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Guideline on clinical investigation of medicinal products for the treatment of peripheral arterial occlusive disease of the lower extremities

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This guideline replaces Note for Guidance on clinical investigation of medicinal products for the treatment of peripheral arterial occlusive disease (CPMP/EWP/714/98 rev 1).

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Guideline on clinical investigation of medicinal products for the treatment of peripheral arterial occlusive disease of the lower extremities

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Executive summary

The main aim of the guideline is to address development of medicinal products for the treatment of atherosclerosis-related chronic ischaemia affecting the lower extremities.

This is the first revision of the Note for Guidance on clinical investigation of medicinal products for the treatment of peripheral arterial occlusive disease (CPMP/EWP/714/98 rev 1).

The main aim of the first revision was to explicitly address clinical development of medicinal products intended to treat arterial lower extremity disease, to reflect updates in disease classification and management, including the angiosome concept, but also provide guidance on the regulatory estimands of interest, definition of clinical endpoints, and cover specific aspects related to the clinical development of advanced medical therapies (ATMPs) in the setting of lower extremity arterial disease.

1. Introduction (background)

Lower extremity ischaemic disease (LEAD) is the most common clinical manifestation of chronic peripheral arterial occlusive disease (PAOD) and is sustained by the obstruction of blood flow within the limb arteries or the aortoiliac tract, commonly recognising atherosclerosis as etiopathogenetic cause.

The wide spectrum of clinical presentation, severity and anatomical distribution of the disease imposes the use of classification schemes for patient management. Historically, the Fontaine and Rutherford classifications have been most widely employed. The Fontaine categorization rates the LEAD in four stages based on signs and symptoms: asymptomatic patients (stage I), intermittent claudication (stage II, with a distinction between stage IIa and IIb referring to claudication at a distance > 200 m and < 200 m, respectively), rest pain (stage III), and trophic lesions including necrosis/gangrene (stage IV). The Rutherford staging is based upon a combination of clinical symptoms and non-invasive haemodynamic measures (i.e. treadmill test, arterial brachial indices). Like the Fontaine scheme, it distinguishes four grades of disease including asymptomatic disease (Grade 0), intermittent claudication (Grade I), rest pain (Grade II) and morphologic lesions (Grade III), with a further subcategorization into six classes, based on objective criteria. With the introduction of the new definition of the Chronic Limb Threatening Ischaemia (CLTI), which refers to the end-stage manifestation of chronic atherosclerosis and better reflects the continuum of occlusive disease and associated symptomatology, also including diabetic patients, the Global Vascular Guidelines (GVG) recommend the application of the Wound, Ischemia, and foot Infection [WIfI] grading score. In candidates for surgery, the GVG suggest the new Global Limb Anatomic Staging System (GLASS) as a classification tool in the planning of revascularisation strategies: through an angiography-based characterisation of the target arterial path (TAP) (that can also incorporate the so-called angiosome concept) and the estimated limb-based patency (LBP), the GLASS rating reflects the probability of success of the procedure and consequent clinical benefit of treatment in terms of limb salvage probability.

The natural course of LEAD shows a high variability. In a trial (Hiatt WR et al, 2017) that mostly recruited patients with Rutherford Grades I-III, the analysis of changes at 12 months from baseline in the Rutherford classification showed that the clinical symptomology remains unchanged in most

patients (63.7%), improves in 25.4% and worsens in 10.9% of the study population. A relevant information for the purpose of preventative strategies came from the analysis of the group of asymptomatic patients that reported disease progression with a rate of 26% over 1 year.

Almost 50% of patients who present with the most advanced stage of disease (i.e. CLTI) have no history of prior LEAD (Nehler MR et al, 2023). These patients are more likely older and male with pre-existing cardiovascular morbidities and renal failure. Because of the neuropathy-associated symptomatology, diabetic patients often remain underdiagnosed, and this explains the highest probability of presenting with de-novo CLTI. Because of the high likelihood of peripheral arterial disease and the elevated prevalence of an asymptomatic manifestation in this population (i.e. around 75%), learned societies now recommend yearly screening in diabetic patients (Global Vascular Guidelines, 2019). Of note, the diabetic status predisposes to a more rapid progression and increased severity of the disease. Data have been reported for a higher rate of major and all amputations in type 1 compared to type 2 diabetics and differences in revascularization strategies between the two groups (Jain N et al, 2022).

Generally, all LEAD patients (even if asymptomatic) are at increased risk of major adverse cardiovascular events such as myocardial infarction, stroke or cardiovascular death and major adverse limb events like amputations, chronic or acute lower limb ischaemia, lower limb revascularization. Subjects not suitable for, or who failed revascularisation using surgical bypass or endovascular methods, who often represent the target population of clinical trials testing advanced therapy medicinal products (ATMPs), are at high risk for amputation and death (Norgren et al 2007).

