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Value from Innovation

WHITE PAPER

Small molecule inhibitors– Unlocking the potential of iPSC-based therapeutics

WITH THEIR GENETIC TRACTABILITY AND VIRTUALLY LIMITLESS CAPACITY FOR EXPANSION, iPSCS OFFER UNPRECENDENTED OPPORTUNITIES FOR PATIENT-SPECIFIC DISEASE MODELING, HIGH-THROUGHPUT DRUG DISCOVERY AND TOXICOLOGY STUDIES, AND REGENERATIVE MEDICINE. DESPITE THEIR PROMISE, CLINICAL TRANSLATION STILL POSES SIGNIFICANT SCALABILITY AND MANUFACTURING CHALLENGES, DEMANDING DEVELOPMENT OF LARGE-SCALE AND COST-EFFECTIVE CELL CULTURE STRATEGIES. FUJIFILM'S NEWLY LAUNCHED COMPLETE CEPT COCKTAIL AND GMP-GRADE Y-27632 ROCK INHIBITOR SUPPORT BOTH UPSTREAM AND DOWNSTREAM PROCESSES, OFFERING COMPREHENSIVE SOLUTIONS FOR ALL STAGES OF iPSC-BASED THERAPY DEVELOPMENT.

Induced Pluripotent Stem Cells – A New Frontier in Therapeutic Development

The ability to directly reprogram terminally differentiated adult somatic cells to a pluripotent state[1] has revolutionized the future of medicine. Like human embryonic stem cells (hESCs),[2] induced pluripotent stem cells (iPSCs) can be expanded in culture indefinitely and differentiated into almost all cell types.[3] More importantly, iPSCs can be derived from any individual patient or healthy subject, and are relatively amenable to genetic engineering, offering reliable and clinically relevant in vitro human models that circumvent the use of animal models or the ethical and immune rejection concerns surrounding human ESCs.

The advent of iPSC technology has introduced endless possibilities for clinical and biomedical applications (Fig. 1). Beyond disease modeling and drug discovery, iPSCs offer an autologous cell source for treatment of devastating degenerative diseases that conventional medicine cannot presently cure, such as ischemic heart failure, diabetes, Parkinson's, Alzheimer's disease, and age-related macular degeneration.[4,5] Emerging applications are also evaluating the utility of iPSC-derived multicellular organoids and organs in disease modeling and regenerative therapy.[5]

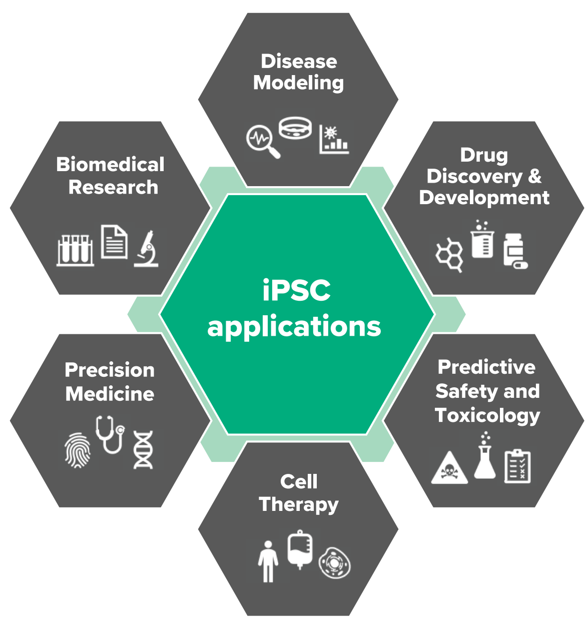

Figure 1. Biomedical applications of iPSCs.

