

Claims

1. A method for assessing the potency of a pharmaceutical product assumed to be effective in treating a disorder, preferably a genetic disorder, wherein the disorder results in an impaired functionality of a physiological bodily function of a subject having the disorder, wherein the method comprises:
 - (i) incubating an engineered tissue with the pharmaceutical product, wherein the engineered tissue is or has been modified to be representative for the disorder;
 - (ii) determining at least one physical parameter of the engineered tissue resulting from step (i), wherein said physical parameter is a measure of the functionality of the physiological bodily function, and
 - (iii) determining whether a value of the at least one physical parameter determined in step (ii) meets a predetermined threshold of said physical parameter, which determines whether the pharmaceutical product has potency, wherein, if said value of the at least one physical parameter exceeds the predetermined threshold, potency of the pharmaceutical product is indicated for treating the disorder by at least partially restoring the functionality of the physiological bodily function, wherein the predetermined threshold is determined based on a reference experiment, wherein the at least one physical parameter of an engineered tissue is determined in the absence of the pharmaceutical product or by comparing values of the at least one physical parameter obtained in the absence and presence of the pharmaceutical product, and wherein said reference experiment encompasses a reference condition and/or reference experiment based on an untreated engineered tissue that is or has been modified to be representative for the disorder.
2. The method of claim 1, wherein the predetermined threshold is based on and/or obtained by a reference experiment using a statistical method, and wherein the predetermined threshold is preferably a value deviating from a mean reference value obtained for the at least one physical parameter in a reference experiment by at least between 0.5x and 2.5x standard deviations (SD) and/or by at least 0.5x SD, 1x SD, 1.5x or 2x SD, optionally expressed as % change of the mean reference value.
3. The method of any one of the preceding claims, wherein i) the engineered tissue comprises one or more of heart tissue, heart muscle, muscle tissue, skeletal muscle tissue, neuronal tissue or connective tissue and/or ii) has been genetically edited to comprise a mutation being seen as causative for the (genetic) disorder and/or iii) has

been subjected to a non-genetic intervention to be representative for the disorder.

4. The method of any one of the preceding claims, wherein the pharmaceutical product is an advanced therapy medicinal product (ATMP) such as a gene therapy medicinal product (GTMP), a cell therapy medicinal product (CTMP), or a tissue-engineered product (TEP); a small molecule, a peptide, a protein, a nucleic acid, synthetic RNA, non-coding RNA, a ribonucleoprotein particle (RNP) complex, or a read-through enhancer, preferably the GTMP is a viral vector, a nanoparticle preferably comprising a nucleic acid and/or proteins, preferably comprised in RNP, or a nucleic acid such as an antisense oligonucleotide, and/or wherein the GTMP is a synthetic or a viral vector, such as a retrovirus such as an adenovirus or an adeno-associated virus (AAV) or a lentivirus, more preferably an AAV is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAVrh10, AAVrh74 or any combination thereof, preferably AAV2 or AAV9.
5. The method of any one of the preceding claims, wherein the pharmaceutical product comprises a nucleic acid encoding a protein for gene editing or comprising an expression product thereof such as an endonuclease, e.g., a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN) or a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) nuclease.
6. The method of any one of claim 4-5, wherein the GTMP comprises an endonuclease such as Cas9 or Cas13, preferably *Streptococcus pyogenes* Cas9 (SpCas9), *S. aureus* Cas9, *S. auricularis* Cas9, *S. lugdunensis* Cas9, or *N. meningitides* Cas9, and a guide RNA (gRNA); or comprises a nucleic acid encoding an endonuclease such as Cas9 or Cas13, preferably *Streptococcus pyogenes* Cas9 (SpCas9), *S. aureus* Cas9, *S. auricularis* Cas9, *S. lugdunensis* Cas9, or *N. meningitides* Cas9, and one or more guide RNA (gRNA).
7. The method of any one of the preceding claims, wherein said physical parameter is i) one or more selected from the group consisting of length, mass, time, electrical current, temperature, luminous intensity, and physical parameters derived from the foregoing, ii) is one or more selected from the group consisting of force, movement, electrical current, mass, tissue structure, preferably swelling/condensation, lucidity, or one or more biomechanical parameters, preferably tissue compaction, contraction, stiffness, ultimate strength, elasticity, extensibility, excitability, resilience, and/or toughness, iii) one or more selected from the group consisting of tissue contraction, stiffness, force development, spontaneous beating frequency, contraction time and/or velocity,

