1. A summary description of project context and objectives, (do not exceed 4000 characters)

Age Related Macular Degeneration (AMD) is a chronic degenerative disease, probably caused by age-associated alterations of the retina that exhibits two distinct types, a slow progressing avascular and the rapidly progressing, blinding neovascular form (nvAMD), which is characterised by the growth of choroidal vessels (CNV) into the subretinal space.

No treatments are available for avascular AMD. nvAMD is treated by intraocular injections of inhibitors of vascular endothelial growth factor (VEGF), which is overexpressed in nvAMD and is responsible for the growth of choroidal blood vessels into the subretinal space. Only about 30% of patients regain significant visual acuity when treated with anti-VEGFs, which to be effective require frequent, often monthly injections, for life.

Pigment epithelium-derived factor (PEDF), secreted by Retinal Pigment Epithelial (RPE) cells, is the natural inhibitor of VEGF. However, treatment with PEDF is not feasible because of its short half-life. An ideal treatment would be the replacement of degenerated RPE cells by cells that overexpress PEDF, e.g., peripheral RPE, Iris Pigment Epithelial (IPE) cells and stem cells. Transplantation of RPE or IPE cells to the subretinal space of nvAMD patients has not shown significant visual improvements, indicating that endogenous PEDF secretion of these cells is not sufficient to arrest CNV.

The goal of TargetAMD is treatment of nvAMD by subretinal transplantation of genetically modified RPE or IPE cells to overexpress PEDF. Specifically, RPE or IE cells isolated from a peripheral retina or iridectomy will be transfected with the *PEDF* gene and transplanted to the subretinal space of the same patient during one surgical session lasting about 60 minutes. The *PEDF* gene will be delivered to the isolated cells using the non-viral *Sleeping Beauty* transposon system, which integrates the gene into the host cell's genome. Transposons are short DNA sequences that can change their location in the genome via a "cut and paste" mechanism. In animal models *Sleeping Beauty* and its hyperactive form *SB100X* have been used successfully to correct genetic defects in hereditary diseases, such as Huntingon's disease and sickle cell anemia. *SB100X* has several advantages for gene delivery to cells, specifically, ability to integrate large inserts, efficient delivery of transgenes, stable integration of transgenes into the host cell's genome and safety, since *SB* does not preferentially integrate into active genes. Both, the *SB* transposase and the *PEDF* gene will be encoded into the novel free of antibiotic resistance marker (pFAR4) miniplamids, thus avoiding the possible transfer of antibiotic resistances to the treated cells.

The TargetAMD project will accomplish the first European in-human clinical trial using the *SB100X* and pFAR plasmids. In preparation for the trial, TargetAMD project will define the criteria to be used for non-viral-based gene therapy clinical trials. Specifically, the following criteria that will be defined: (a) safety by encoding the *SB100X* transposase and the *PEDF* gene into pFAR4 miniplasmids); (b) characterisation of electroporation conditions for the integration of pFAR4 plasmids; (c) determination of efficacy of the system by transfecting and transplanting rat RPE and IPE cells subretinally in a rat model of CNV; (d) exclusion of systemic biodistribution of transfected cell or naked genes in a rabbit model; (e) GMP manufacture of pFAR plasmids; (f) preparation of dossiers containing pre-clinical data and application to regulatory authorities for gene therapy clinical trials approval; (g) completion of phase Ib/IIa clinical trials in which RPE cells from 10 nvAMD patients and IPE cells from 10 nvAMD patients will be isolated, transfected and transplanted into the same patients during a single surgical session; (h) dissemination of results to the scientific community and general public; (i) exploitation of newly developed devices and reagents.

2. A description of the work performed since the beginning of the project and the main results achieved so far, (do not exceed 4000 characters)

To accomplish the planned clinical trials, from the start the consortium has pursued the objectives proposed in the grant. The *SB100X* transposase and the *PEDF* gene encoded in free of antibiotic resistance marker (pFAR4) plasmids have been used to transfect low numbers of human IPE and RPE cells. PEDF copy number integrated, *PEDF* gene expression and protein secretion have been analysed to define efficiency of transgene delivery and expression.