The therapy of LEAD focuses on symptoms relief and the prevention of cardiovascular morbidity, amputation and death. Given the association with conventional cardiovascular risk factors, interventions aiming at controlling smoking, hypertension, dyslipidaemia and diabetes as well as the use of antithrombotic agents, pain controllers and rehabilitation programs as appropriate, all concur to the optimal management of patients undergoing either a medical treatment or surgical approach to therapy (2024 ESC Guidelines or the management of peripheral arterial and aortic diseases).

2. Scope

Guidance is provided on the clinical development program of medicinal products intended to treat lower extremity arterial disease. Acute ischaemia and peripheral vascular disorders of inflammatory or immunologic origin such as Buerger‘s disease and necrotic vasculitis are not considered because these diseases differ from arteriosclerosis obliterans in their clinical picture, in their evolution and in their prognosis.

The current revision concerns the clinical development program of medicinal products, including ATMPs, intended for an indication in LEAD, with specific reference to the estimand of interest including the definition of the study population, and choice of clinical endpoints for inference of efficacy in confirmatory trials.

3. Legal basis and relevant guidelines

This Guideline should be read in conjunction with the introduction and general principles of Annex I to Directive 2001/83/EC, as amended, and all other relevant EU and ICH guidelines. These include, but are not limited to:

- Guideline for good clinical practice (EMA/CHMP/ICH/135/1995 [ICH E6[R2]]);
- ICH Guideline E8 (R1) on general considerations for clinical studies (EMA/CHMP/ICH/544570/1998 Corr*);
- Pharmacokinetic studies in man (1987);
- Note for Guidance on Population Exposure: the extent of population exposure to assess clinical safety (CPMP/ICH/375/95 [ICH E1]);
- Note for Guidance on Dose Response Information to Support Drug Registration (CPMP/ICH/378/95 [ICH E4]);
- Note for Guidance on Statistical Principles for Clinical Trials (CPMP/ICH/363/96 [ICH E9]) and Addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials (EMA/CHMP/ICH/436221/2017 [ICH E9[R1]]);
- Points to consider on switching between superiority and non-inferiority (CPMP/EWP/482/99);
- Guideline on the choice of the non-inferiority margin (EMEA/CPMP/EWP/2158/99); Note for Guidance on choice of control group in clinical trials (CPMP/ICH/364/96);
- Note for Guidance on Studies in Support of Special Populations: Geriatrics - CPMP/ICH/379/95 (ICH E7) and Questions and Answers (EMA/CHMP/ICH/604661/2009 [ICH E7 Q&A]);
- Reflection Paper on assessment of cardiovascular safety profile of medicinal products (EMA/CHMP/50549/2015);
- Guideline on the evaluation of medicinal products for cardiovascular disease prevention (EMEA/CHMP/EWP/311890/2007)
- ICH E11(R1) guideline on clinical investigation of medicinal products in the paediatric population (EMA/CPMP/ICH/2711/1999)
- Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)
- Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009)
- Quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (CHMP/GTWP/671639/2008)
- Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products (EMEA/149995/2008)
- Reflection Paper on the regulatory Guidance for the use of health-related quality of life (HRQL) measures in the evaluation of medicinal products (EMEA/CHMP/EWP/139391/2004)

4. Development strategy

Throughout the clinical development programme, key aspects to be considered pertain to the selection of patients and definition of study objectives, which are expected i) to depend on the development phase of the medicinal product (see section 5 for a detailed guidance on study design) and ii) to provide adequate representativeness of the target indication. This latter point is an important requirement in confirmatory therapeutic studies, for which a disease stage-specific approach can be adopted in the choice of the study population and clinical endpoints, thus making efficacy results fully evaluable. In this context, a distinction between treatment and preventive objectives can be considered, based on the intended indication. A list of acceptable clinical endpoints is reported in this section, while the choice of valid primary and secondary endpoints according with both the development and disease stage is discussed in section 6-7.

4.1. Selection of patients - General considerations

The criteria used for the diagnosis of LEAD in patients recruited for clinical trials must be clearly defined. The diagnosis, type of occlusive lesion (stenosis, complete block) and its location must be confirmed by objective means. Different classification systems are currently available for patient staging, covering both the clinical and anatomical characterization of disease. The choice of the different classification systems and their combination is expected to be adequate to the definition of a study population that should be homogenous in terms of disease severity, localization, and risk of disease progression. While a symptomatology-based grading supports the definition of stage of disease, anatomical classification systems also incorporating, but not limited to the angiosome concept, are essential elements to be considered in revascularization and wound healing treatments. For instance, the angiosome-based approach relies upon the angiographic study of the affected territories and supports the planning of surgical procedures through the analysis of feasibility for either a direct flow restoration (i.e. by re-establishing arterial patency in line to the ischemic area), or the possibility to achieve an indirect revascularisation through collaterals. The success of both techniques is influenced not only by technical aspects related to the surgical intervention, but also to comorbidities (i.e. diabetes, smoking status) that influence the perfusion status.