relaxation time and/or velocity, force of contraction, contractile force, and one or more multi-electrode array (MEA) measures; preferably measured as pole deflection, Young's modulus, EHM shortening, spontaneous beating frequency, contraction time and/or velocity, relaxation time and/or velocity, force of contraction, twitch tension, resting tension, and one or more from the group consisting of Weighted Mean Firing Rate, Burst Frequency (Hz), Interspike Interval in a burst (ms), Network Burst (NB) Frequency, Interspike Interval in a NB (ms), and NB Duration (s), respectively, and/or iv) not a gene or protein expression level.

8. The method of any one of the preceding claims, wherein i) the engineered tissue is human, from a non-human primate such as macaque or marmoset, from a pig, from a rodent such as mouse, rat or guinea pig, preferably wherein the engineered tissue is human, and/or ii) the engineered tissue comprises cells obtained from a patient, who will undergo a treatment with the pharmaceutical product, or iii) the engineered tissue comprises cells not obtained from the patient, who will undergo a treatment with the pharmaceutical product
9. The method of any one of the preceding claims, wherein the method further comprises the analysis of a biomarker such as gene or protein expression of the biomarker.
10. The method of any one of the preceding claims, wherein the engineered tissue is heart muscle, and i) wherein the at least one physical parameter is force of the engineered tissue and/or movement of the engineered tissue, ii) wherein the one or more conditions comprise that the engineered tissue exerts a force of 0.01 mN or more, 0.05 mN or more, 0.1 mN or more, or 1 mN or more, and/or iii) wherein the disorder is a genetic heart condition, preferably a genetic heart condition selected from the group consisting of hereditary heart disease such as hereditary forms of dilated, hypertrophic, and arrhythmogenic cardiomyopathies or fibroblastopathies as well as inborn errors of metabolism-associated cardiomyopathies, more preferably a condition selected from the group consisting of Duchenne muscular dystrophy (DMD), Noonan syndrome, diastolic cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, long and short QT syndrome, Takotsubo syndrome, a lysosomal storage disorder, titinopathy, and Barth syndrome, or wherein the disorder is a non-genetic heart condition, preferably a non-genetic heart condition induced by simulated neurohormonal and/or pharmacological stimulation, preferably by a catecholamine, an angiotensin, and/or transforming growth factor β , by a drug such as doxorubicin, a tyrosine kinase inhibitor and/or a cardiotoxic drug, by mechanical injury such as crush injury, by thermal injury such as freezing or overheating, by a biophysical

injury such as radiation injury, by infection such as a virus infection with cardiotropic virions such as coxsackie, SarsCov, cytomegaly virus and Dengue virus, or a parasitic infection such as Chagas disease with Trypanosoma cruzi, optionally by a bodily fluid such as a serum from a patient with a heart disease, and/or optionally by cells such as blood derived mononuclear cell cytes, preferably T-cells, B-cells, NK-cells, macrophages from patients with heart disease.

