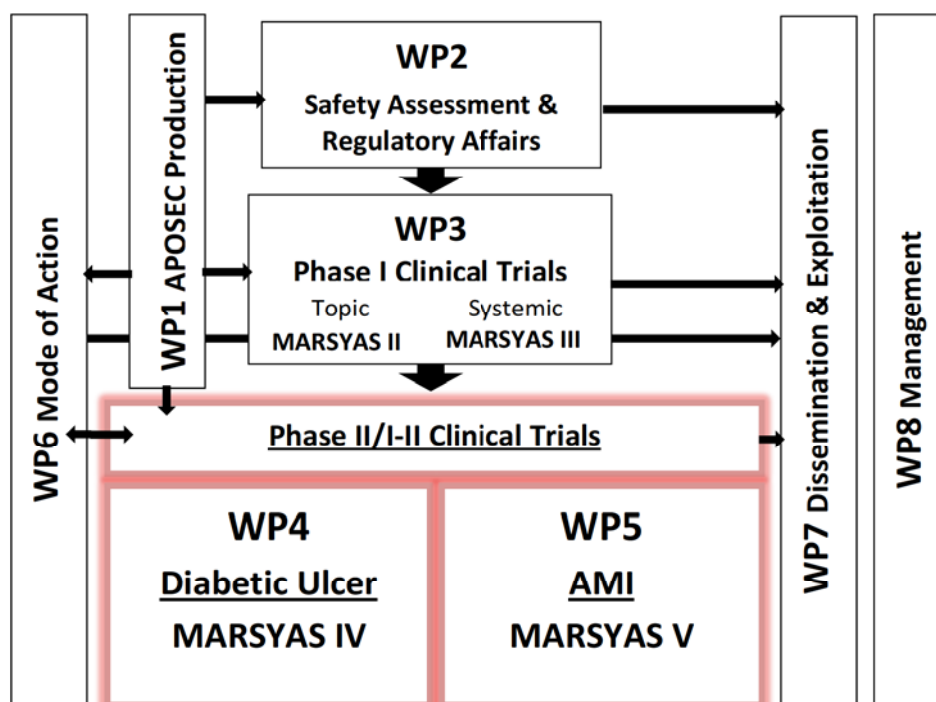
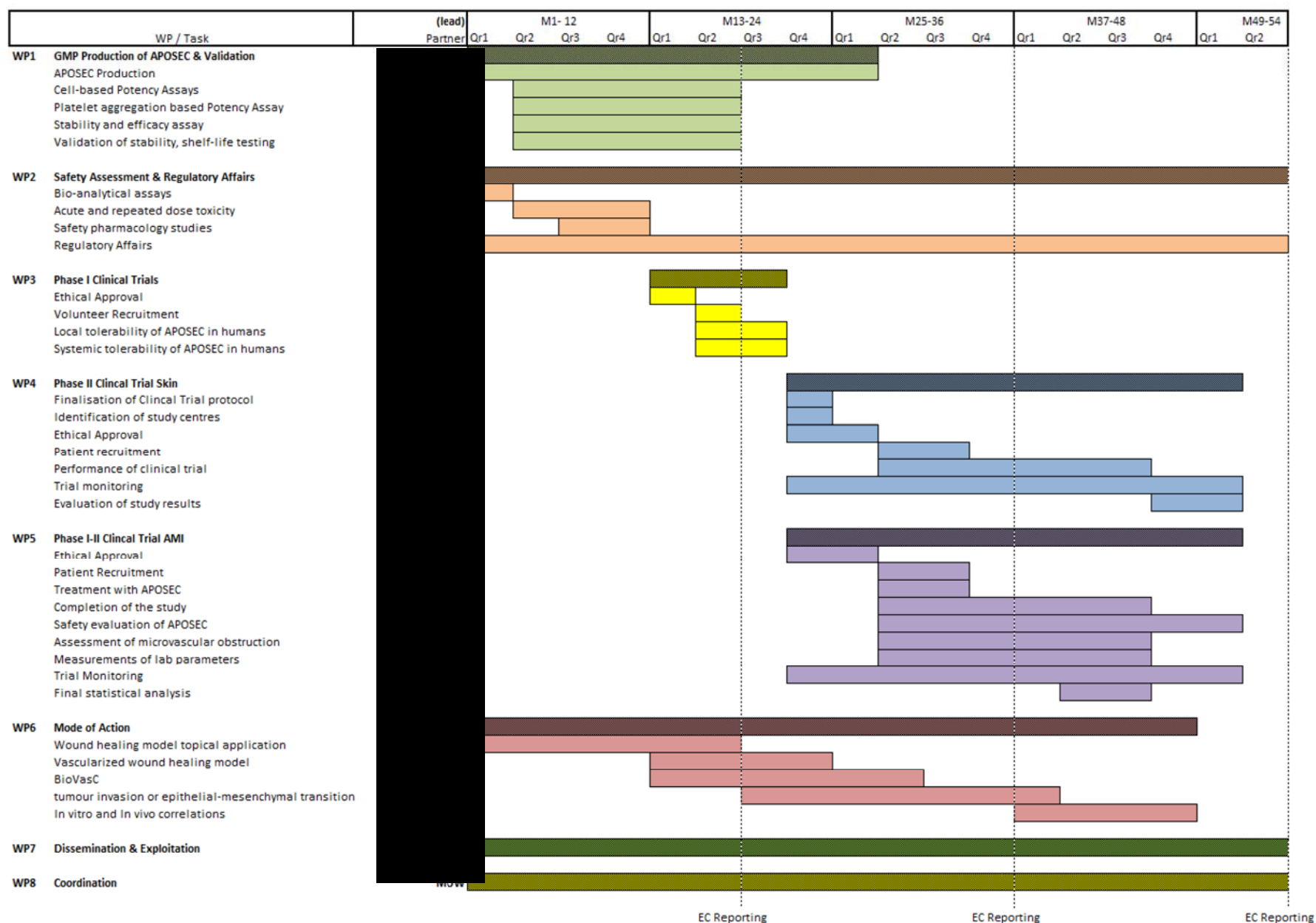


Figure 18: APOSEC Project Overview



3.1.1 Work packages

Table 3.1b: List of work packages

WP No	Work Package Title	Lead Participant No	Lead Participant Short Name	PMs	Start Month	End month
1	GMP Production of APOSEC & Validation	2		101	1	27
2	Safety Assessment & Regulatory Affairs	3		84,5	1	54
3	Phase I Clinical trials – topical & systemic (MARSYAS II, III)	1		37	13	21
4	Phase II Clinical Trial – Skin (MARSYAS IV)	1		12	22	51
5	Phase I-II study in the indication acute myocardial infarction (AMI) (MARSYAS V)	1		153,5	22	51
6	Mode of Action	5		55	1	48
7	D&E	4		66	1	54
8	Management	1		68	1	54
				577		

Work package number	1	Start and end month:					M1 – M27
Work package title	GMP Production of APOSEC & Validation						
Participant number	2						
Participant short name							
Person-months per participant:	101						

WP Leader: █████

Objectives

The focus of WP1 is the development and introduction of potency and stability assays for APOSEC. Furthermore, high amounts of APOSEC will be produced and an adjustment of the production process (scale-up) including process validation is needed.

- 1) To conduct a trial, which identifies the most appropriate proteins suitable for stability assays in topical and systemic solutions.
- 2) To conduct a trial, which identifies the relevant cytokines for effectivity tests.
- 3) To conduct a trial in which ideal and stabile cells are identified for stability and potency assays as well as reference for further clinical and experimental work.
- 4) To establish stability and potency assays.
- 5) To produce APOSEC for all other work packages as a GMP compliant product for clinical trials.
- 6) To produce APOSEC and validate the final process.

Description of work

T1.1 Development of Cell-based potency assay (M4-M18: █████)

Cell lines with known characteristics have to be selected and used in a feasibility trial in which dilutions of APOSEC are applied to the cells in a cell culture setting with appropriate media. One cell line (chosen out of in total four alternative cell lines – all of human origin) will be found for the systemic use of APOSEC and one for topical use. Cell lines are either cardiomyocytes, endothelial cells, lymphocytes, monocytes, fibroblasts, mesenchymal stromal cells and IPS cells. The cell lines in combination with their proliferative properties shall be applicable for monitoring the concentration dependent proliferation of APOSEC. The objective is to identify a procedure in combination with a cell line allowing a stable and robust assay to determine the dose-dependent proliferative activity of APOSEC. The two cell lines should be frozen and kept in a master cell bank. In parallel a stable and robust assay, which allows the determination of a dose dependent proliferative activity of APOSEC has to be established, validated and used in the clinical studies.

Besides validated protocols for cell culture of these selected two cell lines, cell culture proliferation capacity and apoptosis will be evaluated with different methods/parameters: ATP content via luciferase reaction, NO concentration via colorimetric assay Kit (Bio Vision), Annexin V and PI staining (flowcytometry)

T1.2 Development of potency assay based on platelet aggregation (M4-M18: █████)

APOSEC inhibits platelet aggregation. Platelet rich plasma will be supplemented with APOSEC (at different cell concentrations) or with 0.9% NaCl. The aim is to find the optimal APOSEC concentration inhibiting the platelet aggregation. Optical detection (aggregation according BORN) is used for the measurement of platelet aggregation. Platelet aggregation is the additional potency/efficacy assay for platelet inhibition properties of APOSEC.

T1.3 Stability and efficacy assays with cytokines (M4-M18: [REDACTED])

Different cytokines will be tested for concentration in APOSEC with the aim to identify those in close correlation with cell based assays. Testing cytokines will give the opportunity to use a rapid assay in the production process and adjust the batch sizes or mixtures of APOSEC production batches. This shall enable a continuous and equivalent production. Furthermore, cytokines will also be used for stability assays including with worst-case scenarios during production to validate all process steps and the tolerability of processes. In this phase various cytokines (for example ENA-78, IL-8, IL-16 etc.) will be tested with different concentrations of APOSEC under various conditions. A selection of cytokines for efficiency and stability assays will be found.

T1.4 Validation of stability, shelf life determination (M4-M18: [REDACTED])

Samples from production will be analysed in a validated procedure to determine the biological activity of APOSEC. Stability studies comprise details regarding storage conditions, testing frequency and testing of worst-case scenario samples to identify quality control parameter. The maximum shelf life for systemic and topical APOSEC has to be found in this task. Cell based assays and cytokine assays will be used to determine the loss of biological activity as well as the quantity of relevant cytokines.

T1.5 Production of APOSEC and validation of added or amended processes (M1-M27: [REDACTED])

A production process for topical APOSEC has to be attached to the existing and accepted GMP process for the production of APOSEC. As high amounts of APOSEC are needed, adjustment of the production process is inevitable. Regular production of APOSEC and changes in the production process enhancing efficiencies will be introduced together with the cytokine assays to ensure equivalent dosing of APOSEC. The established protocol will be validated according to GMP. Production will provide APOSEC to all partners.

Deliverables

- D1.1 Cell based potency assay for topical and systemic APOSEC (M18)
- D1.2 Platelet inhibition potency assay for APOSEC (M18)
- D1.3 Cytokine assays for in-process controls in APOSEC production (M18)
- D1.4 Stability assay for APOSEC (M18)

Work package number	2	Start Date and End Month				M1 – M54	
Work package title	Safety Assessment & Regulatory Affairs						
Participant number	3	7	10				
Short name of participant							
Person/months per participant:							

Task leader: [REDACTED]

Objectives

The aim of this WP is (1) to conduct toxicological and safety pharmacological animal studies in order to assess the safety and toxicology of the medicinal drug candidate in animal models, and (2) to address preclinical and especially toxicological matters raised by regulatory agencies:

- 1) Development, implementation and validation of bio-analytical methods
- 2) Evaluation of toxicological and safety pharmacological properties of APOSEC as medicinal product for topical and systemic application
- 3) Organization of all regulatory activities, preparation of regulatory documents for entering into clinical Phase I/II studies and submission

Description of work

According to the regulatory framework, APOSEC is a new drug compound that has to comply with specific guidelines. These guidelines are set by drug compounds termed “biologics” or blood derived products. For toxicological and safety evaluation the following guidelines have to be followed:

- ICH M3(R2) and S6 + Addendum;
- Guideline 2001/83/EG;

T2.1 Method development, implementation and validation for the determination of the concentration of lead compounds in the drug formulation and in animal plasma/serum samples (M1-M3: [REDACTED])

The objective of T2.1 is to set up bio-analytical assays (e.g. ELISA) for determination of lead compounds in drug formulation and in animal plasma/serum. These assays must be validated accordingly, e.g.