Since revascularisation should be offered to all CLTI patients, the definition of "no or poor revascularisation options" needs to be tightly defined in the study protocol. Treatment groups should be balanced in respect of patient demography, severity of disease, previous revascularisation procedures and duration of symptoms, as well as concomitant medications and standardized rehabilitation programs, as appropriate. This is usually ensured by adequate randomisation procedures. Influences of potential confounders such as cardiovascular risk factors (e.g. smoking, hypertension, hyperlipidaemia, diabetes mellitus) should be carefully taken into consideration in the analysis plan, and respective therapeutic measures should not be changed during the course of the trial unless ethically or clinically indicated (e.g. for participant safety) keeping in mind that an appropriate strategy dealing with intercurrent events should be specified in the study protocol (see section 5). A distinction between type 1 and type 2 diabetes should also be considered, based on the reported differences in terms of clinical outcomes and therapeutic management between the two populations.

4.2. Assessment of efficacy/ Methods to assess efficacy

4.2.1. Treatment endpoints

4.2.1.1. Improvement of walking capacity

Claudication distances should be assessed using a standardised, reproducible test methodology (i.e. treadmill test or 6MWD test). There are two internationally accepted treadmill protocols, i.e. the constant workload protocol using a constant speed and grade (mostly 3.2 km/h and 12% grade), and the graded test where the speed is kept constant, but the grade is varied, starting horizontally but then increasing in predefined steps (e.g. 2%) at predefined intervals (e.g. 2 min). The two tests differ, in that the relationship between workload and walking time follows a linear function with the constant test but a curvilinear function with the graded test.

Both tests can be equally recommended for use in clinical trials but cannot be used in an interchangeable way; a decision on the treadmill protocol and the treadmill settings must be made beforehand and should not be altered.

The 6MWD can also be considered as a method to assess clinical efficacy. It has been demonstrated to be representative of daily life walking functionality and is correlated with daily physical activity in contrast to the treadmill test (McDermott 2014).

The 6MWD and treadmill test are not interchangeable and the choice between methodologies should take into account, among other parameters, the studied population in terms of disease stage and concomitant CV conditions, as well as accessibility to the different rehabilitation programs (either supervised treadmill or home-based exercise), especially in global trials, which can all distinctly affect the measured outcomes (McDermott 2020; A Clinical European Consensus Document on PAD training 2024). Should the 6MWD and treadmill test be proposed for efficacy assessment in the same trial, results from both measures are expected to be consistent with a treatment-dependent functional improvement.

Treadmill testing

Initial claudication distance (ICD)

From a clinical point of view, ICD compared to absolute claudication distance (ACD) as symptomatic endpoint may be the more important variable, since patients seldomly force themselves to the extreme of ACD. On the other hand, ICD is more subjective.

If ICD is chosen as primary endpoint, ACD should be evaluated as a secondary endpoint.

Absolute claudication distance (ACD)

ACD can be used alternatively. However, if a graded treadmill protocol is used, ACD should be the primary efficacy variable. The reproducibility of ACD is superior to ICD with graded protocols.

If ACD is chosen as primary endpoint, ICD should be evaluated as a secondary endpoint.

Six Minute Walk Distance Test (6MWD)

The 6MWD can be used as a primary endpoint, considering it has been demonstrated to be representative enough of daily life walking functionality, and is even more correlated with daily physical activity than the historically more often used treadmill testing (McDermott 2014). The 6MWD was found also to correlate with mortality outcomes (McDermott 2011).

The minimal clinically important difference (MCID) that is intended to be used in the inference of efficacy of treatment requires to be pre-specified in the study protocol and is expected to be justified and relevant to the specific targeted population.

4.2.1.2. Improvement of pain

Pain (if existing) should be quantified. The intensity of the pain should be assessed by means of standardised methods (e. g. visual analogue scale). A responder analysis requires a pre-specification of changes in pain intensity ratings that will be of clinical importance. Since pain at rest is an endpoint influenced by variables such as mood, motivation, and other factors, the standardisation of the trial methodology is of utmost importance. This does not only refer to the methodology used to quantify pain but does include factors such as the time of pain assessment (same time of the day, preferably at drug trough levels), the personnel taking the measurement (which should not change), and the assessment of analgesic consumption. The consumption and the type of analgesics should be measured and documented, although the comparison of patients on different analgesics schemes may be difficult.

4.2.1.3. Healing of ulcers

Ulcer healing must be defined as healing of all ischaemic ulcers in both legs (all ulcers epithelialized as assessed by an independent physician and documented by photography). Since quantification of partial healing may be difficult to assess objectively and since the clinical relevance of partial healing remains unclear, only total healing of lesions should be reported as main efficacy criterion.

4.2.1.4. Interventional/surgical procedures

Clinical parameters that should be considered include the rate of revascularisation procedures, minor amputations as well as frequency of major amputations.

