Assess safety of intra-arterial autologous myogenic stem cell therapy for m.3243A>G mutation carriers

SUMMARY

Rationale: Mitochondrial disorders are progressive, often fatal multisystem disorders, in 20-25% of the cases caused by heteroplasmic mutations in the mitochondrial DNA (mtDNA). Epidemiological studies have shown that mtDNA disorders affect about 1 in 10,000 of the general population, inducing significant morbidity and mortality and high health and societal costs. Clinical manifestations are most prominent in organs with a high energy demand, like muscle and brain. At this moment, there is no effective treatment known to influence the disease process or manifestation. Myogenic stem cell-based therapies complementing defective muscle cells and fibres, are highly promising to combat the myopathy and exercise intolerance which affect >50% of heteroplasmic mtDNA mutation carriers. Myogenic stem cells called mesoangioblasts (MABs), are currently the only myogenic precursors that fulfil all criteria to be used as advanced therapy medicinal product (ATMP) for systemic treatment, namely good *ex vivo* proliferation capacity, high myogenic capacity and a capability to cross blood vessels, allowing intra-arterial (systemic) delivery towards affected muscle. Both genetically corrected autologous and allogeneic MABs transplantation has been performed in mice and dog models, but only allogeneic MABs transplantation has been performed in patients with Duchene muscular dystrophy (DMD). Treatment with *ex-vivo* expanded MABs resulted in significant regeneration of DMD positive muscle fibers in both mice and dog models. Intra-arterial delivery of allogeneic MABs in DMD boys (phase I/IIa clinical study) demonstrated that the treatment was relatively safe, and that some dystrophin was produced by the new muscle fibers, although not sufficient for functional improvement. Our approach has key advantages as we use autologous MABs, which do not require an immunosuppressive regime. Also, mitochondrial function is partly preserved in mtDNA mutation carriers and partial supplementation by healthy fibres should suffice to ameliorate mitochondrial function. We have demonstrated that MABs of most m.3243A>G carriers contain no or only a low amount (<15%) of the mtDNA mutation, allowing direct *ex vivo* expansion of patient-derived MABs. The overall aim is to induce muscle regeneration using these autologous MABs with a mutation load of <15%, as an advanced therapy medicinal product (ATMP). This proposal covers the first phase I/IIa trial.

Objective: The phase I/IIa trial will consist of an intra-arterial injection (via catheter in femoral artery) of the autologous MABs in the left lower leg of 5 m.3243A>G patients. The primary objective is assessing safety of administration of autologous MABs, which have not been used

as treatment before in humans. Secondary objectives are (1) to assess homing of the labelled autologous MABs to the tibialis anterior muscle 24 hours after i.a. delivery, and (2) assess effectiveness at the tissue level by measuring myogenesis and mtDNA mutation load of treated tibialis anterior muscle compared with untreated muscle from the contralateral leg.

Study design: Mono-center prospective open label intra-subject controlled phase I/IIa clinical study.

Study population: 15 adult m.3243A>G patients, of which 5 will be enrolled in the clinical study.

Intervention: All 15 adult m.3243A>G patients will undergo a ~30mg m. vastus lateralis muscle biopsy at visit 1. From these 15 patients, five patients will enrol the clinical study based on their m.3243A>G mutation load in skeletal muscle and mesoangioblasts. These 5 patients will visit the MUMC for four additional times. From each patient, during visit 2 till 5, in total five muscle biopsies will be collected (1x ~100 mg m.vastus lateralis both legs at visit 2, 2x ~30mg m. tibialis anterior both legs at visit 4 and 2x ~30mg m. tibialis anterior both legs at visit 5). At visit 4, the tibialis anterior muscle of one leg will be treated with 5*10E7/kg autologous MABs via tibial anterior artery delivery. A bout of maximal eccentric exercise will be executed at visit 3. Venous blood samples will be taken at all visits. See figure 1 for overview.