11. The method of any one of claims 1-9, wherein the engineered tissue is neuronal tissue, preferably a neuronal organoid, and i) wherein the at least one physical parameter is electrical current and/or activity, ii) wherein the one or more conditions comprise local and coordinated electrical bursts, and/or clusters, and/or iii) wherein the disorder is a neuronal condition, preferably selected from the group consisting of neurodegenerative diseases, preferably dementia, Parkinson disease, M. Huntington, neuroinflammatory diseases, preferably multiple sclerosis, brain inflammation disorders, preferably meningitis), channelopathy, preferably epilepsy) and psychiatric diseases including autism and schizophrenia.
12. The method of any one of claims 1-9, wherein the engineered tissue is muscle tissue, preferably skeletal muscle tissue, and i) wherein the at least one physical parameter is force or movement, ii) wherein the one or more conditions comprise that the engineered tissue exerts a force of 0.01 mN or more, 0.05 mN or more, 0.1 mN or more, or 1 mN or more, and/or iii) wherein the disorder is a genetic muscular disorder, preferably a genetic muscular disorder selected from the group consisting of Duchenne muscular dystrophy, Facioscapulohumeral dystrophy, Becker dystrophy, Emery-Dreifuss dystrophy, myotonic dystrophy, limb-girdle dystrophy, oculopharyngeal muscular dystrophy, congenital dystrophies, congenital myopathies, myotonia congenita, familial periodic paralysis, and a heritable connective tissue disease such as Marfan syndrome, Ehlers-Danlos syndrome or Loeys-Dietz syndrome
13. The method of any one of claims 1-9 and 12, wherein the engineered tissue is a composition of different types of tissues, preferably a composition of:
 - (i) neurons and skeletal muscle, or
 - (ii) neurons and heart muscle;
 wherein more preferably the composition of different types of tissues forms one or more neuro-muscular junction.
14. The method of any one of claims 1-9, wherein the engineered tissue is heart muscle or skeletal muscle,

the pharmaceutical product is an AAV2 and/or AAV9 viral vector comprising a nucleic acid encoding Cas9, preferably *S. aureus* Cas9, *S. auricularis* Cas9, *S. lugdunensis* Cas9, or *N. meningitides* Cas9, and one or two guide RNA(s) (gRNA), wherein the Cas9 gene is under the control of a muscle-specific promoter such as CK8e or TNNT2,

the disorder is Duchenne muscular dystrophy, and

said physical parameter is force,

preferably wherein the engineered tissue comprises cells obtained from a patient, who will undergo a treatment with the pharmaceutical product,

optionally wherein the engineered tissue is connective tissue, and i) wherein the at least one physical parameter is a biomechanical parameter such as tissue compaction, contraction, stiffness, ultimate strength, elasticity, extensibility, excitability, resilience, or toughness and/or ii) wherein the disorder is (i) a heart disease which presents with cardiac fibrosis, such as a heart failure, preferably of unknown and/or genetic cause, with reduced or preserved ejection fraction, myocardial infarction, a congenital heart disease, a cardiomyopathy with one or more known mutation, age- and/or senescence-induced cardiac fibrosis, a drug-induced cardiac disorder, a metabolic cardiac disorder, a, preferably monogenetic or polygenetic, disorder with contribution of the heart and cardiac fibrosis, a disease involving reactive or replacement fibrosis in an organ such as kidney, liver, lung, and/or skin, etc., (ii) a disorder with impaired scarring and wound healing such as Diabetes mellitus, and/or (iii) a heritable connective tissue disease such as Marfan syndrome, Ehlers-Danlos syndrome or Loeys-Dietz syndrome.

15. The method of any one of the preceding claims, wherein a) the reference (experiment) further encompasses a reference experiment and/or reference condition based on
 - i) an engineered tissue and/or engineered organoid that is derived from ia) a healthy individual and/or ib) well-characterized iPSC representing a healthy wildtype and/or ic) well-characterized iPSC representing a disease genotype and/or id) well-characterized iPSC representing a disease phenotype, and/or
 - ii) an engineered tissue and/or engineered organoid with a permanent genetic repair of a disorder-associated, preferably disorder-causing, mutation and/or
 b) the predetermined threshold is
 - i) determined based on and/or obtained by a reference (experiment), wherein said reference (experiment) is performed in at least one replicate, and/or
 - ii) based on and/or obtained by a reference (experiment) using a statistical method, wherein the reference (experiment) is performed in at least one replicate.