4.2.1.5. Quality of Life (QoL) outcomes

Clinical studies to support regulatory submissions are encouraged to use disease-specific carefully validated tools. For generic considerations, reference is made to the *Reflection paper on the regulatory guidance for the use of health-related quality of life (HRQL) measures in the evaluation of medicinal products (EMEA/CHMP/EWP/139391/2004)*

4.2.2. Prevention endpoints

4.2.2.1. Prevention of disease progression

Amputation

The rate of major amputations can be considered as an efficacy endpoint or as a component of a composite efficacy endpoint.

Only major amputations, above the ankle, should be counted (unlike minor amputations, i.e below the ankle). Both legs must be considered for the assessment of amputation rates.

The criteria for major amputations are to be specified à priori in the study protocol to avoid relevant centre-related effects (e.g. a more conservative or a more progressive attitude towards the indication for amputation).

4.2.2.2. Prevention of CV/ischemic events

Mortality

All-cause mortality or cardiovascular mortality can be considered as an efficacy endpoint or as a component of a composite efficacy endpoint.

Cardiovascular morbidity

Cardiovascular morbidity alone should not serve as a single primary endpoint, but it could be incorporated into a composite primary endpoint which also includes mortality.

Composite Endpoints

The most adequate endpoint in prevention studies is a composite endpoint, if specified à priori, and if consisting of clinically relevant components. Such an endpoint may include cardiovascular morbidity (e.g. stroke, myocardial infarction) and all-cause mortality. Major amputation as a component of a composite endpoint can also be considered and should be generally adopted in more severe stages (stages III/IV, CLTI).

5. Study design

5.1. Pharmacokinetics and Pharmacodynamics

For the purpose of investigating the pharmacokinetics and pharmacodynamics of a new investigational medicinal product intended for the treatment of peripheral arterial disease, reference is made to the available and general EMA guidance on the different aspects of clinical pharmacology (see section 3).

Pharmacodynamic endpoints should be product-specific, defined based on the mechanism of action of the investigational medicinal product with the intention to provide a "proof-of-concept" and evidence of the pharmacological activity of the drug, as well as a characterisation of the exposure-response relationships.

5.2. Exploratory therapeutic studies/dose finding

The purpose of this development phase is to prove the therapeutic activity of the drug under investigation and to establish suitable therapeutic dose ranges.

The dose and therapeutic schedule should be selected according to the results of previous studies. These studies should be carried out in selected patients with strict inclusion and exclusion criteria (see section 4 for general considerations on patient selection).

A randomised, double-blind, parallel group, placebo-controlled design is recommended. Primary assessment criteria depend on the aim of the study (e.g. walking distance in claudication trials, relief of rest pain and ulcer healing as symptomatic endpoints in critical limb ischaemia).

In general, the treatment period should be in the range of 2 to 3 months. However, the overall duration of dose response studies may vary and should be properly justified considering the mechanism of action and the main endpoint of the study. A run-in period is recommended to verify the stability of the patient's conditions, e.g. comedication, stability of the claudication distances.

5.3. Confirmatory therapeutic studies

5.3.1. General statistical aspects

Studies aiming at the proof of efficacy must have a confirmatory statistical approach – e.g. a demonstration of superiority, equivalence or non-inferiority must be pre-specified in the protocol.

The design and analysis should be performed in accordance with the available methodology guidelines.

Generally, studies which investigate the possibility to reduce the risk of several serious events (prevention studies) and which therefore may use a composite endpoint as a primary variable should have a superiority hypothesis or be designed as non-inferiority studies with suitable comparators in the event that established therapies in the pursued indication are available.

However, efficacy in the composite endpoint should be coupled with evidence that none of the components is negatively influenced.

It is expected that the pivotal trials robustly demonstrate statistically significant effects of a relevant clinical magnitude.

The duration of the trial depends on the aim of the study and the endpoint(s) chosen.

However, the length of exposure to the drug should be sufficient to investigate the potential of tolerance developing.

Generally, efforts should be made to collect all relevant data for the primary and important other estimands to minimize the need to rely on untestable assumptions in the analysis and interpretation of the study results. Data obtained after discontinuation of treatment or other intercurrent events are of interest when a treatment-policy strategy is used in the estimand. In case data are missing after treatment discontinuation, it is not plausible that (all of) the treatment benefit is retained, and imputation approaches that are sufficiently robust under plausible clinical scenarios should be used that are unlikely to overestimate the benefit of the new treatment or underestimate the variability of the estimated treatment effect.

5.3.2. Confounding factors to be considered in study design

There are several confounding factors which could influence the results of therapeutic clinicaltrials in LEAD.

Regular physical exercise improves symptoms of intermittent claudication. Thus, the frequent use of repeated exercise testing in clinical trials may lead to an improvement in exercise capacity independent of drug treatment. This should be considered in the design and analysis of such trials. At the same time, adherence of patients to standardized supervised rehabilitation programs should be taken into account. To this respect, exclusion criteria may limit recruitment to those with training capacity based on respiratory or cardiovascular conditions, or major gait disturbance.