- set up of calibration standards
- exploration of lower and upper limit of quantification (LLOQ and ULOQ)
- within-run accuracy and precision using quality control samples
- between-run accuracy and precision using quality control samples
- system reliability

T2.2 Conducting acute and repeated dose toxicity animal studies in rats (M4-M12: [REDACTED])

- (1) Single dose toxicity in rats, intravenous administration, at least 4 groups, 3 dose groups, 1 control group, 5 male and 5 female animals per group, observation period 14 days, test parameter: body weight, clinical observations, mortality/lethality, gross pathology.
- (2) 4-week repeated dose toxicity in rats, intravenous administration 3 times weekly, at least 6 groups, 3 dose groups, 1 control group, 2 recovery groups (high dose and control), 10 male and 10 female animals per group, recovery period 14 days, test parameter: body weight, food consumption, clinical observations and local tolerance, mortality/lethality, haematology, clinical biochemistry, coagulation, gross pathology, organ weights, histopathology.
- (3) 4-week repeated dose toxicity in rats, topical administration 3 times weekly, at least 6 groups, 3 dose groups, 1 control group, 2 recovery groups (high dose and control), 10 male and 10 female animals per group, recovery period 14 days, test parameter: body weight, food consumption, clinical observations and local tolerance, mortality/lethality, haematology, clinical biochemistry, coagulation, gross pathology, organ weights, histopathology.

T2.3 Conduction safety pharmacology studies in rats and dogs (M7-M12: [REDACTED])

- (1) Modified Irwin test in rats, 5 groups, 3 dose groups, negative and positive control, 6 male and 6 female animals per group, single intravenous injection, observations up to 72 h p.a., test parameter: body weight, clinical observations, mortality/lethality, test parameter Irwin.
- (2) Cardiovascular effects and potential for QT interval prolongation after intravenous administration of APOSEC in telemetered dogs, 3 dosages, control, 2 male and 2 female animals, cross over design: day 1 – day 8 – day 15- day 22, test parameter: body weight, clinical observation, systemic and local tolerance, body temperature; blood pressure, heart rate, and ECG and monitoring continuously from 1 h before up to 72 hours after dosing; haemodynamic and electrocardiographic data – recording pre-dose and up to 240 min after dosing.
- (3) Respiratory function in conscious rats using whole body plethysmography after single intravenous administration, 5 groups, 3 dosages, positive and negative control, 8 male and 8 female animals per group, test parameter: body weight, clinical observations, mortality/lethality, whole body plethysmography measurements.

T2.4 Regulatory Affairs (M1-M13: APO)

Preparing of nonclinical documents for First-in-Man: Nonclinical Overview, Nonclinical Written and Tabulated Summaries, Investigator's brochure and/or *Investigational Medicinal Product Dossier (IMPD)*.

- (1) CTD Module 2.4 Nonclinical Overview covers all non-clinical study results regarding Primary and Secondary Pharmacology, Pharmacokinetics, and Toxicology. The Nonclinical Overview presents an integrated and critical assessment of the pharmacologic, pharmacokinetic, and toxicological evaluation of the pharmaceutical.
- (2) CTD Module 2.6 Nonclinical Written and Tabulated Summaries covers all non-clinical study results regarding Primary and Secondary Pharmacology, Pharmacokinetics, and Toxicology. It is recommended that written summaries and summary tables for the nonclinical information in the Common Technical Document be provided in the format outlined according to the guidance M4S – The CTD Safety (Aug 2001, ICH).
- (3) CTD Module 4 Nonclinical Study Reports.
- (4) Investigator's brochure and/or *Investigational Medicinal Product Dossier (IMPD)* – *Nonclinical Pharmacology and Toxicology Data*. In drug development, the Investigator's Brochure (IB) is a comprehensive document summarizing the body of information about an investigational product ("IP" or "study drug") obtained during a drug trial. The IB is a document of critical importance throughout the drug development process and is updated with new information as it becomes available. The purpose of the IB is to compile data relevant to studies of the IP in human subjects gathered during preclinical and other clinical trials. The Investigational Medicinal Product Dossier is the basis for approval of clinical trials by the competent authorities in the EU. See also Clinical Trials Directive - 2001/20/EC.

All these documents must be prepared before clinical Phase I.

Deliverables

- D2.1 Development and Validation Reports on bio-analytical assays for APOSEC (M3)
- D2.2 Study Report - Single dose study on APOSEC (M5)
- D2.3 Study Report - Dose toxicity study on APOSEC, intravenously (M11)
- D2.4 Study Report - Dose toxicity study on APOSEC, topical (M12)
- D2.5 Study Reports on Safety pharmacology studies on APOSEC (M12)
- D2.6 CTD Module 2.4 Nonclinical Overview, CTD Module 2.6 Nonclinical written and tabulated summaries, CTD Module 4 Nonclinical Study Reports, IB and/or IMPD (M13)

Work package number	3	Start Date and End Month					M13 – M21
Work package title	Phase I Clinical trials – topical & systemic (MARSYAS II, III)						
Participant number	1						
Short name of participant							
Person/months per participant:	37						

Task leader: ██████

Objectives

- 1) To conduct a first-in-humans study on local tolerability of APOSEC in healthy subjects with artificial skin wounds
- 2) To assess preliminary safety and toxicity of APOSEC administered intravenously as a single dose in healthy male and female subjects
- 3) To establish a safe systemic intravenous dose for studies in patients with AMI

After toxicological evaluation and positive mandate of an internal review board phase I studies with allogeneic APOSEC can be conducted. The following guidelines for phase I studies have to be followed:

- This trial will follow the relevant CHMP guideline, GUIDELINE ON REQUIREMENTS FOR FIRST-IN-MAN CLINICAL TRIALS FOR POTENTIAL HIGH-RISK MEDICINAL PRODUCTS (EMA/CHMP/SWP/28367/2007)
- All studies will be conducted in agreement with regulatory requirements, ICH-GCP, and the Declaration of Helsinki;
- All studies will be registered in a publicly available registry;
- A data safety monitoring board DSMB will be established to review safety data throughout the trial.

Human phase I trials, testing the safety and tolerability in a topical and systemic application of APOSEC in healthy human probands are central to the process of progressing of clinical phase II trials. Phase I trials will lead to a recommendation for phase II trials in diseased patients utilizing topical or systemic APOSEC. The aim of this work package is (1) to assess the safety and tolerability of APOSEC in repeated topical and (2) one single systemic application.

Further details on exclusion criteria, inclusion criteria and study conduct of MARSYAS II and III can be found in the Annex ‘APOSEC – Essential Information on Clinical Trials’.

T3.1 Approval of the study by local Ethical Committees and other regulatory authorities (M13-M15: ██████)

The study Steering Committee will approve the Study Protocol, Patient informed consent and Investigator Brochure. These documents will be submitted to local Ethical Committee, hospital and university regulatory authorities and Ministry of Health for approval. The study will be registered at EudraCT and clinicaltrials.com homepage.

T3.2 Recruitment of volunteers (M16-M18: ██████)

10 and 30 healthy male volunteers respectively will be recruited according to inclusion and exclusion criteria.

T3.3 Local tolerability of APOSEC in humans (MARSYAS II) (M16-M21: [REDACTED])

A monocentric clinical trial is planned to investigate the effect of APOSEC on wound healing (skin puncture wounds on the forearm, diameter 4 mm) and to establish local tolerability of the investigational product. To this end, the investigational product will be applied over a period of 7 days on skin puncture wounds. Local reactions and healing will be compared to control wound conditions which are assessed on the contralateral arm. The study will follow a dose-escalation protocol employing 6 healthy subjects per dose group with interim analysis.

T3.4 Systemic tolerability of APOSEC in humans (MARSYAS III) (M16-M21: [REDACTED])

A double blind, placebo-controlled, block randomized single ascending dose phase I trial investigating the tolerability and safety of intravenous infusion of APOSEC in healthy volunteers will be performed. A single ascending dose trial will investigate the safety of a systemic intravenous dose and will describe the potential adverse effects of APOSEC in humans. The trial will be designed as a double blind, placebo controlled, block randomized clinical trial. Uneven block randomization will be carried out at ratios 2:1 and 4:1 (active treatment vs. placebo). The two initial dose groups will contain ten subjects, and later dose groups will contain ten subjects. In total, in the systemic study 30 probands will be involved.

Deliverables

D3.1 Study report Phase I clinical trial - topical APOSEC (M21)

D3.2 Study report Phase I clinical trial - systemic APOSEC (M21)

enrolled subjects, examination of eCRF for plausibility, completeness of conformity with the study protocol, examination of adverse events (AE), source data verification and checking of the investigator file. The CRO will document and report all details of the monitoring visit and of any up-action that may be required.

Task 4.5 Preparation of the ethics applications to the local ethics committees (M22-27: CRO)

The study Steering Committee will approve the Study Protocol, Patient informed consent (translated to relevant languages) and Investigator Brochure. These documents will be submitted to local Ethical Committee, hospital and university regulatory authorities and Ministry of Health for approval. An electronic case report form (eCRF) will be constructed and established at each clinical site. Site initiation visit will be performed before study start. The study will be registered at EudraCT and clinicaltrials.com homepage.

Task 4.3 Recruitment of 160 patients with diabetic ulcer (M28-33: CRO)

160 patients will be recruited with diabetic ulcers in the participating centres according to inclusion and exclusion criteria.

Task 4.6 Initiation and performance of the topical phase II study in 160 patients (M28-45: CRO)

The inclusion phase of the APOSEC – Diabetic ulcer trial is approximately 6 months, with the latest follow-up investigation up to 18 months.

After inclusion of 60% of the study probands, preliminary assessment of the data will be performed and the safety and efficacy of the data will be reported.

Task 4.7 Trial monitoring by an independent DSMB (M22-51: █████, CRO)

The independent data safety board (DSMB) will monitor the reported adverse events to relate to the drug procedure.

Task 4.8 Evaluation of the study results (M46-51: █████, CRO)

After finalizing the trial, the DSMB will assess the safety and observed clinical results of the investigated study population. Moreover, clinical observations will be delivered in a blinded fashion to an independent core lab/CRO, blinded to baseline and follow-up investigations and centres. The results will be reported in international meetings and journals.

Deliverables

D4.1 Identification of study centres by the CRO (M24)

D4.2 Approval of the ethics applications by the local ethics committees (M27)

D4.3 Interim report of the DSMB after completion of 60% of the study probands (M39)

D4.4 Final statistics and study report (M51)

Work package number	5	Start date or End Month				M22-51
Work package title	Phase I-II study in the indication acute myocardial infarction (AMI) (MARSYAS V)					
Participant number	1	6	8	9		
Short name of participant						
Person-months per participant	39	19	29	66,5		

Task leader: [REDACTED]

Objectives:

- 1) Determine safety of the single intravenous application of APOSEC in patients with ST-elevation myocardial infarction (STEMI) as an upstream therapy before primary percutaneous coronary intervention (PCI);
- 2) Measure the blood biomarkers indicating myocardial infarction of the patients included in the study;
- 3) Determine microvascular obstruction, infarct size and cardiac function of patients included in the study at 3 day and at 6 months follow-up by cardiac late enhancement magnetic resonance imaging (MRI).

Description of work

After successful toxicological evaluation and completion of systemic phase I study, we aim in this WP (MARSYAS V, AMI study part of the proposal) to conduct a Phase I-II First-In-Men non-randomized multicenter trial to investigate the safety (primary end point) of the APOSEC upstream therapy in 35 consecutive patients with STEMI treated with primary PCI.

The anticipated number of patients completed the study with all follow-ups (FUP) is 30. The secondary endpoints of the trial are the assessment of the efficacy of APOSEC on microvascular obstruction, cardiac function, myocardial perfusion and infarct size evaluated by cardiac MRI with late enhancement (LE-MRI). Biomarkers of the blood will be measured, and cardiac rhythm disturbances will be recorded.

The Principle Investigator of the MARSYAS V study is Mariann Pavone-Gyöngyösi MD (leader of the Steering Committee), at the Dept. of Internal Medicine at the MUW. She will organize and coordinate the clinical trial. After inclusion of the first 15 patients, a preliminary assessment of the data will be performed and the safety and efficacy will be reported.

Further details on exclusion criteria, inclusion criteria and study conduct of MARSYAS V can be found in the Annex 'APOSEC – Essential Information on Clinical Trials'.

