

Human Gene Therapy With Myoblast Transfer

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CELL THERAPY Research Foundation (CTRF) is completing phase II clinical trials of myoblast transfer therapy (MTT, US Patent #5,130,141) on Duchenne's muscular dystrophy (DMD) under an investigational new drug application approved by the US Food and Drug Administration (FDA). MTT is a platform technology of *in vivo* gene transfer, and cell repair and replacement. As such, it is a combined somatic cell therapy and gene therapy. Begun on February 15, 1990, MTT is the world's first human gene therapy.¹

Myoblasts are muscle-building cells endogenous to the human body. Contained within the nucleus of the myoblast are more than 100,000 normal genes that determine cell normality and cell characteristics.

During myogenesis and muscle regeneration, mononucleated myoblasts fuse spontaneously to form multinucleated myotubes, sharing their nuclei in a common gene pool. On establishing neuronal, vascular, and tendon connections, these myotubes will deposit additional contractile filaments and develop into genetically normal myofibers. When this occurs inside a dystrophic muscle, the process of normal myoblast fusion can replace degenerated dystrophic myofibers with genetically normal ones.

When cultured normal myoblasts are injected into dystrophic muscles, they fuse also with dystrophic myofibers, inserting their nuclei to effect genetic repair at the cellular level.² There is no break-in or breakdown of the nuclear membrane like the sperm and the ovum. There is no tetraploidy formation. Normal myonuclei simply take up permanent residence in the dystrophic myofibers that have now turned genetically mosaic, and transcribe the production of the missing protein which, in DMD, is called dystrophin.

Thus, through natural cell fusion, the donor myoblasts transduce all normal genes within their nuclei into the multinucleated dystrophic myofibers. The transfer of genetic material occurs *in vivo* with the myoblasts serve as the source and the vehicles of gene transfer. MTT is therefore a gene therapy, and because myoblasts are cells, it is also a cell therapy.

Myoblasts can be used as an universal gene transfer vehicle by virtue of their ability to transduce full complement of normal genes instead of a single gene. Besides

being in clinical trials of neuromuscular diseases, basic MTT studies have been published on cardiomyopathy,^{3,4} bone degeneration,⁵ anemia,⁶ human growth hormone deficiency,⁷ hemophilia,⁸ and islet allograft rejection.⁹ Conceptually, MTT can find application in type II diabetes and cancer, and other hereditary degenerative diseases.

According to the FDA, gene therapy is defined as a medical intervention based on alteration of the genetic material of living cells, either cells to be administered to humans, or human cells genetically modified *in vivo*. The practice of MTT using normal, not genetically modified myoblasts pertains to the latter category. MTT using transduced myoblasts qualifies for both.

The FDA is concerned about sterility, endotoxins, identity, potency, and purity of cell cultures used in cell therapy. Cell viability immediately before transplants must be shown in view of nonoptimal conditions encountered in transportation and delay. Most cells do not survive when tightly packaged in shipping media.

Myoblasts are readily identified by their abilities to migrate, divide, align, fuse, and to deposit actin and myosin to form sarcomeres.¹⁰ They lose their major histocompatibility complex-class I (MHC-I) antigens after fusion. Mature myofibers do not express MHC-I antigens. Thus MTT subjects do not require life-long immunosuppression.

CTRF myoblast cultures are at least 98% pure. Viability demonstrated immediately before transplants is 98% using Erythrocin B (Sigma), 0.1%, at pH 7.23.

An often-asked question is how long can myoblasts survive if they succeed in engrafting. To this end, the world's first MTT subject was re-biopsied 6 years after receiving MTT. Dystrophin was present in more than 95% of the myofibers of the myoblast-injected muscle but was absent in the sham-injected muscle.¹¹ The result indicates that donor myoblasts survived, developed, and produced

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Myoblast Transfer Therapy

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