Regular physical exercise and cessation of smoking are of much importance in the treatment of intermittent claudication and have significant impact also on the outcomes of revascularisation. Advice on smoking cessation and physical exercise should be given before patients are included in a clinical trial. Respective effects should be documented.

Even if claudication distances were considered clinically stable and stability was proven during the run-in phase of a clinical trial in LEAD stage II patients, a marked placebo effect cannot be avoided.

Experiences from previous trials indicate that observed variability of claudication distances between trials varies considerably. In addition, the distribution of these endpoints is often skewed. These factors should be considered when calculating the sample size and planningthe analysis strategy (e.g. considering logarithmic transformation).

In patients hospitalised for critical limb ischaemia there is a high response rate as regards bothrest pain and ulcer healing during placebo treatment. If this situation is not accounted for, the number of patients enrolled to a clinical trial may be inadequate for inference of treatment effect relative to placebo.

5.3.3. Estimand of interest

An estimand is a precise description of the treatment effect reflecting the clinical question posed by a given clinical trial objective. A disease-specific approach should be adopted for the definition of the primary and (key) secondary estimands, with the definition of the main clinical endpoints to be driven by the intended use of an investigational drug and target population, as defined by disease stage and anatomical localization. As a general consideration for LEAD patients, the primary outcome should be either the symptomatic relief or a preventative effect on cardiovascular events and amputations, differences between the active treatment and control arms as the summary measure.

Intercurrent events should be considered in the estimation of the effect through the selection of appropriate strategies as follows.

Intercurrent events expected to be potential modifiers of treatment effect in the context of LEAD include drug discontinuation, changes in background therapies with effects on the perfusion status or drug-to-drug interactions, as well as terminal events (i.e. death or leg amputation when not included in the clinical study endpoints) or unplanned revascularisation procedures. The nature of the specific

intercurrent events and their chance to occur vary depending on the target population, as defined by disease severity, anatomical distribution, and presence of comorbidities. It is expected that the study protocol identifies relevant intercurrent events and clearly defines strategies to handle them. As a general consideration, a treatment policy strategy should be regarded as the preferred regulatory approach for intercurrent events related to changes in the background treatment, additional treatment, treatment discontinuation or unplanned revascularization procedures. However, this may not apply to studies testing a non-inferiority or equivalence hypothesis, which therefore must be considered in the selection of the appropriate strategy. For terminal events, a composite strategy should be implemented that considers the event as a suitably undesirable outcome in itself. While for composite endpoints this intercurrent event is considered in itself informative of the outcome and is therefore incorporated into the definition of the estimand, this is not straightforward to implement for continuous endpoints (i.e., walking capacity, relief of pain, healing). Due to the methodological issues involved, it is recommended to seek scientific advice in order to define the appropriate strategy for estimand composition. Reference is also made to relevant guidelines (ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials).

6. Studies evaluating symptomatic treatment

For studies evaluating treatment of symptoms of LEAD, the specific claim of the clinical benefit put forward in the product information is expected to be clearly supported by relevant study population and primary endpoints.

6.1. Design elements

A randomised parallel group, double-blind, placebo-controlled design is generally required. Placebo should be used for the control group since suitable reference substances have not yet been established in the symptomatic treatment of intermittent claudication, as well as in more advanced stages of disease. Active drug-controlled trials without a placebo arm may only be considered if the comparator drug has consistently shown superiority over placebo (assay sensitivity, see ICH E10).

A run-in phase of 2-6 weeks is recommended to verify the stability of the patient's conditions, e.g. comedication, stability of the claudication distances.

The active treatment phase should normally last for a minimum of 6 months for administration of an investigational medicinal product intended for chronic therapy. Shorter lengths are expected to be adequately justified depending on the nature of the product, mode of action, intended indication.

Depending on the duration of active treatment, the length of the follow-up period may vary. Generally, the cumulative duration of active treatment phase and follow-up period should not be less than 6 months for a controlled trial. Double blinding should be maintained during the whole period.

The allowed medication during the active treatment phase and follow-up period should be standardised as much as possible.

The follow-up period should be specified à priori in the study protocol.

A disease stage-specific approach should be adopted. Across the spectrum of CLTI patients, the unequivocal characterization of disease stage in the study population should be provided to estimate treatment effect (i.e. revascularisation success).

6.2. Patient selection/target population

The stage of disease should be clearly outlined in the study protocol using appropriate classification scoring systems.

For claudicant patients, a history of typical intermittent claudication lasting for at least 6 months to ensure clinical stability is expected. The clinical diagnosis of LEAD should be confirmed by objective evidence (e. g. reduced ankle systolic blood pressure). It is recommended that patients with high variability in the walking distance be excluded. For this purpose, at least two treadmill tests should be performed with a timeinterval of $\geq$ 1 week. The maximum change in the claudication distance should not exceed a predefined threshold [e.g. 25 % for the absolute claudication distance (ACD)]. Walking training is considered the first treatment option in this patient population. Therefore, it is expected that this therapeutic measure is tried for all patients before they are considered for entering the trial, unless otherwise justified. If walking training is applied during the study, some advantages may exist to use supervised, structured protocols.