T5.1 Approval of the study by local Ethical Committees and other regulatory authorities (M19 - M24:

The study Steering Committee will approve the Study Protocol, Patient informed consent (translated to Polish, Danish and Rumanian) and Investigator Brochure. These documents will be submitted to local Ethical Committee, hospital and university regulatory authorities and Ministry of Health for approval. An external Clinical Research Organisation (CRO) will be subcontracted, and electronic case report form (eCRF) will be constructed and established at each clinical site. Site initiation visit will be performed before study start. The study will be registered at EudraCT and clinicaltrials.com homepage.

T5.2. Recruitment of patients (M25 - M30: [REDACTED])

Thirty-five patients will be recruited with STEMI and primary PCI in 3 participating centers (Region Hovedstaden, Denmark, Universitatea de Medicina si Farmacie Tg Mures, Romania, Silesian Medical University in Katowice, Poland) according to inclusion and exclusion criteria. The recruitment phase of the MARSYAS V trial is approximately 6 months, with the latest follow-up investigations up to 1 year.

T5.4 Treatment with APOSEC (M25 – M30: [REDACTED])

The treatment with a single intravenous infusion of APOSEC will be performed as an upstream therapy to

primary PCI, additional to the current medications and procedures recommended by current ESC and AHA/ACC guidelines. The APOSEC powder will be diluted in isotonic solution up to 500 ml infusion and will be administered with 15 ml/min infusion rate.

T5.4. Completion of the study (M25 – M42: [REDACTED])

All patients complete the study with all planned follow-up.

T5.5 Safety evaluation of APOSEC (M25 – M48: [REDACTED])

The intravenous APOSEC treatment in STEMI patients will be evaluated for safety, regarding occurrence of major adverse cardiac and cerebrovascular events, including re-infarction, all-cause death, re-intervention of the infarct-related artery and major bleeding during the 1-year follow up. The safety evaluation will be performed by the DSMB and the PI.

T5.6 Assessment of microvascular obstruction and cardiac function by LE-MRI (M25 – M42: [REDACTED])

At day 3 cardiac LE-MRI will be performed and area at risk and microvascular obstruction will be measured. At 6 month follow-up, cardiac LE-MRI will be repeated to evaluate myocardial perfusion and infarct size, left ventricular systolic and diastolic function, myocardial fibrosis and right ventricular function. Transthoracic echocardiography (TTE) and 24h ECG monitoring will be performed 1 day after APOSEC administration. TTE will be repeated at the 6-month clinical visit. Independent Core laboratory (will be subcontracted) will analyze the TTE, 24h ECG and LE-MRI.

T5.7 Measurements of laboratory parameters (M25 – M42: [REDACTED])

Before APOSEC treatment, and after primary PCI, at day 1, 2 and 3, at week 6, 6 and 12 months follow up, blood samples will be collected to measure routine blood parameters (such as creatine kinase, troponin T, N-terminal proBNP). Additional blood sample will be collected for specific biomarkers as an indicator of the APOSEC treatment.

T5.8 Trial monitoring by the independent CRO (M19 – M42: [REDACTED], CRO)

The CRO will perform the study monitoring. Regular monitoring visits will include check of informed consent, subject registration of all enrolled subjects, control of eCRS, completeness of conformity with the study protocol, examination of adverse events, source data verification and checking of the investigator file. The CRO will be responsible for document communication, set up of workflows, timelines, project specific procedures and tracking tools, and storage of the files according to the agreed standard operation procedures (SOPs).

T5.9 Final statistical analysis of the study and final study report (M36 – M42: [REDACTED], CRO)

After the final follow-up, and collection of all data, the study results will be evaluated by an independent statistician. Primary and secondary endpoints will be analyzed and reported. Final study report will be summarized and distributed for all partners for approval.

Deliverables

D5.1 Inclusion of thirty-five patients in the trial (M33)

D5.2 All patients completed the study (M45)

D5.3 Analyses of the safety data (primary endpoint) by the independent DSMB (M51)

D5.4 Assessment of the secondary end points by the Core Labs and investigators (M51)

D5.5 Final report of the study (M51)

Work package number	6	Start Date and End Month					M1 – M48
Work package title	Mode of Action						
Participant number	5						
Short name of participant	████						
Person/months per participant:	55						

Task leader: █████

Objectives

APOSEC has a positive influence on damaged cells and tissues and is over time inducing regeneration. The all over aim of this work package is to understand the mode of action. This knowledge can then be used to optimize the concentration and the frequency of application for phase I and II and III clinical trials.

- 1) Mode of action on a wound healing model based on a human full skin equivalent simulating the topical application in the planed clinical trials
- 2) Mode of action on a wound healing model based on a vascularized human full skin equivalent. Focus of the experiments is the induction of angiogenesis.
- 3) Mode of action on a wound healing model based on a human myocardial equivalent simulating the application in the planed clinical trials
- 4) Mode of action in a human vascularized tumour model simulating the intravenous application of APOSEC

Description of work

T6.1 Wound healing model of the skin simulating the topical application of APOSEC (M1-18: █████)

We have established different complex human skin models, including a technology to injure automatically the skin in a defined manner on a high-standardized level (wound healing model).

Human full skin equivalents (n=200) will be engineered from 20 different donors varying in gender and age. The skin models will be automatically injured with the punch and the wound will be filled with a hydrogel containing APOSEC in a concentration of 12,5M/ml or 25M/ml analogous to the clinical trial Phase I. After 1, 2, 3, and 4, 8 and 14 days, skin models will be fixed and the influence of APOSEC on cellular behaviours and wound healing mechanisms will be examined by histological and immuno-histological methods and on molecular and metabolomics level. Another focus of the read out will be the capability of APOSEC components to bind on the extracellular matrix of the tissues in the human organisms. Knowledge regarding this possible mode of action is necessary to control the dose that will bind to the ECM and calculate and define a sustained release of APOSEC in the wound of patients.

An advantage of the complex human tissue models is that the cells can be isolated after the exposure with APOSEC and DNA, mRNA and proteins can be isolated from the exposed tissue. A comparison of molecular regulations of cells of the tissue models without the incubation with APOSEC and with dose dependent contact with APOSEC in different concentrations will be performed to understand the mechanism of action. The cell culture media of the different skin models will be used for metabolomics and compared with the results of the bio-analytics of WP3. The results of all these mode of action experiments are important to classify APOSEC as it represents an intermediate level of complexity in between molecular mechanisms and physiological outcomes, especially when the exact molecular target/s has/have not yet been elucidated.

Macrophages will be added to the skin model. The same experiments will be conducted and the amount of Macrophage type I (chronic wound) and type II (healing wound) will be examined to understand the mode of action of APOSEC in different wound types.

Bacteria as *Staphylococcus epidermidis* or *Staphylococcus aureus* are applied to the skin wound healing

model including macrophages and the tests described above will be carried out.

T6.2 Vascularized wound healing model (M13-M24: █████)

We have established a vascularized complex human skin model, including a feeding artery, a capillary network and a vein. This vascularized human skin model will be injured automatically in a defined manner on a high-standardized level (vascularized wound healing model).

Human full skin equivalents (n=20) will be built from 4 different donors varying in gender and age. The vascularized skin model will be automatically injured with the punch and the wound will be filled with a hydrogel containing APOSEC in a concentration of 12,5M/ml or 25M/ml analogous to the clinical trial Phase I. After 4, 12, 28, and 56 days skin models will be fixed and the influence of APOSEC on cellular behaviours, wound healing and induction of angiogenesis will be examined by histological and immunohistological methods and on molecular and metabolomics level. The release of ECM bound APOSEC components into the circulation of the vascularized skin model will be analysed over 56 days.

The cell culture media of the different skin models will be used for metabolomics and compared with the results of the bio-analytics of WP3 and Task 6.1.

Macrophages will be added to the skin model using the vasculature. The same experiments will be conducted and the amount of Macrophage type I (chronic wound) and type II (healing wound) will be examined to understand the mode of action of APOSEC in different wound types.

Bacteria as *Staphylococcus epidermidis* or *Staphylococcus aureus* are applied to the skin wound healing model including macrophages and the tests described in 6.2 and 6.1 will be carried out.

T6.3 Complex human myocardial tissue model on vascularized scaffold (BioVaSc) (M13-M32: █████)

In a first step a standardized injuring technique will be established. In the injured myocardial tissue model APOSEC will be applied through the blood vessel system and the analysis established in WP 3 and T6.1 will be used to determine, if APOSEC components reach the cells surrounding the wound and which metabolic pathways in the cardiomyocytes are activated after 1, 3 or 6 week after APOSEC application.

T6.4 The potential of APOSEC to induce tumour invasion or epithelial-mesenchymal transition (EMT) (M19-M40: █████)

The Fraunhofer Project group will supply us with the technology to set up human vascularized tumour models. These human vascularised tumour tissues will be incubated with APOSEC through the blood vessel system with different concentration of APOSEC over time. The invasion and EMT capability will be compared with the standardized induction of invasion and EMT by TGF- β . Transformed tumour/healthy cells will be isolated by laser capture technology to evaluate the APOSEC induced signalling networks. A promising new approach to study cellular signalling networks and their changes through targeted agents in tumour models that have specific mutations. Through this network analysis, modes of drug action can be understood.

Deliverables

D 6.1 Mode of action after topical application in a human wound (M18)

D 6.2 Mode of action after system application in a human skin wound (M24)

D 6.3 Mode of action in an injured human myocardium (M32)

D 6.4 Critical dose inducing tumour cell invasion (M36)

D 6.5 Critical dose inducing EMT (M40)

D 6.6 In vitro and in vivo correlation in skin wound healing (M42)

D 6.7 In vitro and in vivo correlation in AMI study (M48)

Work package number	7	Start and end month:					M1 – M54
Work package title	Dissemination and Exploitation						
Participant number	1	2	3	4	5	6	7
Participant short name							
Person-months per participant:	7	1	14	38	1	1	1
Participant number	8	9	10				
Participant short name							
Person-months per participant:	1	1	1				

WP Leader: [REDACTED]

Objectives

Dissemination and Exploitation (D&E) covers both internal knowledge management and external communications / dialogue with a range of stakeholders as described in Section 2.2 (Measure to maximise impact) as well as transparently managing IPR to optimize exploitation opportunities. The overall WP objective is to promote the APOSEC project and its results widely and as effectively to relevant stakeholder groups to fulfil both dissemination and exploitation strategies. Based on a D&E strategy and implementation plan, initiated in the proposal and elaborated T7.1, a range of dissemination tools such as a public website, social media, flyers, posters, factsheets and publications for various stakeholder groups (see section 2.2.). Furthermore, animated whiteboard videos are planned to communicate the ‘Invisible World of APOSEC™ Regenerative Medicine’ to non-specialists. Other D&E activities include representing the project in the media, participating in congresses, conferences and fairs and defining exploitation models to in preparation for commercialisation of results by industrial and SME partners. The WP is led by [REDACTED], a specialised SME without research tasks in the project, whose mission is to professionally - and importantly - objectively manage all D&E aspects of the project. All partners will have at least 1 person month to be able to contribute to any of the tasks described below.

Description of work

T7.1 D&E Strategy and Management (M1-M54: [REDACTED], All)

A basic stakeholder analysis and D&E strategy is already provided within Sect. 2.2. Upon project commencement, this will be refined into operative details of event planning, specifying stakeholder needs and requirements and setting up D&E control mechanisms. The operative D&E plan will be reviewed and updated each period (including dissemination reporting) in the context of the project progress and evolving market, stakeholder and policy related issues. In order to professionally manage stakeholder relations over the project duration, a stakeholder database will be set up so as to manage contacts (between various stakeholder groups and press & media), dissemination of material, event invitations and participation and to generally record interaction with the project.

Task 7.2 Dissemination Tools and Channels (Project Identity, Public website, intranet, printed dissemination material) (M1-M54: [REDACTED], All)

The dissemination tools will have professionally designed project identity (logo & associated colour scheme etc) with the project website as the central means of public dissemination. It will contain usual project information but will also be linked with selected interactive social media channels and keep press & media as well as stakeholders updated on project progress & results. The (password protected) intranet will facilitate document management within the consortium and with selected stakeholders. A series of professionally developed printed dissemination material such as flyers, marketing and scientific posters,

policy briefs, newsletters etc. will be produced to reflect the current stage of project progress and will be made available to all partners for dissemination in their own countries. Material may be translated by the partners themselves based on the approved English version. The partners will be actively involved in content generation for the website and dissemination materials.