Banking on the future of iPSC technology

iPSC-based therapies have been deemed safe and effective in preclinical in vitro studies and early clinical trials,[6] leading to a steady rise in clinical applications for an expanding range of conditions.[7] In fact, more than 80 clinical trials involving the use of iPSCs for disease intervention, generation of iPSC-based products, or high-throughput screening of new pharmacological candidates have been completed or are currently underway globally (Fig. 2).[6] Clinical translation of stem cell interventions is therefore a major factor driving iPSC demand, and although this flood of clinical trials has resulted in regulatory approval delays, updated regulatory guidelines and internal reorganization initiatives[8,9] are expected to streamline and expedite the approval process, further driving demand.

Additionally, efforts to stem the growing incidence of chronic diseases like cancer, stroke, diabetes, and heart disease[10,11] are relying more and more on the use of iPSCs as research tools. iPSCs offer significant advantages over other available alternatives, including the elimination of animal models and ethical considerations associated with ESCs, which has boosted capital investment in iPSC-related research[12] and further propelled market growth. Indeed, according to industry analysis reports, the global iPSC market is predicted to grow at an estimated ~9.3% CAGR over the next five years[13] and reach $3.57 billion by 2026,[14] with the regenerative medicine sector undergoing the fastest growth.[15]

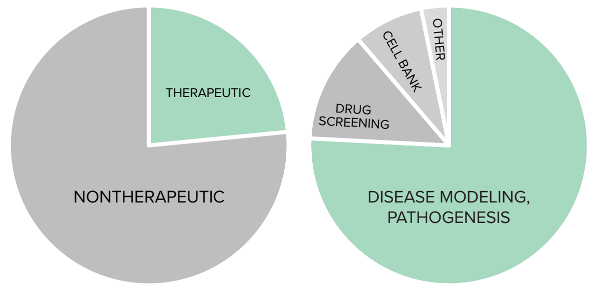

Figure 2. Distribution of global clinical trials involving iPSCs. “Nontherapeutic” studies primarily utilize iPSCs for disease modeling, drug screening, and creating cell banks. “Therapeutic” studies are clinical trials in which iPSCs were (re)transplanted into patients in an effort to develop treatments. (Adapted from Kim et al. 2022, Stem Cell Rev Rep).

Translational prospects and scalability challenges

Despite their immense therapeutic potential, few iPSC-based therapies evaluated in clinical trials have actually translated into treatment strategies.[6] Clinically applied regimens may require billions of cells per dose per patient,[16] demanding large-scale cell line generation, expansion, cryopreservation, and directed differentiation strategies that are safe, reproducible, and efficient.[17] iPSC production remains a long and complex process, however, plagued with significant manufacturing challenges that stem from the same characteristics that make iPSCs attractive disease models and therapies in the first place.

Even though pluripotent cells are capable of unlimited self-renewal, their inherent sensitivity to microenvironment perturbations can lead to poor survival during cell culture and cell manipulations, considerably limiting their scalability.[5] Indeed, routine cell passaging can lead to massive cell death, a fact that has been known for years and that represents a major obstacle to clinical translation of iPSCs.[18] Although significant progress has been made, iPSC culture remains variable and suboptimal,[19] and the lack of efficient protocols is particularly evident in single-cell conditions required for expansion, cryopreservation, or cloning experiments.

iPSCs are adhesion-dependent cells that require cultivation in colonies for maintenance of pluripotency, cell expansion, and subsequent differentiation.[20,21] Conversely, efficient and robust cloning, cryopreservation, and differentiation protocols are best realized in single-cell cultures, where loss of cell-cell and cell-matrix interactions leads to extensive apoptosis, even under favorable culture conditions. Furthermore, the adaptation to animal- and feeder-free conditions, a shift from conventional iPSC reprogramming and culture systems that is gaining traction in the field as a means to address labor, scaling, cost, and safety concerns, can also impact differentiation and apoptosis.[22] Finally, poor recovery of cryopreserved iPSCs due to increased susceptibility to apoptosis is also a significant obstacle to large-scale manufacture of clinical grade iPSCs.[23]

SMALL MOLECULE INHIBITORS – Blocks that propel stem cell-based technology

Bridging the translational gap between preclinical and clinical settings requires that we achieve iPSC production in vastly greater volumes than our present capabilities allow. In addition, success is highly dependent on skill, something that cannot be easily taught or embedded into automated manufacture protocols.