Main study parameters/endpoints: The primary endpoint is to assess safety will be by monitoring infusion (angiography), monitoring for acute adverse effects (24hrs), blood sampling and muscle sampling to assess local and systemic inflammation and muscle markers (CK). Secondary endpoints are assessment of effectiveness at the tissue level: namely, migration of the IC-Green labelled mesoangioblasts from the bloodstream into the tibialis anterior muscle (24 hrs after infusion) and formation of new muscle fibers and m.3243A>G mutation load (28 days after infusion).

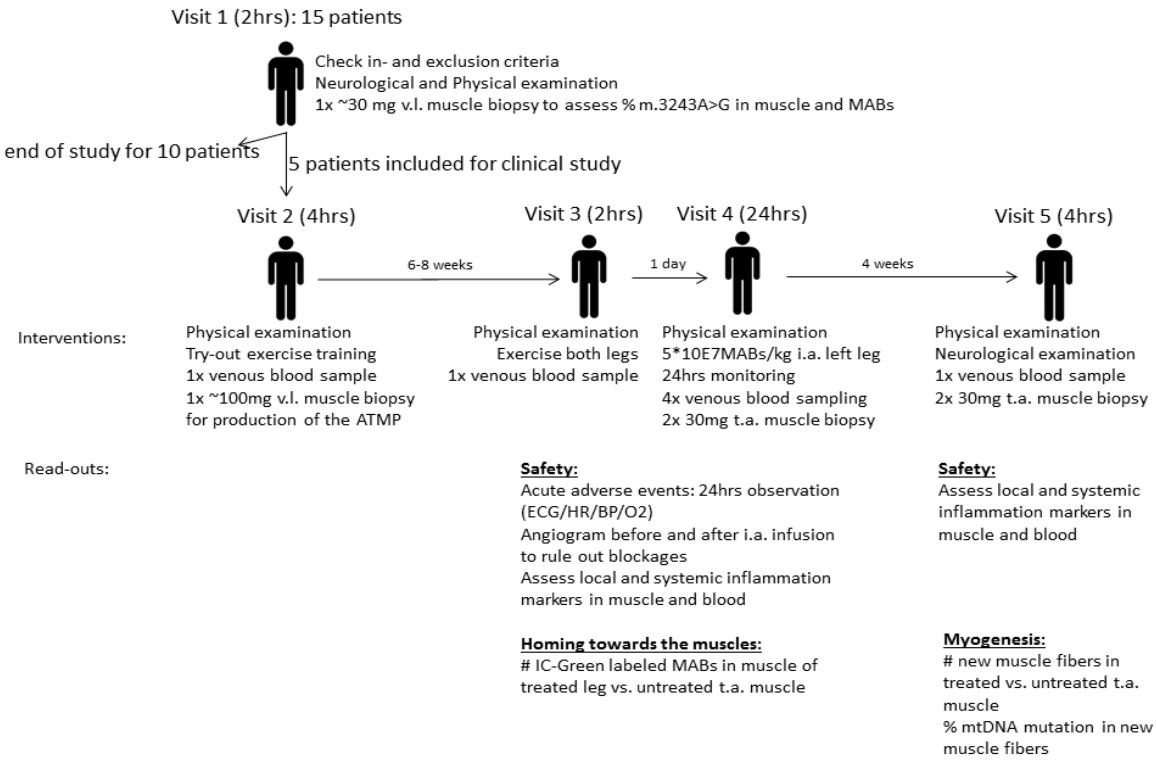

Figure 1. Study design

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Written informed consent
- Age: 18+
- Sex: male/female
- Patients with the m.3243A>G mutation

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Use of anti-coagulants, anti-thrombotics and other medication influencing coagulation
- Have a weekly alcohol intake of ≥ 35 units (men) or ≥ 24 units (women)
- Current history of drug abuse
- Deficient immune system or autoimmune disease
- Significant concurrent illness

- Ongoing participation in other clinical trials
- Major surgery within 4 weeks of the visit
- Vaccination within 4 weeks of the visit
- Pregnant or lactating women
- Psychiatric or other disorders likely to impact on informed consent
- Patients unable and/or unwilling to comply with treatment and study instructions
- Any other factor that in the opinion of the investigator excludes the patient from the study
- A history of strokes
- Allergy for contrast fluid
- Peripheral signs of ischemia or vasculopathy