T7.4 Production of animated project videos (M19-M54: █████, All)

In order to better promote the APOSEC project, the development and production of animated videos (e.g. white board animation) is planned at different stages of the project. These videos present an important tool in order to provide a better understanding of the mechanism of action underlying APOSEC™ i.e. the ‘Invisible World of APOSEC™ Regenerative Medicine’ and will be designed in a way that is suitable for non-specialist stakeholder groups. The produced videos will be made available to all partners for dissemination and can e.g. be embedded in the project and partner websites as well as in scientific presentations at conferences.

T7.4 Dissemination and training events (M1-M54: █████, All)

As part of dissemination, the project will organise dissemination events, which can include internal training, stakeholder workshops and demonstration events typically back to back with project meetings. The specifics of these events will correspond to the planned work and results as they become available and defined in the D&E Plan (D7.1) at the beginning of the project and updated again at the end of each period. Furthermore, partners may present APOSEC and their particular research interests at conferences, science days etc. once it is clarified that sensitive IP (e.g. with patent/commercial potential) will not be prematurely disclosed. It is anticipated that exhibition presence at international fair and conferences will attract potential end-users, customers and technology licensors of the developed results. Events will be budgeted for individually with cost shared between the benefiting partners with possibility of seeking third party sponsorship being examined.

T7.5 IPR and Innovation Management (M1– M54: APO, █████, All)

IP and innovation aspects need to be managed throughout the full project life cycle and has already begun in the proposal phase to indentify background IP brought in by the partners, define a fundamental IP and benefits strategy to protect and promote the interests of the commercial partners followed by dissemination. In this context, IP claims to will be reviewed based on the provisions in the Grant and the Consortium Agreements. RTDS will introduce transparent IP management procedures with task being implemented and led by the SME APO as the main exploitation partner. The IP documentation will be made available to the Executive Board for analysis which will include; cost-benefit analysis of results c.f. existing treatments, possibilities and costs of IP protection, joint vs. single ownership, ownership transfers and overall business models and investment needs. The IPR management scheme is elaborated on in the section 2.2.

Deliverables

D7.1 D&E Strategy and Period Operative Plan incl. website (M4)

D7.2 Period 1 Report on dissemination events, materials and exploitation of results (M18)

D7.3 Period 2 D&E Operative Plan (M18)

D7.4 Period 2 Report on dissemination events, materials and exploitation of results (M36)

D7.5 Period 3 D&E Operative Plan (M36)

D7.6 Period 3 Report on dissemination events, materials and exploitation of results (M54)

Work package number	8	Start and end month:					M1 – M54
Work package title	Management						
Participant number	1	2	3	4	5		
Participant short name							
Person-months per participant:	48	1	6	12	1		

WP Leader: [REDACTED]

Objectives

The responsibilities of the co-ordinator have been described in Section 3.2, which also includes details of the governance structure and procedures. The main objectives of this work package are therefore;

- to provide resources for effective coordination of all project activities and maintenance of contact with Commission representatives as described in Section 3.2 of the proposal
- to undertake quality assessment of project progress, results and impact
- to achieve timely submission of progress and financial reports to the Commission
- to ensure the description of work and the Consortium agreement is maintained and updated where necessary
- To ensure transparent CRO subcontracting overall performance fulfilment

This WP comprises the overall coordination and management of the project in order to meet the objectives of the project in terms of the project plan and professional, transparent administration.

Description of work

Task 8.1 Overall Coordination and Organisation of Meetings ([REDACTED])

The two decision making bodies are the General Assembly (GA) as the ultimate decision body of the project and the Executive Board as the operational management body responsible of monitoring the project. The EB is chaired by the coordinator [REDACTED] who will arrange the EB meetings in connection with project meetings twice a year. GA meetings are foreseen during the project beginning with Kick-off, then at 9-month intervals (mid and end of period). The EB meetings can also be arranged as teleconferences on approximately 6 week intervals. The coordination also includes receiving (from the EC) and distributing the Grant to partners as well as amending the Grant and/or Consortium Agreements as required.

Task 8.2 Work Package Leadership [REDACTED] APO, [REDACTED]

Work package leaders are responsible for the activity planning and implementation of the work in their respective work packages. The WP Activity Plans are on a detailed level below tasks and will be worked and update on a six monthly basis for internal project communication. WP Leaders will ensure that the work is carried out according to the work plan and the progress of their WP is reported to the coordinator. It should be noted that while MUW is leading 3 research WPs besides Coordination, these WPs are led by three very experienced senior researchers; WP3 – Phase I [REDACTED], WP4 Phase II Skin (Prof. Ankersmit, the Principle Investigator and Coordinator of the project) and WP5 – AMI ([REDACTED]). This is an clear necessity because [REDACTED] plays a pivotal role in the clinical trials, central top the project and with an emphasis on lean project management.

Task 8.3 Quality assurance and submission of reports & deliverables ([REDACTED], APO)

The project coordinator will manage submission of periodic reports and deliverables to the EC via the Portal. As Coordinator and Principal Investigator of the project, [REDACTED] will assure quality of the (scientific) progress reporting and deliverables, taking up contact as necessary to the responsible partners. The reports are prepared in collaboration with project partners and the work package leaders.

Task 8.4 Administration and Cost reporting [REDACTED]

This task includes setting up of suitable templates and tools (could be on-line) plus guidelines and training to the partners on reporting and administration issues. The task leader will provide support to the partner in producing their cost reports and in-house documentation and will collect and consolidate the cost reports for internal (EB) reviews and EC periodic reporting.

Task 8.5 CRO subcontract tendering and performance evaluation (APO)

APOSEC as a clinical trial project, have significant subcontracting, mainly for the CRO (Clinical Research Organisation). The contracting partner (APO) is clearly responsible for the tendering process and the subsequent implementation of their respective subcontracts. However, the project success is highly dependent on the CRO subcontracting, so the EB needs to be involved in the CRO selection process, as well as the performance evaluation, so this specific management task has designed for this purpose, so that the EB is fully informed on the CRO subcontracting.

Deliverables

D8.1 Period 1 General Assembly Meeting minutes (M18)

D8.2 Period 2 General Assembly Meeting minutes (M36)

D8.3 Period 3 General Assembly Meeting minutes (M54)

Periodic and final reports are obligatory and are therefore, not listed as deliverables.

3.1.2 Deliverables

Table 3.1c: List of Deliverables

Deliverable number	Deliverable name	WP No	Short name of lead participant	Type	Dissemination level	Delivery date
2.1	Development and Validation Reports on bio-analytical assays for APOSEC	2		R	CO	M3
7.1	D&E Strategy and Period Operative Plan incl. website	7		R, DEC	PU	M4
2.2	Study Report - Single dose study on APOSEC	2		R	CO	M5
2.3	Study Report - Dose toxicity study on APOSEC, intravenously	2		R	CO	M11
2.4	Study Report - Dose toxicity study on APOSEC, topical	2		R	CO	M12
2.5	Study Reports on Safety pharmacology studies on APOSEC	2		R	CO	M12
2.6	CTD Module 2.4 Nonclinical Overview, CTD Module 2.6 Nonclinical written and tabulated summaries, CTD Module 4 Nonclinical Study Reports, IB and/or IMPD	2		R, OTHER	CO	M13
1.1	Cell based potency assay for topical and systemic APOSEC	1		R	CO	M18
1.2	Platelet inhibition potency assay for APOSEC	1		R	CO	M18
1.3	Cytokine assays for in-process controls in APOSEC production	1		R	CO	M18
1.4	Stability assay for APOSEC	1		R	CO	M18
6.1	Mode of action after topical application in a human wound	6		R	CO	M18
7.2	Report on dissemination events, materials and exploitation of results	7		R	CO	M18
7.3	Period 2 D&E Operative Plan	7		R	CO	M18
8.1	Period 1 General Assembly Meeting minute	8		R	CO	M18
4.1	Identification of study centres by the CRO	4		R	CO	M24
3.1	Study report Phase I clinical trial - topical APOSEC	3		R	CO	M21
3.2	Study report Phase I clinical trial - systemic APOSEC	3		R	CO	M21
6.2	Mode of action after system application in a human skin wound	6		R	CO	M24
4.2	Approval of the ethics applications by the local ethics committees	4		R	CO	M27
5.1	Inclusion of thirty-five patients in the trial	5		OTHER	CO	M33
6.3	Mode of action in an injured human myocardium	6		R	CO	M32
6.5	Critical dose inducing tumour cell invasion	6		R	CO	M36

7.4	Period 2 Report on dissemination events, materials and exploitation of results	7		R	CO	M36
7.5	Period 3 D&E Operative Plan	7		R	CO	M36
8.2	Period 2 General Assembly Meeting minutes	8		R	CO	M36
4.3	Interim report of the DSMB after completion of 60% of the study probands	4		R	CO	M39
6.6	Critical dose inducing EMT	6		R	CO	M40
6.7	In vitro and in vivo correlation in skin wound healing	6		R	CO	M42
5.2	All patients completed the study	5		OTHER	CO	M45
6.8	In vitro and in vivo correlation in AMI study	6		R	CO	M48
4.4	Final statistics and study report	4		R	CO	M51
5.3	Assessment of the secondary end points by the Core Labs and investigators	5		R	CO	M51
5.4	Assessment of the secondary end points by the Core Labs and investigators	5		R	CO	M51
5.5	Final report of the study	5		R	CO	M51
7.6	Period 3 Report on dissemination events, materials and exploitation of results	7		R	CO	M54
8.3	Period 3 General Assembly Meeting minutes	8		R	CO	M54

3.2 Management structure and procedures

The APOSEC project has 10 partners (=beneficiary) including six HE partners, two industry partners and three SMEs including one not-for-profit association. The management structure is based on the governance structure for medium and large projects as defined in the **DESCA H2020 V1 Consortium Agreement**, comprising two decision-making bodies; the General Assembly (GA) and the Executive Board (EB).

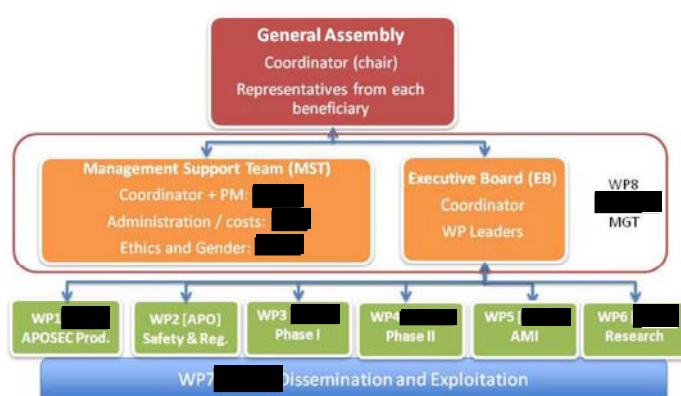


Figure 19: Management structure of APOSEC

The **General Assembly (GA)** is the ultimate decision-making body of the project. GA will decide e.g. on issues related to the content, finances, intellectual property rights, publishing project results and evolution of the consortium if required. The GA consists of one representative of each participating organisation and is chaired by the coordinator.

The **Executive Board (EB)** is the operational management body of the project, responsible for efficient implementation of the project and comprises the WP leaders, whereby [REDACTED] is leader of 4 WPs including coordination, so this is a total of 5 partners. The WP leadership may appear unbalanced in most collaborative projects, but actually makes sense in this project because of the pivotal role MUW has in the clinical trials, central to this project. EB will make proposals and report to GA for project level decision making such as possible changes to the work plan and if needed, budget reallocation as well as approving deliverables and project publications and discussing project business models. The EB has the double function of being an Exploitation Committee i.e. for IP and innovation management as described in Task 7.5 in the D&E work package. EB consists of the Coordinator & WP leaders with the coordinator being chairman.

The **operational procedures** of EB as well as GA shall be laid down in the Consortium Agreement, but also include a specific task relating to the CRO subcontract (T8.5) as this is an essential part of the clinical trial. The internal division of roles and responsibilities of each beneficiary, including the Coordinator, is defined in Article 41 of the Grant Agreement. The EB functions to ensure interlinking between the work packages (WP), whereas the WP Leaders should manage the overall implementation aspects including management of Task Leaders and the timely production of deliverables from their respective work packages.