Claudication studies should not include patients suffering from illnesses limiting their exercise capacity to a relevant degree.

Only patients with rest pain due to chronic critical limb ischaemia, that is defined as persistent recurrent pain at rest requiring analgesics for more than 2 weeks should be considered.

Generally, patients eligible for surgical/interventional reconstruction should not be included. However, patients with a high perioperative/periinterventional risk for ischaemic complications may be included, provided that the study design guarantees that necessary invasive procedures are notdelayed.

As per all patients with CLTI, the diagnosis currently requires objectively documented atherosclerosis, based on a combined evaluation of pressure measurements and Doppler arterial waveforms corroborated by the vascular imaging, combined with rest pain for at least 2 weeks.

In diabetic patients, macroangiopathy (rather than microangiopathy or neuropathy) should be the leading cause for the lesion(s). Patients with skin lesions of mixed arterio-venous origin or patients suffering from a vasculitis should not be included.

It is strongly recommended to study diabetic patients and non-diabetic patients in separate trials or to use appropriate stratification.

For CLTI patients, since revascularisation should be offered and be prioritized with respect to experimental treatments, conditions leading to "poor or no revascularisation option" needs to be tightly defined in the study protocol. To this end, it should be considered that treatment decisions also reflect the local expertise of clinical specialists, patient's access to specific surgical techniques and technologies, as well as the availability of a multidisciplinary and interdisciplinary team-based care to optimize patient's outcomes at a given facility. In this context, a centralized independent adjudication

committee that would revise individual clinical cases may be considered under certain circumstances to ensure consistency in the recruitment process and fully adherence to the inclusion criteria across study centres.

6.3. Choice of endpoints

It is recommended that a disease stage-specific approach is adopted for the choice of the clinical endpoints to be used in confirmatory studies evaluating symptomatic outcomes, also depending on the intended drug indication. The chosen endpoints should be clinically meaningful and consistent with the expected drug effect according to its mechanism of action.

For specific methodological aspects regarding these clinical endpoints, reference is made to the sections 5.

6.3.1. Primary Endpoints

Acceptable primary efficacy endpoints include walking capacity, control of pain, and wound healing.

For claudication studies, walking distance should be the primary symptomatic endpoint.

For patients with rest pain, the main symptomatic efficacy endpoint is the relief of pain at rest. It must be shown that the investigational medicinal product has no analgesic properties in terms of mode of action, although pain reduction is the downstream effect of its pharmacological action.

In the presence of skin lesions, the main symptomatic efficacy endpoint is complete healing of all necrosis and ulcerations.

Wound healing, pain control and limb salvage should be the primary goal in patients with CLTI who have no options for revascularization.

A response-based approach can be applied, for instance. endpoints can be defined as the patient being alive, having both legs, having no wound or pain, and being off analgesics. This endpoint concept should consider the time period for which the response can be maintained and is intended to study the overall medium-term/long-term outcome. Clinical response can also be defined by a change in stage as defined by classification scoring systems.

6.3.2. Secondary Endpoints

Secondary endpoints should focus on clinically relevant data supporting the study aim. These include walking distance, haemodynamic measures, interventional/surgical procedures, quality of life, consumption of analgesics.

7. Studies evaluating prevention of disease progression/prevention of ischemic events

7.1. Design elements

A randomised, parallel group, double-blind, controlled study design is generally required. Placebo and/or active drug-controlled trials may be adequate.

Treatment should last for a minimum of 12 months, but longer periods are recommended. For specific considerations, reference is made to the *Guideline on the evaluation of medicinal products for cardiovascular disease prevention (EMEA/CHMP/EWP/311890/2007).*

7.2. Patient selection/target population

In general, all patients with a proven diagnosis of LEAD are eligible for clinical trials of the prevention of ischaemic events. Thus, patients may present with a history of intermittent claudication, previous peripheral (lower extremity) vascular intervention such as surgical endarterectomy, bypass grafting or abdominal aortic aneurysm repair, transcutaneous endoluminal procedures (PTA, stenting), minor or major amputations because of LEAD, or may be asymptomatic if LEAD has been proven by objective means (e.g. haemodynamic and non-invasive imaging studies or angiography).

Regarding clinical trials focused on cardiovascular prognosis, it is recommended that a disease stage-based approach is followed in the selection of the study population. Patients with intermittent claudication and CLTI should be studied separately, or appropriate stratification techniques should be applied; within the CLTI group and depending on the aim of the investigational treatment (i.e. post-procedural or peri-procedural interventions or treatment in patients not suitable for revascularization), additional clinical and anatomical disease classifications should be adopted for an unequivocal description of the study population to be recruited.