Although the *SB100X* system is inherently safer than viral vectors, additional safety features were tested, specifically (1) the use of *SB100X* as mRNA to avoid self-integration; (2) addition of insulator sequences to minimise the risk of transactivation of genes near the integration site; (3) integrate sequences direct into "safe harbours" sites of the genome. Based on the results of these experiments the consortium decided that the final constructs of the medical product would be pFAR4-ITRs CMV PEDF BGH, and pFAR4-ITRs CMV SB100X. A new partner charged with the manufacture of GMP plasmids joined the consortium and has begun the manufacture of pre-clinical and clinical batches. Transfection of 5'000 to 10'000 primary IPE and RPE cells, isolated from biopsies of human donor eyes has been successfully established and a significant number of replicate transfections have been done and additional replicates are being done to prove that the transfection protocol is reproducible for use in patients. In all replicate experiments level of PEDF expression and secretion was determined. Samples for analysis of numbers of integrated copies have been collected and will be done in the very near future. For use in humans it is mandatory that the electroporation buffer is free of animal-derived components and is produced under GMP conditions. A novel buffer, developed for the specific use of TargetAMD, has been shown to be suitable for the efficient transfection of primary human RPE and IPE cells. Likewise it has been necessary to modify the Clinoporator™ to meet the requirements of TargetAMD and since the number of cells to be transfected is small, micro-cuvettes have been developed. The modified Cliniporator™ has all the required approvals and has been installed in the GMP facility of UNIGE.

In vivo, efficacy studies in a rat model of CNV have shown that transplantation of PEDF-transfected cells inhibits neovascularization. Safety of the treatment was evaluated by the lack of growth of transfected cells in soft agar and by the random integration profile of the *PEDF* gene. Completion of a biodistribution study will exclude the risk of systemic distribution of the cells or the DNA.

Standard Operating Procedures were developed to insure standardisation and transparency of the process. Measures have been implemented to ensure sterility of production and high quality of the final product. A Patient Information brochure, an Informed Consent Form and a Case Report Form have been completed. A preliminary version of the Investigator's Brochure and the Investigational Medicinal Product Dossier are available and are being continuously updated with pre-clinical data. Additionally, the consortium met with the Swiss Regulatory Authority to discuss the clinical trial (reference ID no.: 26663-KLV) and develop mandatory in-process and quality controls.

The TargetAMD website (http://www.targetamd.eu and http://www.targetamd-project.eu), published during the first project period, is continuously updated. The TargetAMD logo is used on the website and on all publications, to define corporate identify and increase visibility of the project.

Figure 1 The TargetAMD logo

During the last 18 months TargetAMD consortium has been active at dissemination activities with presentations at numerous international congresses and at local conferences, with student lectures and with information events complemented. A number of peer-reviewed publications have been submitted or are in preparation.

3. The expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project so far), (do not exceed 4000 characters)

The results presented here show that the final goal of the grant, "the successful completion of a phase Ib/IIa clinical trial for the treatment of AMD using transposon-based gene therapy technology" will be accomplished. The first obstacle, namely the transfection of low cell numbers and of freshly isolated low numbers of human RPE and IPE cells has been successfully mastered, using plasmids free of antibiotic resistance markers, a modified electroporation device, especially developed cuvettes and buffers.

Efficacy studies in a rat model of choroidal neovascularization have demonstrated that PEDF-transfected cells transplanted subretinally overexpress PEDF and inhibit choroidal neovascularization, without any observed harmful side effects.

Two phase Ib/IIa clinical trials are scheduled in TargetAMD. In the first clinical trial, RPE cells are harvested from the peripheral retina of nvAMD patients. In the second clinical trial, IPE cells will be isolated from an iris biopsy. In both cases, the cells will be isolated and immediately transfected with the *PEDF* gene and transplanted subretinally in the same surgical session, which will last about 60 minutes. Comparison of the results of both clinical trials will be important, since the harvesting of RPE cells is a traumatic intervention and it is possible that the peripheral RPE carry the defect causing the CNV in the patient whereas IPE cells, a suitable substitute for RPE cells since they share the embryonic origin, morphological and phenotypical similarities with RPE cells, are easy to obtain without any significant trauma to the patient.

The two clinical trials will provide information not only as to the benefit for the treatment of nvAMD, but will provide information on the use and benefits of these novel technologies for the treatment of other diseases. For the nvAMD patient the successful trial will pave a novel and possible life-long treatment for the disease. Current treatments require life-long, frequent, intravitreal injections of anti-VEGFs. And since one injection of Lucentis® or other approved drug is $1'000.00 or more per injection, the costs per patient per year is a minimum of $12'000.00, a substantial burden for industrialised and unaffordable for poor countries. Moreover, the risk of side effects is increased by the repetitive injections, and is a logistic problem for elderly and blind patients. These problems can be solved by one single surgical intervention comprising the harvesting and transplantation of PEDF-transfected cells as proposed by TargetAMD, which would prevent choroidal neovascularization for an extended period of time and possible for the life of the patient. Since the protocol for the clinical trials comprise techniques available to most ophthalmologists it should be possible that the procedure will become routine such that any ophthalmic surgeon would be able to perform it.

Beyond the clinical trial for nvAMD the procedures and protocol developed by TargetAMD will increase awareness of the use of *SB100X* and transposons in general and their possible use in gene therapy clinical trials, thus avoiding the difficulties inherent with the use of viral vectors, especially in conjunction with the ability to deliver genes efficiently to very few cells.