3.2.1 Roles and Responsibilities

Coordinator

The coordinator [REDACTED] is the main point of contact between the Funding Agency (=European Commission - EC) and the consortium. In its function, the coordinator:

- Signs the Grant Agreement with the EC after authorisation by the parties of the project
- Finalises, with the support of the EB members, the reports and project documents (including Financial Certificates as required) required by the EC
- Ensures prompt delivery of all deliverables to the EC after approval by the EB
- Pursuant to the contract, the coordinator is responsible for the following tasks and functions:
 - Financial transaction of the EC grants to the beneficiaries, and
 - Preparation for, and documentation of GA/EB meetings

The coordinator is dependent on and instructed by the General Assembly (GA) and any decision taken by the project coordinator can be overruled by the GA. [REDACTED] will be the responsible partner for project coordination. [REDACTED] as an organization is very experienced and has (and partially still is) participated in approximately 105 FP7 projects, 15 of which as coordinator. **The Project Coordinator, Prof. Dr. H. Jan Ankersmit**, is responsible for the overall administrative, financial, technical/scientific and organisational management of the project as a whole.

Management Support Team (MST)

The MST supports the Coordinator in the day-to-day management of the project which comprises:

- Project Manager at the MUW, which will maintain an overview of the scientific and technical implementation of the project, keeping the Coordinator and relevant partners informed on the current and upcoming project issues.
- [REDACTED] will support the Coordinator and consortium on management and cost reporting procedures including for collecting and consolidating costs for mid-period controlling and periodic reporting. [REDACTED] has extensive experience in EU project administration, both as partner and coordinator.
- [REDACTED] will support and advise the Coordinator in management of Ethics and Gender issues.

Together, the Coordinator and the Management Support Team are responsible for:

- Interfacing between the EC and consortium, both for technical and administrative/financial matters
- Coordination of the overall work plan according to the predefined time schedule
- Supporting and monitoring the cost and progress reports from the partners and the appropriate consolidation to produce the Periodic Reports for the EC
- Organization of GA meetings: Kick Off, mid-period, end of period 18 month and the final meeting
- Planning, implementation and reporting of Ethics and gender related issues

Responsibilities of the WP leaders

- Participate at the Executive Board Meetings (every 6 months)
- Compile and update six-monthly activity reports of detail plans with task leaders and contributors
- Reporting of WP results, progress and observed problems to the EB
- To suggest solutions or adaptations to solve problems within WPs (avoiding deviation)
- Monitoring WP progress towards objectives and milestones
- Facilitating partner interaction within a Work Package as well as to other Work Packages

3.2.2 Communication strategy

The communication strategy will be based on three pillars: the day-to-day communication, the web-based communication and the project meetings. The day-to-day communication between the partners will mainly take place by telephone and e-mail. The coordinator will facilitate smooth communication and interaction between all researchers involved in the project. The web-based communication will consist of an external, public website and an internal, password restricted web-based shared working environment (e.g. SharePoint-based) hosting all relevant project documents (reports, meeting minutes, presentations, etc.) where partners can down-and upload data and documents. The following project meetings will be organised:

- GA meetings; Kick off, mid-period, end of period, Final meeting
- EB meetings; 6-8 teleconferences per year with, face-to-face meetings at GA meetings and as needed
- WP meetings, whenever considered necessary for the progress of the WP; mostly by Teleconference
- Technical Review meetings (to be organised by coordinator as required with the EC Project Officer).

In order to obtain maximum efficiency, the various meetings will as much as possible be organised in conjunction, e.g. GA and EB meetings will be combined and where appropriate, with dissemination events.

3.2.3 Management of knowledge: innovation, intellectual property and publications

Knowledge, intellectual property rights (exploitation) and publications are operatively managed within the Dissemination and Exploitation (D&E) WP but detailed analysis use of *results* are discussed at EB level in context of innovation, cost benefit analyses and business planning. The H2020 rules for ownership of results are defined in the GA and elaborated upon in the CA, but details of further steps and investments for post-project, commercial use of results will need to be defined. This includes attracting the investors necessary for Phase III clinical trials, but also examining exploitation potential of other results before dissemination. Although the EB has the double role as an Exploitation Committee, the creators i.e. owners of the results are the decision makers on business models, including eventual transfer of ownership which can include other consortium members beyond the EB. All partners (i.e. the GA) are party to the Consortium Agreement and will be involved in innovation management i.e. to both recognise and utilise market and technical problems, with a goal of successfully implementing appropriate creative ideas.

3.2.4 Methods for monitoring of project success and eventual conflict resolution mechanisms

The success of the project will be evaluated against clearly defined and measurable parameters:

- Milestones (Table 3.2a)
- Deliverables (Table 3.1c)
- Patent applications, exploitation potential
- Scientific outcome: scientific publications, conference presentations, academic theses

These elements will be critically assessed during the meetings. If deviations from the plans are evident, the reasons will be elaborated further and a contingency plan made to overcome the difficulties. The project progress and the quality of results will be reviewed by the EB and compared with the work plan targets. A change in the work plan (e.g. to improve quality of results) will be proposed if needed, with a final decision being taken at GA level. Any problems or conflicts will be tackled quickly to minimise their negative effect. Should any problems be foreseen or arise, the respective participant will report this via the work package leader to the Coordinator, who will decide on the necessary steps and conclusions taking into account the opinions of the related partners and will inform the Executive Board and if deemed necessary i.e. having impact at project level, seek decision making at GA level.

Table 3.2a: List of milestones

MS	Milestone name	Related WPs	Estimated date	Means of verification
1	Sufficient quantity of APOSEC produced for Toxicology	1	M04	██████████ report the quantities received from RED
2	Completed, positive Toxicology tests	2	M12	Positive toxicology report
3	MARSYAS II, III ethical approvals positively completed	3	M15	Positive ethic votes obtained
4	Completed, positive Potency assays	1	M18	Positive potency assay report
5	Completed, positive Phase I Trial systemic MARSYAS III	3	M21	Study report phase I systemic
6	Completed, positive Phase I Trial topical MARSYAS II	3	M21	Study report phase I topical
7	MARSYAS IV ethical approval positively completed	4	M27	Positive ethic votes obtained
8	MARSYAS V ethical approval positively completed	5	M27	Positive ethic votes obtained
9	Sufficient patients recruited for MARSYAS IV, Phase II Skin	4	M33	CRO reports this fact
10	Sufficient patients recruited for MARSYAS V, Phase I-II AMI	5	M33	Partners ██████████ and ██████████ report this fact
11	Interim Reports of trial review committee completed and approved for MARSYAS IV	4	M39	Positive Interim Report of Data Safety Management Board (DSMB)
12	Interim Reports of trial review committee completed and approved	5	M39	Positive Interim Report of Data Safety Management

	for MARSYAS V			Board (DSMB)
13	MARSYAS IV completed	4	M51	Study report Marsyas IV
14	MARSYAS V completed	5	M51	Study report Marsyas V

Table 3.2b: Critical risks for implementation

Description of risk	WP(s) involved	Proposed risk-mitigation measures
Insufficient APOSEC produced for Toxicology testing	1	Increase production by short-term employment of additional lab technician
Failed toxicology	2	Low probability; technical review of project; decision on project continuation to be taken
Failed Phase I topical – MARSYAS II Occurrence of adverse effects	3	Very low probability; technical review of project part on ‘DFU’; decision on continuation regarding MARSYAS IV to be taken, MARSYAS V continues as planned
Failed Phase I systemic – MARSYAS III Occurrence of adverse effects	3	Very low probability; technical review of project part on ‘AMI’; decision on continuation regarding MARSYAS V to be taken, MARSYAS IV continues as planned
Failed Phase II ‘DFU’ – MARSYAS IV No significant therapeutic efficacy detected	4	Low probability; the indication DFU will not be subjected to the next level of clinical testing utilising APOSEC™
Failed Phase I-II ‘AMI’ – MARSYAS V	5	Low probability; the indication AMI will not be subjected to the next level of clinical testing utilising APOSEC™

3.3 Consortium as a whole

The consortium consists of ten partners from seven EU Member States, namely Austria, Denmark, Germany, Poland, Romania, Slovakia and Spain. The partner types represented are four universities, one university hospital, two SMEs, two large enterprises and one non-profit association.

██████████ as the coordinator of the APOSEC project, is also coordinating and/or conducting all clinical trials planned in the APOSEC project. The ██████████ researchers directly involved in these trials, Dr. Hendrik Jan Ankersmit, ██████████ have proven track records in conducting clinical research and trials.

For the clinical phase I-II trial on AMI (MARASYAS V), three renowned European clinical centres for cardiac research joined the consortium: [REDACTED]

[REDACTED] All three principal investigators involved [REDACTED] are well-known in their field and have already extensive experience in the conduction of clinical trials, also within European collaborative projects.

Further necessary research on APOSEC will be performed by the Chair [REDACTED] [REDACTED] at the [REDACTED] lead by [REDACTED]. Their extensive knowledge and know-how on tissue models are key to the generation of relevant research data on the mode of action and tumorigenicity of APOSEC.

The participation of the Aposcience AG (APO), as the owner of the APOSEC patents, and the [REDACTED] of the [REDACTED], as the only GMP facility approved for the production of APOSEC, is without doubt crucial. Previous close collaborations between APO, [REDACTED] and [REDACTED] ensure a good communication within the project.

██████████ ██████████ and ██████████ are two large enterprises well experienced in pharmaceutical research and bring their know-how on toxicology testing of new drugs into the consortium. The SME ██████████ completes the consortium with extensive experience and a proven track record in dissemination and exploitation management as well as administration of European collaborative projects.

All partners have an international orientation and experience in joint projects in the field of medical research and/or clinical trials. This will facilitate the necessary communication and cooperation during the project and possibly later in the exploitation phase. Figure 20 shows the complementarities and roles of the different partners in the APOSEC Project.

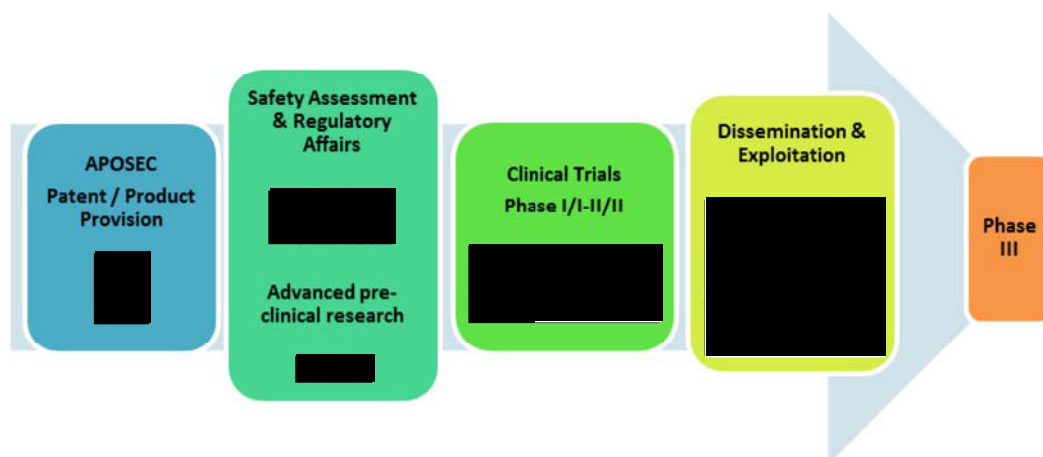


Figure 20: Complementarities and roles of the different partners in the APOSEC Project

3.4 Resources to be committed

3.4.1 Overview Person/months required

The table below provides an overview of the total person/months per participant and per WP.

It should be noted that WP8 – Management not only includes person/months for overall project coordination and reporting but also person/months for innovation management as well as the tendering process of the high volume subcontracts.

Since dissemination and exploitation is to some extent a joint effort, every participant is included with at least one person/month in WP7.

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	Total Person/ Months per Participant
1			37	12	39		7	48	143
2	101						1	1	103
3		10					14	6	30
4							38	12	50
5						55	1	1	57
6					19		1		20
7		14					1		15
8					29		1		30
9					66,5		1		67,5
10		60,5					1		61,5
Total Person/ Months	101	84,5	37	12	153,5	55	66	68	

3.4.2 Other direct costs

Below, tables are shown for each participant (all, except [REDACTED] and [REDACTED]) where the sum of other direct costs, incl. subcontracting, exceeds 15% of the personnel costs for that participant.