Diabetics and non-diabetics should be studied separately or, if included in the same study, this distinction should serve as stratification factor.

Background therapy

Vasoactive substances other than the test drug, haemodilution or rheological therapy may be considered prohibited medications as per protocol, if clinically justifiable, and any use be recorded during the study. Other pharmacotherapy which is considered relevant for the treatment of LEAD or relevant for the prevention of cardiovascular events in general must be documented. It should be maintained during the course of the study. If this is not possible, e.g.during follow-up or deterioration, the study design must consider this appropriately (for specific considerations. reference is made to the statistical section on estimands). All other medicinal products can be given, as long as they have no established effect on the investigated parameters. However, their administration must be fully documented.

In CLTI, basic local treatment (e. g. local wound treatment, removal of necrotic tissue, antibiotics) must be documented and should be standardised as much as possible.

Because LEAD patients have a high risk for cardiovascular events, it is recommended to use antiplatelet agents and statins as background therapy as well as to optimise diabetic control and implement smoking cessation in patients prior to study entry. It is desirable to avoid any change in medication.

7.3. Choice of endpoints

7.3.1. Primary endpoints

Since the goal of preventative trials is the reduction of atherosclerosis-associated morbidity and mortality events, cardiovascular morbidity (e.g. myocardial infarction, stroke), major amputation and death are the clinically most meaningful endpoints. They may be used in isolation or in form of a composite endpoint. The components of a composite endpoint will depend on the clinical stage of LEAD. Whereas cardiovascular morbidity/mortality and all-cause mortality will be the most appropriate components for trials in mild to moderately severe diseased patients (i.e. Fontaine stages I and II), the rate of major amputations should also be considered in trials including CLTI patients (i.e. Fontaine stages III and IV).

The question on whether to use all-cause mortality or cardiovascular mortality as a component of a composite endpoint will depend on the estimated frequency and the possibility to identify cardiovascular death. The trial hypothesis, whether this is superiority on non-inferiority, may also play a role, particularly if the incidence of cardiovascular- and non-cardiovascular death does differ substantially. However, generally, all-cause mortality should be given preference as long as there are no persuading arguments for the use of cardiovascular mortality. If a composite endpoint is used and significantly influenced by the drug under investigation, it is expected that the single components will move in the same direction. However, a significant effect in the composite endpoint should be coupled with evidence that none of the components is negatively influenced.

7.3.2. Secondary Endpoints

Components of the composite endpoint

If the primary endpoint is a composite endpoint, the components of this composite endpoint should be evaluated as secondary endpoints.

In addition, walking distance, haemodynamic measures, interventional/surgical procedures, quality of life, consumption of analgesics can be considered.

8. Safety aspects

All adverse effects occurring during clinical trials should be fully documented. Any groups especially at-risk should be identified. Special efforts should be made to assess potential adverse effects that are characteristic of the class of drug being investigated.

Adverse drug events occurring during the treatment should be carefully recordedthroughout all study phases, including data about their nature, frequency, intensity, andrelevance.

Particular attention should be paid to the following specific side effects:

8.1. Increase in Blood pressure and heart rate

This may be either symptomatic or asymptomatic. Special attention should be paid to orthostasis and first-dose phenomenon.

8.2. Neurohumoral activation and pro-arrhythmic and/or pro-anginal effects

Depending on the particular pharmacodynamic properties of the new agent, measurement of effects on neurohumoral compensatory mechanisms, heart rate, ECG and Holter monitoring should be performed at frequent intervals throughout the study.

Effects on cardiac conduction (PR, QRS, QT and QTc) should be documented.

8.3. Rebound, withdrawal phenomena

Withdrawal phenomena, especially rebound phenomena should be studied in selected cases depending on the mode of action of the investigational product and specific concerns.

8.4. Mortality, cardiovascular morbidity

If not investigated as efficacy endpoint, a separate analysis on all-cause mortality, cardiovascular morbidity / and vascular death should be made on basis of the pivotal clinical trials. A new agent in LEAD is only acceptable for registration if there is no negative impact on mortality and cardiovascular morbidity.

9. Studies in special populations

9.1. Studies in elderly patients

The prevalence of LEAD increases with age in both sexes, with the prevailing group of patients being in the elderly population. It is relevant to generate evidence on clinical pharmacology, efficacy and safety that are representative of this subgroup and the different old age categories, considering that it will constitute the predominant target population.

9.2. Studies in paediatric patients

Atherosclerosis-related peripheral vascular disease is very rare in paediatric patients but can affect children with specific underlying predisposing conditions leading to an early disease manifestation also within the paediatric age (i.e. nephrotic syndrome, type 1 diabetes, familial hypercholesterolaemia) (Lavie G et al. 2014; Virani SS et al. 2020; Akinyosoye G et al. 2022 and 2023). The epidemiological scenario in this setting is still under definition and might change over time. Extrapolating data from the adults to the paediatric setting is a possibility that requires considerations on a case-by-case basis (Reflection paper on the use of extrapolation in the development of medicines for paediatrics). The general requirements for a safe, efficient and ethical study of medicinal product in the paediatric

population should be satisfied, as outlined in relevant EMA guidance on paediatric drug development (ICH E11(R1) guideline on clinical investigation of medicinal products in the paediatric population).