Partner 1 MUW	Cost (€)	Justification
Travel	[REDACTED]	Project coordinator incl. 4 other Principal Investigators of clinical trials planned in the APOSEC project
Clinical Trial MARSYAS I + II	[REDACTED]	Regulatory submissions, Volunteer remuneration, Trial insurance, safety lab costs, test kits
Clinical Trial MARSYAS V	[REDACTED]	Laboratory tests and consumables (e.g. ELISA, DNA expression analyses)
Open access costs	[REDACTED]	Multiple scientific publications anticipated after clinical trials
Dissemination costs	[REDACTED]	Event organisation and conference participation
Subcontracting	[REDACTED]	Data archiving (15 years) Clinical trial MARSYAS II + III
Subcontracting	[REDACTED]	Financial Audit Certificate
Subcontracting	[REDACTED]	Independent Core Lab for Clinical trial MARSYAY V
Total	[REDACTED]	

Partner 2 RED	Cost (€)	Justification
Travel		4,5 year project duration, approx. 4 consortium meetings
Consumables		Material costs Production Process
Consumables		Material costs Potency Assays
Consumables		Material costs Stability Testing
Subcontracting		Radiation treatment during APOSEC production
Subcontracting		Financial Audit Certificate
Subcontracting		Support Potency Assays
Total		

Partner 3 APO	Cost (€)	Justification
Travel		4,5 year project duration, approx. 4 consortium meetings
Subcontracting		Financial Audit Certificate
Subcontracting		Support Regulatory Affairs
Subcontracting		Monitoring CT MARSYAS V via CRO
Subcontracting		Support in Conduct, Monitoring CT MARSYAS IV via CRO
Total		

Partner 4 RTDS	Cost (€)	Justification
Travel		4,5 year project duration, approx. 4 consortium meetings
Consumables		Graphics purchase, Print, Management tool licence
Subcontracting		Financial Audit Certificate
Subcontracting		Website programming and maintenance
Subcontracting		Event organisation, catering etc.
Total		

Partner 5 UKW	Cost (€)	Justification
Travel		4,5 year project duration, approx. 4 consortium meetings
Consumables		Consumables over 3 years for cell culture + molecular biology, ELISA kits and antibodies, biochemical analyses
Technology		Bioreactor technology for tissue models
Subcontracting		Financial Audit Certificate
Subcontracting		Technology Development, [REDACTED]
Total		

Partner 7 HAR	Cost (€)	Justification
Travel		Meeting participation
Laboratory animals		Necessary for toxicity testing in WP2
Subcontracting		ECGs assessment
Subcontracting		Histopathology assessment
Total		

Partner 8 UMF	Cost (€)	Justification
Travel		4,5 year project duration, approx. 4 consortium meetings
Consumables		Catheter sets for 10 patients
Total		

Partner 9 SUM	Cost (€)	Justification
Travel		4,5 year project duration, approx. 4 consortium meetings
Open access		Publication anticipated following clinical trial
Subcontracting		Trial insurance
Third Party		(MRIs, non-invasive and laboratory tests, ambulatory visits)
Total		

References

- American College of Foot and Ankle Surgeons (ACFAS). **Foot Ulcers Affect 15 Percent of All Diabetic Patients**. 2006. (http://www.footphysicians.com/diabetes/diabetes_foot_ulcers.htm). footphysicians.com.
- American Society of Plastic Surgeons (ASPS). **Evidence-based Clinical Practice Guideline: Chronic Wounds of the Lower Extremity**. 2007. <http://www.plasticsurgery.org/Documents/medical-professionals/health-policy/evidence-practice/Evidence-based-Clinical-Practice-Guideline-Chronic-Wounds-of-the-Lower-Extremity.pdf>
- Ankersmit HJ, Hoetzenecker K, Dietl W, Soleiman A, Horvat R, Wolfsberger M, Gerner C, Hacker S, Mildner M, Moser B, Lichtenauer M, Podesser BK. **Irradiated cultured apoptotic peripheral blood mononuclear cells regenerate infarcted myocardium**. Eur J Clin Invest. 2009 Jun;39(6):445-56. doi: 10.1111/j.1365-2362.2009.02111.x. Epub 2009 Apr 16.
- Barshes NR, Sigireddi M, Wrobel JS, Mahankali A, Robbins JM, Koungias P, Armstrong DG. **The system of care for the diabetic foot: objectives, outcomes, and opportunities**. Diabet Foot Ankle 2013 Oct 10;4.
- Bittencourt MC1, Perruche S, Contassot E, Fresnay S, Baron MH, Angonin R, Aubin F, Hervé P, Tiberghien P, Saas P. **Intravenous injection of apoptotic leukocytes enhances bone marrow engraftment across major histocompatibility barriers**. Blood 2001;98:224-30.
- Blanquer M, Moraleda JM, Iniesta F, Gómez-Espuch J, Meca-Lallana J, Villaverde R, Pérez-Espejo MÁ, Ruíz-López FJ, García Santos JM, Bleda P, Izura V, Sáez M, De Mingo P, Vivancos L, Carles R, Jiménez J, Hernández J, Guardiola J, Del Rio ST, Antúnez C, De la Rosa P, Majado MJ, Sánchez-Salinas A, López J, Martínez-Lage JF, Martínez S. **Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: a pilot safety study**. Stem Cells. 2012 Jun;30(6):1277-85.
- Branski LK, Mittermayr R, Herndon DN, Norbury WB, Masters OE, Hofmann M, Traber DL, Redl H, Jeschke MG. **A porcine model of full-thickness burn, excision and skin autografting**. Burns. 2008 Dec;34(8):1119-27
- Braunschweig F, Cowie MR, Auricchio A. **What are the costs of heart failure?**. Europace 2011 May;13 Suppl 2:iii13-7.
- Cannon CL, Neal PJ, Southee JA, Kubilus J, Klausner M. **New epidermal model for dermal irritancy testing**. Toxicol In Vitro. 1994 Aug;8(4):889-91.
- Di Santo S, Yang Z, Wyler von Ballmoos M, Voelzmann J, Diehm N, Baumgartner I, Kalka C. **Novel cell-free strategy for therapeutic angiogenesis: in vitro generated conditioned medium can replace progenitor cell transplantation**. PLoS One. 2009 May 21;4(5):e5643.

- Devaux M, de Looper M. **Income-related inequalities in health service utilisation in 19 OECD countries 2008-2009**. OECD Health working papers 58, 2012
- Dissemond J. *Ulcus cruris – Genese, Diagnostik und Therapie*. 2007, 2. Aufl. Bremen: UNI-MED.
- Dominguez-Rodriguez A, Abreu-Gonzalez P, Reiter RJ. **Cardioprotection and pharmacological therapies in acute myocardial infarction: Challenges in the current era**. World J Cardiol. 2014 Mar 26;6(3):100-106.
- European Commission (EC), 2012. **Commission Communication — Annual Growth Survey 2013**. COM(2012) 750 final, 28.11.2012.
- European Commission (EC), 2013. Infographic on ‘Investing in Health’. http://ec.europa.eu/health/strategy/docs/investing_in_health_infograph_en.pdf
- European Commission (EC), 2014. **Health Strategy Objectives**. http://ec.europa.eu/health/strategy/objectives/index_en.htm
- Fox KAA, Goodman SG, Anderson Jr FA, Granger CB, Moscucci M, Flather MD, Spencer F, Budaj A, Dabbous OH, Gore JM on behalf of the GRACE investigators, **From guidelines to clinical practice: the impact of hospital and geographic characteristics on temporal trends in the management of acute coronary syndromes: The Global Registry of Acute Coronary Events (GRACE)**. Eur. Heart J 2003; 24:1414-1424.
- Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. **Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells**. Nat Med. 2005 Apr;11(4):367-8.
- Gottrup F, Apelqvist J, Price P, European Wound Management Association Patient Outcome Group. **Outcomes in controlled and comparative studies on non-healing wounds: recommendations to improve the quality of evidence in wound management**. J Wound Care 2010 Jun;19(6):237-68.
- Hankin CS, Knispel J, Lopes M, Bronstone A, Maus E. **Clinical and Cost Efficacy of Advanced Wound Care Matrices for Venous Ulcers**. Vol. 18, No. 5 June 2012 JMCP Journal of Managed Care Pharmacy, p. 375-384.
- Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. **Circulating Endothelial Progenitor Cells, Vascular Function, and Cardiovascular Risk**. N Engl J Med 2003;348:593-600.
- Hoetzenecker K, Assinger A, Lichtenauer M, Mildner M, Schweiger T, Starlinger P, Jakab A, Berényi E, Pavo N, Zimmermann M, Gabriel C, Plass C, Gyöngyösi M, Volf I, Ankersmit HJ. **Secretome of apoptotic peripheral blood cells (APOSEC) attenuates microvascular obstruction in a porcine closed chest reperfused acute myocardial infarction model: role of platelet aggregation and vasodilation**. Basic Res Cardiol. 2012 Sep;107(5):292.
- Hoetzenecker K, Zimmermann M, Hoetzenecker W, Schweiger T, Kollmann D, Mildner M, Hegedus B, Mitterbauer A, Hacker S, Birner P, Gabriel C, Gyöngyösi M, Blyszczuk P, Eriksson U, Ankersmit HJ. **Mononuclear cell secretome protects from experimental autoimmune myocarditis**. Eur Heart J. 2013 Jan 14. [Epub ahead of print]
- Holzinger C, Zuckermann A, Kopp C, Schöllhammer A, Imhof M, Zwölfer W, Baumgartner I, Magometchnigg H, Weissinger E, Wolner E. **Treatment of Non-healing Skin Ulcers with Autologous Activated Mononuclear Cells**. Eur J Vasc Surg 8, 351-356 (1994).
- Icks A, Haastert B, Trautner C, Giani G, Glaeske G, Hoffmann F. **Incidence of lower-limb amputations in the diabetic compared to the non-diabetic population. Findings from nationwide insurance data, Germany, 2005-2007**. Exp Clin Endocrinol Diabetes. 2009 Oct;117(9):500-4.
- International Diabetes Federation (IDF). **IDF Diabetes Atlas, 6th Edn**. 2013. ISBN: 2-930229-85-3
- Jayaraman P, Nathan P, Vasanthan P, Musa S, Govindasamy V. **Stem cells conditioned medium: a new approach to skin wound healing management**. Cell Biol Int. 2013 Oct;37(10):1122-8.
- Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, Bulte JW, Petrou P, Ben-Hur T, Abramsky O, Slavin S. **Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis**. Arch Neurol. 2010 Oct;67(10):1187-94.
- Kocher AA, Schlechta B, Gasparovicova A, Wolner E, Bonaros N, Laufer G. **Stem cells and cardiac regeneration**. Transpl Int. 2007 Sep;20(9):731-46.
- Kocher AA, Schuster MD, Bonaros N, Lietz K, Xiang G, Martens TP, Kurlansky PA, Sondermeijer H, Witkowski P, Boyle A, Homma S, Wang SF, Itescu S. **Myocardial homing and neovascularization by**

- human bone marrow angioblasts is regulated by IL-8/Gro CXC chemokines.** *J Mol Cell Cardiol.* 2006 Apr;40(4):455-64.
- Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. **Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function.** *Nat Med.* 2001 Apr;7(4):430-6.
- Korf-Klingebiel M, Kempf T, Sauer T, Brinkmann E, Fischer P, Meyer GP, Ganser A, Drexler H, Wollert KC. **Bone marrow cells are a rich source of growth factors and cytokines: implications for cell therapy trials after myocardial infarction.** *Eur Heart J.* 2008 Dec;29(23):2851-8.
- Langer A, Rogowski W. **Systematic review of economic evaluations of human cell-derived wound care products for the treatment of venous leg and diabetic foot ulcers.** *BMC Health Services Research* 2009, 9:115.
- Larose E, Rodés-Cabau J, Pibarot P, Rinfret S, Proulx G, Nguyen CM, Déry JP, Gleeton O, Roy L, Noël B, Barbeau G, Rouleau J, Boudreault JR, Amyot M, De Larochellière R, Bertrand OF. **Predicting late myocardial recovery and outcomes in the early hours of ST-segment elevation myocardial infarction traditional measures compared with microvascular obstruction, salvaged myocardium, and necrosis characteristics by cardiovascular magnetic resonance.** *J Am Coll Cardiol.* 2010 Jun 1;55(22):2459-69.
- Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KKL, Murabito JM, Vasan RS, **Long-term trends in the incidence of and survival with heart failure.** *N Engl J Med* 2002; 347:1397-1402
- Lichtenauer M, Mildner M, Baumgartner A, Hasun M, Werba G, Beer L, Altmann P, Roth G, Gyöngyösi M, Podesser BK, Ankersmit HJ. **Intravenous and intramyocardial injection of apoptotic white blood cell suspensions prevents ventricular remodelling by increasing elastin expression in cardiac scar tissue after myocardial infarction.** *Basic Res Cardiol.* 2011 Jun;106(4):645-55.
- Lichtenauer M, Mildner M, Hoetzenecker K, Zimmermann M, Podesser BK, Sipos W, Berényi E, Dworschak M, Tschachler E, Gyöngyösi M, Ankersmit HJ. **Secretome of apoptotic peripheral blood cells (APOSEC) confers cytoprotection to cardiomyocytes and inhibits tissue remodelling after acute myocardial infarction: a preclinical study.** *Basic Res Cardiol.* 2011 Nov;106(6):1283-97.
- Lu D, Chen B, Liang Z, Deng W, Jiang Y, Li S, Xu J, Wu Q, Zhang Z, Xie B, Chen S. **Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: a double-blind, randomized, controlled trial.** *Diabetes Res Clin Pract.* 2011 Apr;92(1):26-36.
- Mildner M, Hacker S, Haider T, Gschwandtner M, Werba G, Barresi C, Zimmermann M, Golabi B, Tschachler E, Ankersmit HJ. **Secretome of peripheral blood mononuclear cells enhances wound healing.** *PLoS One.* 2013;8(3):e60103.
- Nichols M, Townsend N, Luengo-Fernandez R, Leal J, Gray A, Scarborough P, Rayner M. **European Cardiovascular Disease Statistics 2012.** European Heart Network 2012, Brussels, European Society of Cardiology, Sophia Antipolis
- Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. **Bone marrow cells regenerate infarcted myocardium.** *Nature.* 2001 Apr 5;410(6829):701-5.
- Pavo N, Zimmermann M, Pils D, Mildner M, Petrás Z, Petneházy O, Fuzik J, Jakab A, Gabriel C, Sipos W, Maurer G, Gyöngyösi M, Ankersmit HJ. **Long-acting beneficial effect of percutaneously intramyocardially delivered secretome of apoptotic peripheral blood cells on porcine chronic ischemic left ventricular dysfunction.** *Biomaterials.* 2014 Apr;35(11):3541-50. doi: 10.1016/j.biomaterials.2013.12.071. Epub 2014 Jan 16.
- Prompers L, Huijberts M, Schaper N, Apelqvist J, Bakker K, Edmonds M, Holstein P, Jude E, Jirkovska A, Mauricio D, Piaggese A, Reike H, Spraul M, Van Acker K, Van Baal S, Van Merode F, Uccioli L, Urbancic V, Ragnarson Tennvall G. **Resource utilization and costs associated with the treatment of diabetic foot ulcer. Prospective data from the Eurodiale Study.** *Diabetologia* 2008 Oct;51(10):1826-34.
- Roger VL, Weston SA, Redfield MM, Hellerman-Homan JP, Killian J, Yawn BP, Jacobsen SJ, **Trends in heart failure incidence and survival in a community-based population.** *JAMA* 2004; 292:344-350
- Rogers LC, Lavery LA, Armstrong DG. **The right to bear legs--an amendment to healthcare: how preventing amputations can save billions for the US Health-care System.** *J Am Podiatr Med Assoc.* 2008 Mar-Apr;98(2):166-8.

- Schaper NC. **Lessons from Eurodiale.** Diabetes Metab Res Rev 2012; 28 (Suppl 1): 21–26.
- Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC, Longaker MT. **Human Skin Wounds: A Major and Snowballing Threat to Public Health and the Economy.** Wound Repair Regen. 2009 Nov-Dec;17(6):763-71.
- Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, López CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Jeschke MG, Klein MB, Gamelli RL, Gibran NS, Brownstein BH, Miller-Graziano C, Calvano SE, Mason PH, Cobb JP, Rahme LG, Lowry SF, Maier RV, Moldawer LL, Herndon DN, Davis RW, Xiao W, Tompkins RG. **Genomic responses in mouse models poorly mimic human inflammatory diseases.** Proc Natl Acad Sci U S A. 2013 Feb 26;110(9):3507
- Singer AJ, Clark RAF. **Cutaneous Wound Healing.** N Engl J Med, Sept. 2 1999.
- Singh N, Armstrong DG, Lipsky BA. **Preventing Foot Ulcers in Patients With Diabetes.** JAMA, January 12, 2005—Vol. 293, No. 2
- Spencer FA, Allegro J, Goldberg J, Gore JM, Fox KAA, Granger CB, Mehta RH, Brieger D on behalf of the GRACE investigators, **Association of statin therapy with outcomes of acute coronary syndromes: the GRACE study.** Ann Int Med 2004; 140:857-866
- Strauer BE1, Brehm M, Zeus T, Gattermann N, Hernandez A, Sorg RV, Kögler G, Wernet P. **[Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction].** Dtsch Med Wochenschr. 2001 Aug 24;126(34-35):932-8. [Article in German]
- Tanaka R, Vayntrub M, Masuda H, Ito R, Kobori M, Miyasaka M, Mizuno H, Warren SM, Asahara T. **Quality-control culture system restores diabetic endothelial progenitor cell vasculogenesis and accelerates wound closure.** Diabetes. 2013 Sep;62(9):3207-17.
- Tasso R, Ilengo C, Quarto R, Cancedda R, Caspi RR, Pennesi G. **Mesenchymal stem cells induce functionally active T-regulatory lymphocytes in a paracrine fashion and ameliorate experimental autoimmune uveitis.** Invest Ophthalmol Vis Sci. 2012 Feb 16;53(2):786-93.
- Timmers L, Lim SK, Arslan F, Armstrong JS, Hoefer IE, Doevendans PA, Pick JJ, El Oakley RM, Choo A, Lee CN, Pasterkamp G, de Kleijn DPV. **Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium.** Stem Cell Res 2008; 1, 129-137
- Uccelli A, Mancardi G. **Stem cell transplantation in multiple sclerosis.** Curr Opin Neurol. 2010 Jun;23(3):218-25.
- Velagaleti RS, Pencina MJ, Murabito JM, Wang TJ, Parikh NI, D'Agostino RB, Levy D, Kannel WB, Vasan RS. **Long-Term Trends in the Incidence of Heart Failure After Myocardial Infarction.** Circulation 2008 Nov 11;118(20):2057-62.
- Visiongain, 2013. **The Top 20 Companies in the Advanced Wound Care Market 2013-2023.** London, United Kingdom
- Willmann JK, van Bruggen N, Dinkelborg LM, Gambhir SS. **Molecular imaging in drug development.** Nature Review Drug Discovery. 2008 Jul;7(7):591-607
- Wollert KC, Drexler H. **Cell therapy for the treatment of coronary heart disease: a critical appraisal.** Nat Rev Cardiol. 2010 Apr;7(4):204-15.
- Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. **Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial.** Lancet. 2004 Jul 10-16;364(9429):141-8.
- World Health Organisation (WHO). **Fact sheet No. 312: The top 10 causes of death.** Update 2013. <http://www.who.int/mediacentre/factsheets/fs312/en/>
- Yang Z, von Ballmoos MW, Faessler D, Voelzmann J, Ortmann J, Diehm N, Kalka-Moll W, Baumgartner I, Di Santo S, Kalka C. **Paracrine factors secreted by endothelial progenitor cells prevent oxidative stress-induced apoptosis of mature endothelial cells.** Atherosclerosis. 2010 Jul;211(1):103-9.
- Yeh RW, Chandra M, Sorel M, Selby JV, Go AS. **Population trends in the incidence and outcomes of acute myocardial infarction.** N Engl J Med 2010; 362:2155-65
- Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, Nichols G. **Global healthcare expenditure on diabetes for 2010 and 2030.** Diabetes Res Clin Pract. 2010 Mar;87(3):293-301.

4 Members of the APOSEC consortium

Participant No.		Participant organisation name	Country
1 (Coord.)			Austria
2			Austria
3	APO	Aposcience AG	Austria
4			Austria
5			Germany
6			Denmark
7			Spain
8			Romania
9			Poland
10			Slovakia

Partner No. 3 / SME: Aposcience AG (APO), Austria

Aposcience AG is a Vienna based biotechnology company. It was founded in 2008. Aposcience AG is the company partner of the Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration, a public-private partnership situated at the MUW, where all preclinical evaluations of APOSEC™, including small and large animal studies, were performed. Aposcience AG is the patent holder of all patents concerning APOSEC (EP2201954, WO2010070105-A1 for the treatment of internal hypoxia induced inflammation, and EP0967534 for wound healing).

Contribution to the Project

APOSCIENCE AG is the European SME that is intended to benefit from positive results in the planned clinical phase I and II studies and will transform it to a major player in the field of regenerative medicine. The company contributes to the project by disseminating the trial results and searching for partners for clinical phase III trials, and financing additional projects. For the current project, [REDACTED] a renowned toxicology expert, will be employed as freelance in-house consultant for toxicology issues by Aposcience AG. A clinical research organization (CRO) will be subcontracted by Aposcience AG to conduct clinical phase II studies in the indication diabetic ulcer, and to monitor clinical phase I-II studies in acute myocardial infarction (AMI).

Relevant Patents

- WO2010070105 “Pharmaceutical preparation for treatment of conditions associated with ischemia”
- WO2010079086 “Pharmaceutical preparation comprising supernatant of blood mononuclear cell culture”

Key personnel

[REDACTED] (M) provides rich professional experience from working for major pharmaceutical companies as well as a CEO for biopharmaceutical companies, including the supervision of clinical studies. Since 2011 he is CEO of Aposcience AG and, concomitantly, of [REDACTED] another Vienna based biopharmaceutical company.

[REDACTED] (M) is especially experienced in

- preclinical drug development (safety pharmacology, toxicology, pharmacokinetics);
- regulatory toxicology and international guidelines (ICH, FDA, EMA, OECD);
- international chemical laws (REACH);
- Laboratory animal science and animal welfare.

He was head of several R&D departments and study director in various pharmaceutical and research companies. Since July 2012 he is [REDACTED] Vienna, Austria. Furthermore he is working as independent contractor and professional/freelancer for Aposcience AG.

Project manager, N.N.

4.2 Third parties involved in the project (including use of third party resources)

Partner 1, [REDACTED]

Does the participant plan to subcontract certain tasks (please note that core tasks of the project should not be sub-contracted)	YES
Data Archiving (15 years) for clinical trial documentation for clinical trial MARSYAS II + III Issuing of a Financial Audit Certificate In WP5, subcontracting is related to the following task: T5.6 Assessment of microvascular obstruction and cardiac function by LE-MRI (M25 – M42) An independent Core laboratory will be subcontracted to analyse two Transthoracic echocardiographs (TTE), 24h ECG and two LE-MRIs per patient included in the trial.	
Does the participant envisage that part of its work is performed by linked third parties	NO
<i>If yes, please describe the third party, the link of the participant to the third party, and describe and justify the foreseen tasks to be performed by the third party</i>	
Does the participant envisage the use of contributions in kind provided by third parties (Articles 11 and 12 of the General Model Grant Agreement)	NO
<i>If yes, please describe the third party and their contributions</i>	

Partner 2, [REDACTED]

Does the participant plan to subcontract certain tasks (please note that core tasks of the project should not be sub-contracted)	YES
Necessary radiation treatment during APOSEC production to induce cell apoptosis as well as achieve viral clearance Issuing of a Financial Audit Certificate Support in the development of potency assays; subcontracted work related to the following tasks: T1.1 Development of Cell-based potency assay T1.2 Development of potency assay based on platelet aggregation	
Does the participant envisage that part of its work is performed by linked third parties	NO
<i>If yes, please describe the third party, the link of the participant to the third party, and describe and justify the foreseen tasks to be performed by the third party</i>	
Does the participant envisage the use of contributions in kind provided by third parties (Articles 11 and 12 of the General Model Grant Agreement)	NO
<i>If yes, please describe the third party and their contributions</i>	

Partner 3, APO

Does the participant plan to subcontract certain tasks (please note that core tasks of the project should not be sub-contracted)	YES
Issuing of a Financial Audit Certificate Support Regulatory Affairs; subcontracted work related to the following tasks: T2.4 Regulatory Affairs Subcontracted work will include engagement with the European Medicines Agency as well as consulting on specific regulatory issues on 'Biologicals' In WP4, subcontracting related to the clinical trial and therefore the following tasks: T4.2 Identification of study centres by the CRO (M22-M24: CRO) The study sites shall involve six to eight university hospitals in Romania and Germany, which will be determined by the subcontracted CRO. The CRO will perform (a) site qualifications, (b) be responsible for the collection of documents for all study centres, (c) for lab accreditation and (d) curricula vitae of the local principal investigators, (e) the preparation and submission of the application of the concerned local ethics committees and other local authorities, and (f) and the organisation of investigator meetings. The CRO will further develop a detailed monitoring plan, perform site selection visits, perform site initiation visits, perform regular interim monitor visits, and perform site close-out visits. Regular monitor visits include check of informed consent, subject registration of all enrolled subjects, examination of eCRF for plausibility, completeness of conformity with the study protocol, examination of adverse events (AE), source data verification and checking of the investigator file. The CRO will document and report all details of the monitoring visit and of any up-action that may be required. Task 4.5 Preparation of the ethics applications to the local ethics committees (M22-27: CRO) The study Steering Committee will approve the Study Protocol, Patient informed consent (translated to relevant languages) and Investigator Brochure. These documents will be submitted to local Ethical Committee, hospital and university regulatory authorities and Ministry of Health for approval. An electronic case report form (eCRF) will be constructed and established at each clinical site. Site initiation visit will be performed before study start. The study will be registered at EudraCT and clinicaltrials.com homepage. Task 4.3 Recruitment of 160 patients with diabetic ulcer (M28-33: CRO) 160 patients will be recruited with diabetic ulcers in the participating centres according to inclusion and exclusion criteria. Task 4.6 Initiation and performance of the topical phase II study in 160 patients (M28-45: CRO) The inclusion phase of the APOSEC – Diabetic ulcer trial is approximately 6 months, with the latest follow-up investigation up to 18 months. After inclusion of 60% of the study probands, preliminary assessment of the data will be performed and the safety and efficacy of the data will be reported. Task 4.7 Trial monitoring by an independent DSMB (M22-51: MUW, CRO) The independent data safety board (DSMB) will monitor the reported adverse events to relate to the drug procedure.	