10. Advanced Therapy Medicinal Products (ATMPs)

ATMPs comprise gene therapy, somatic cell therapy medicinal products and tissue engineered products. In the setting of peripheral arterial disease, regenerative treatment aims at promoting limb perfusion and skin lesion healing through restoration of the vascular function (i.e. vasodilation) and structure, as well as phenomena of vascular regeneration mediated by angiogenesis, arteriogenesis, and vasculogenesis. The regulation of the inflammatory milieu and tissue regeneration are additional mechanisms of action potentially contributing to the regenerative function of ATMPs in the CLTI condition.

While the general recommendations on study design already provided in other sections of this guidance all apply to ATMP products tested in this clinical condition, ATMP-related specificities can be recognised pertaining to the study design, patient selection and clinical endpoints that warrant specific considerations. For the general principles of quality and clinical experimentation of ATMPs, reference is made to the relevant EMA guidelines that should be consulted in conjunction with the present recommendations.

10.1. Study Design

Whilst it is acknowledged that dose-dependency generally does not apply to ATMPs, the chosen therapeutic dose should be fully substantiated.

Certain ATMPs can undergo a single administration; however, the follow-up period should not be less than 6 months in controlled trials. The length of follow-up may vary but should be appropriate for the assessment of functionality and structural aspects of the repaired and/or regenerated tissue (target limb arteries), as well as its persistence in the human body which is expected to be considerably longer than 6 months. The follow-up should also enable assessment of secondary efficacy endpoints or important safety variables, such as the frequency of reconstructive measures, morbidity and mortality, and the rate of major amputations. Double-blindness should be maintained during the whole period.

10.2. Patient selection

Across the spectrum of CLTI patients, an unequivocal characterization of disease stage in the study population should be provided to estimate treatment effect (i.e. revascularisation success). An anatomical disease characterization, also adopting the angiosome terminology, should be considered in the study design to ensure a standardised route of drug administration, and may support the assessment of the regenerative properties of tested ATMPs, even though it is worth noting that for previously re-vascularised patients the angiosome concept might not be directly applicable given the post-surgery anatomical rearrangement.

In addition to the diabetic status, the smoking habit could impact on the regenerative effect of ATMPs. It is strongly recommended to study diabetic and non-diabetic as well as smokers and non-smokers in separate trials or to use appropriate stratification schemes.

10.3. Criteria for Efficacy

10.3.1. Primary Endpoints

Wound healing, pain control and limb salvage should be the primary goal for ATMPs in patients with CLTI who have no options for revascularization. For specific methodological aspects regarding these clinical endpoints, reference is made to the relevant sections 4-7. For prognostic endpoints including amputation reference is made to the section on prevention trials.

10.3.2. Secondary Endpoints

In addition to the secondary endpoints listed in the section 6 and 7, demonstration of to the vascular regenerative properties and the mode-of-action of ATMPs appears particularly relevant to ATMPs trials. To this end, vascular imaging modalities are regarded as particularly valuable in the confirmation of clinical efficacy. It is acknowledged that objective imaging-based methodologies are not available for quantification of vascular changes. However, it is expected that all relevant measures will be taken to support the claimed mechanistic model.

10.4. Criteria for Safety

ATMPs carry additional risks that should be addressed according to current relevant EMA recommendations (*Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products [EMEA/149995/2008 rev.1]*). Moreover, adverse events of special interest should be identified and pre-specified in the study design, including but not limited to allergic/immunologic reactions, severe infections, adverse events of CRP increases, specific AEs of MACE, MI/UA, strokes, AEs of rest pain increase and/or ulcer worsening, incidence of tumours. An appropriate risk management plan is always required to monitor events in the post-marketing phase, considering the limited length of follow-up in registration clinical trials.

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12. Definitions

ACD: Absolute Claudication Distance;

ATMP: Advanced Therapy Medicinal Products;

CRP: C-reactive Protein;

CLTI: Chronic Limb Threatening Ischaemia;

ECG: Electrocardiography;

GVG: Global Vascular Guidelines;

GLASS: Global Limb Anatomic Staging System;

ICD: Initial Claudication Distance;

LBP: Limb-Based Patency;

LEAD: Lower Extremity Ischaemic Disease;

MCID: Minimal Clinically Important Difference;

6MWD: 6-Minute Walk Distance Test;

PAOD: Peripheral Arterial Occlusive Disease;

PAD: Peripheral Arterial Disease;

PTA: Percutaneous Transluminal Angioplasty;

QoL: Quality of Life;

TAP: Target Arterial Path;

WIfI: Wound, Ischemia, and foot Infection (grading score).