In WP5, subcontracting is related to the monitoring of the clinical trial and the following tasks: T5.1 Approval of the study by local Ethical Committees and other regulatory authorities (M19 - M24) An external Clinical Research Organisation (CRO) will be subcontracted, and electronic case report form (eCRF) will be constructed and established at each clinical site. Site initiation visit will be performed before study start. T5.8 Trial monitoring by the independent CRO The CRO will perform the study monitoring. Regular monitoring visits will include check of informed consent, subject registration of all enrolled subjects, control of eCRS, completeness of conformity with the study protocol, examination of adverse events, source data verification and checking of the investigator file. The CRO will be responsible for document communication, set up of workflows, timelines, project specific procedures and tracking tools, and storage of the files according to the agreed standard operation procedures (SOPs).	
Does the participant envisage that part of its work is performed by linked third parties	NO
<i>If yes, please describe the third party, the link of the participant to the third party, and describe and justify the foreseen tasks to be performed by the third party</i>	
Does the participant envisage the use of contributions in kind provided by third parties (Articles 11 and 12 of the General Model Grant Agreement)	NO
<i>If yes, please describe the third party and their contributions</i>	

Partner 4, [REDACTED]

Does the participant plan to subcontract certain tasks (please note that core tasks of the project should not be sub-contracted)	YES
Issuing of a Financial Audit Certificate Work to be subcontracted is related to the following tasks: Task 7.2 Dissemination Tools and Channels Printing of dissemination material (e.g. flyers, posters, fact sheets etc.), project website programming and maintenance T7.4 Dissemination and training events Necessary subcontracting related to event organisation, catering etc.	
Does the participant envisage that part of its work is performed by linked third parties	NO
<i>If yes, please describe the third party, the link of the participant to the third party, and describe and justify the foreseen tasks to be performed by the third party</i>	
Does the participant envisage the use of contributions in kind provided by third parties (Articles 11 and 12 of the General Model Grant Agreement)	NO

If yes, please describe the third party and their contributions

Partner 5, UKW

Does the participant plan to subcontract certain tasks (please note that core tasks of the project should not be sub-contracted)	YES
Issuing of a Financial Audit Certificate Subcontracted work related to the following task: T6.4 The potential of APOSEC to induce tumour invasion or epithelial-mesenchymal transition (EMT) The [REDACTED] will supply [REDACTED] with the technology to set up human vascularized tumour models. These human vascularised tumour tissues will be incubated with APOSEC through the blood vessel system with different concentration of APOSEC over time. The invasion and EMT capability will be compared with the standardized induction of invasion and EMT by TGF-β. Transformed tumour/healthy cells will be isolated by laser capture technology to evaluate the APOSEC induced signalling networks. A promising new approach to study cellular signalling networks and their changes through targeted agents in tumour models that have specific mutations. Through this network analysis, modes of drug action can be understood.	
Does the participant envisage that part of its work is performed by linked third parties	NO
<i>If yes, please describe the third party, the link of the participant to the third party, and describe and justify the foreseen tasks to be performed by the third party</i>	
Does the participant envisage the use of contributions in kind provided by third parties (Articles 11 and 12 of the General Model Grant Agreement)	NO
<i>If yes, please describe the third party and their contributions</i>	

Partner 6, [REDACTED]

No third parties involved.

Partner 7, [REDACTED]

Does the participant plan to subcontract certain tasks (please note that core tasks of the project should not be sub-contracted)	YES
Subcontracted work related to the following tasks: T2.2 Conducting acute and repeated dose toxicity animal studies in rats T2.3 Conduction safety pharmacology studies in rats and dogs ECG assessment Histopathology assessment	
Does the participant envisage that part of its work is performed by linked third parties	NO

<i>If yes, please describe the third party, the link of the participant to the third party, and describe and justify the foreseen tasks to be performed by the third party</i>	
Does the participant envisage the use of contributions in kind provided by third parties (Articles 11 and 12 of the General Model Grant Agreement)	NO
<i>If yes, please describe the third party and their contributions</i>	

Partner 8, [REDACTED]

No third parties involved.

Partner 9, [REDACTED]

Does the participant plan to subcontract certain tasks (please note that core tasks of the project should not be sub-contracted)	YES
Subcontracted work related to Clinical Trial MARSYAS V and the following tasks: T5.4 Treatment with APOSEC T5.4. Completion of the study Trial insurance	
Does the participant envisage that part of its work is performed by linked third parties	YES
The [REDACTED] is the university hospital linked to the [REDACTED] They are two separate legal entities, although their work is closely intertwined. The treatments of the patients recruited at [REDACTED] for the clinical trial MARSYAS V will be performed at the [REDACTED] as the necessary facilities are owned by the [REDACTED] and not [REDACTED]	
Does the participant envisage the use of contributions in kind provided by third parties (Articles 11 and 12 of the General Model Grant Agreement)	NO
<i>If yes, please describe the third party and their contributions</i>	

Partner 10, HAM

No third parties involved.

5 Ethics and Security

5.1 Ethics

Clinical Trials

In total, four clinical trials will be performed in different clinical centres and countries:

- | | |
|----------------|--|
| 1. MARSYAS II | allogeneic GMP APOSEC PHASE I topical |
| 2. MARSYAS III | allogeneic GMP APOSEC PHASE I systemic |
| 3. MARSYAS IV | allogeneic GMP APOSEC PHASE II Diabetic foot ulcer |
| 4. MARSYAS V | allogeneic GMP APOSEC PHASE I-II AMI |

The information provided below represents a summary of ethics relevant issues related to all four clinical trials. More detailed information, also on conduct and data management, can be found in the respective annex 'APOSEC – Essential Information on Clinical Trials'.

The study centres participating in the clinical trials performed within the APOSEC project are engaged to respect the current legislation and regulations in their countries. For each clinical trial, a corresponding study protocol, informed consent and Investigator brochure will be constructed and submitted to the local Ethical Committees, regulatory authorities of the universities, hospitals and Ministry of Health.

The final approval of the human topical and intravenous application of the investigational medicinal product (IMP) APOSEC will be done by the National Medicines and Healthcare products Regulatory Agency (AGES). The documents submitted will include for the Regulatory Agency the final protocol, the Investigational Medicinal Product Dossier (IMPD) and Clinical Trial Application (CTA). After receiving the final approval, the clinical trial participants will discuss the finalization of the protocol and clinical study management and data management according to local specific issues.

The study documents including study protocol, patient informed consent, investigator brochure and filled out local ethical form will be submitted to the local Ethics Committee for approval. Patient information and informed consent will be translated to the corresponding language.

All data will be included in the eCRF anonymously and kept in this secure database. Nominated Monitor Agency [REDACTED] Austria for MARSYAS II and III studies, and a nominated CRO for the international multicentre studies MARSYAS IV and V) will control the course of the study, data collection, the adverse events and ethical issues.

The clinical trials performed within the APOSEC project include healthy volunteers in MARSYAS II and III and two types of specific patient populations: 1) the diabetic patients with leg ulcer in MARSYAS IV and 2) patients with acute myocardial infarction in MARSYAS V.

Informed consent will be given freely by the volunteer/patient after he/she has been informed of the nature, significance, implications and risks of the study, including anonymization of data; and consent is evidenced in writing, dated and signed, or otherwise marked, by that person so as to indicate his/her consent, prior to the start of the study. The nature of the informed consent will comply with the current version of the Declaration of Helsinki, the current requirements of Good Clinical Practice (CPMP/ICH/135/95) and local regulations.

Indemnity and Compensation

In accordance with EU Clinical Trials Directive 2000/20/EC Article 3 (2f), provision is to be made for the indemnity or compensation in the event of injury or death attributable to the clinical trial; and insurance or indemnity to cover the liability of the Investigator or Sponsor.

Trial participants will be informed about the transfer of their anonymized data within the framework of the documentation and notification required according to §7, paragraph 2, no. 15 and §12 and §13 of the GCP regulations. Study participants who do not agree to the transfer, will not be included in the clinical trial.

The monitoring agency as well as the primary investigators of the trial will control the correct administration mode of the drug. The local Institutional Pharmacy and the monitoring agency will control the drugs, expiry date and the collection of the rest of the medicines. Any deviation in production, storage or delivery will be reported to the independent Data Safety and Monitoring Board (DSMB) and the Principal Investigators will be informed. Any consequences regarding the patient treatment will be reported to the relevant Ethics Committee.

The AGES and the European Medicine Agency (after EMA approval) and the GMP facility will control the

- manufacturing process
- the product stability,
- product manufacturing, characterization
- delivery of the APOSEC medicine to the clinical centres,
- blood donors, ensure blood from approved blood bank and donor criteria
- tests to show absences of relevant human viruses
- qualification process
- Verification of the source, safety, and sterility,
- Process timing, intermediate storage
- Define methods of cell collection conditions
- Transportation and storage conditions
- Good Tissue Practice rules

Toxicity and safety pharmacology studies in animals

In accordance with guidelines and directives issued by international health regulation authorities and harmonisation bodies (EMA, ICH; FDA), acute and repeated dose toxicity studies as well as safety pharmacology studies have to be performed for allogeneic APOSEC before clinical trials can commence. The studies will be conducted in line with the Community Code relating to medicinal products for human use (Directive 2001/83/EC) and guidelines such as ICH M3(R2), ICH S6 plus addendum, ICH S7A, EMEA/CHMP/SWP/488313/2007, CPMP/SWP/1042/99 (EMA) and the CDER Guidance for Single Dose Acute Toxicity Testing.

The animal studies within the APOSEC project will be carefully planned on the basis of all research data obtained so far and the 3R principles (replace/reduce/refine) will be taken into account wherever possible.

All standards and requirements regarding the protection of animals used for scientific purposes, laid down in Directive 2010/63/EU, will be met and any additional national legislation in place will be followed by the participant conducting the animal studies.

5.2 Security¹

Please indicate if your project will involve:

- activities or results raising security issues: NO
- 'EU-classified information' as background or results: NO

¹ Article 37.1 of Model Grant Agreement. *Before disclosing results of activities raising security issues to a third party (including affiliated entities), a beneficiary must inform the coordinator — which must request written approval from the Commission/Agency; Article 37. Activities related to 'classified deliverables' must comply with the 'security requirements' until they are declassified; Action tasks related to classified deliverables may not be subcontracted without prior explicit written approval from the Commission/Agency.; The beneficiaries must inform the coordinator — which must immediately inform the Commission/Agency — of any changes in the security context and — if necessary — request for Annex 1 to be amended (see Article 55)*



This electronic receipt is a digitally signed version of the document submitted by your organisation. Both the content of the document and a set of metadata have been digitally sealed.

This digital signature mechanism, using a public-private key pair mechanism, uniquely binds this eReceipt to the modules of the Participant Portal of the European Commission, to the transaction for which it was generated and ensures its full integrity. Therefore a complete digitally signed trail of the transaction is available both for your organisation and for the issuer of the eReceipt.

Any attempt to modify the content will lead to a break of the integrity of the electronic signature, which can be verified at any time by clicking on the eReceipt validation symbol.

More info about eReceipts can be found in the FAQ page of the Participant Portal. (<http://ec.europa.eu/research/participants/portal/page/faq>)