ROCK inhibition for enhanced iPSC survival

This issue has been partly addressed with the inhibition of Rho-associated protein kinase (ROCK), a serine-threonine kinase that regulates cytoskeletal contraction and rearrangement. ROCK activity is involved in a variety of cellular processes including apoptosis, migration, proliferation, and differentiation,[22] and its inhibition promotes stem cell survival.[24] Y-27632, the most effective and selective ROCK inhibitor,[25] was first shown to have beneficial effects on human ESC survival, differentiation potential, and differentiation capabilities in a landmark study published in 2007 (Fig. 3).[26] Subsequent work recapitulated Y-27632 utility in the recovery and passage of iPSCs,[22] and supplementation of iPSC culture media with 10 µM Y-27632 prior to single-cell dissociation has since become standard practice in the iPSC field.

Y-27632 improves cell survival by blocking cell contraction and the ensuing apoptosis- and anoikis-mediated cell death.[27] Y-27632 supplementation can therefore increase single cell survival rate, improve plating and cloning efficiency, support undifferentiated growth of dissociated cells or cells passaged in feeder-free conditions, promote embryoid body formation, and enhance cryopreservation and differentiation processes,[28] making it an invaluable tool for iPSC cultivation.

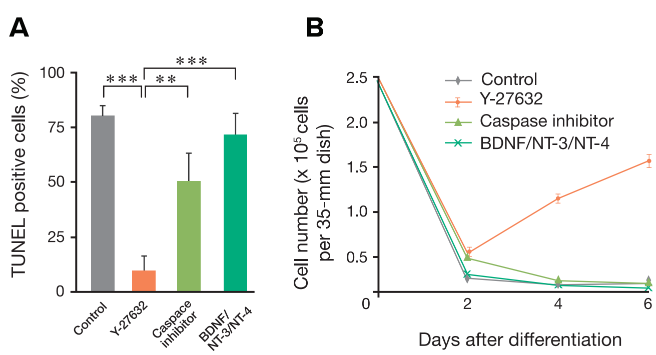

Figure 3. Y-27632 promotes survival of dissociated human ESCs. A, Effects of Y-27632, caspase inhibitor, and neurotrophin cocktail (BDNF/NT-3/NT-4) on percentages of apoptotic cells. **B,** Cell numbers 2, 4, and 6 days after culturing dissociated cells. (From Wantanabe et al. 2007, Nat Biotechnol).

CEPT: the new kid on the block

Although a significant advance, Y-27632-mediated ROCK inhibition does not protect iPSCs from stress mechanisms that can otherwise impact cellular homeostasis, structure, and function.[17] To overcome these challenges, researchers at the National Center for Advancing Translational Sciences (NCATS) developed a four-component cocktail of cytoprotective compounds identified in a targeted high-throughput screen of >15,000 small molecules.[17] CEPT combines Chroman 1[29]– a selective ROCK inhibitor that is more potent than Y-27632, the pan-caspase inhibitor Emricasan, the integrated stress response (ISR) inhibitor Trans-ISRIB, and a polyamine solution to synergistically improve iPSC survival in basic and translational research and downstream applications.

Published results[17,30-33] (Fig. 4) show that the CEPT cocktail is fast acting and has unique cytoprotective properties, overcoming cellular stressors intrinsic to stem cell workflows that induce DNA damage and cause cell death. CEPT supplementation supports the structural and functional integrity of dissociated cells and is therefore particularly useful for applications requiring single-cell isolation, preserving pluripotency, karyotype, and differentiation ability in long-term single-cell passaging and cryopreservation of iPSC lines. Furthermore, CEPT enhances survival of cryopreserved differentiated cells and improves the functionality of derived cell types. Finally, CEPT is more potent and has better target selectivity than Y-27632 and other commercially available reagents (CloneR, RevitaCell, SMC4), providing superior cytoprotective effects at a fraction of the cost.

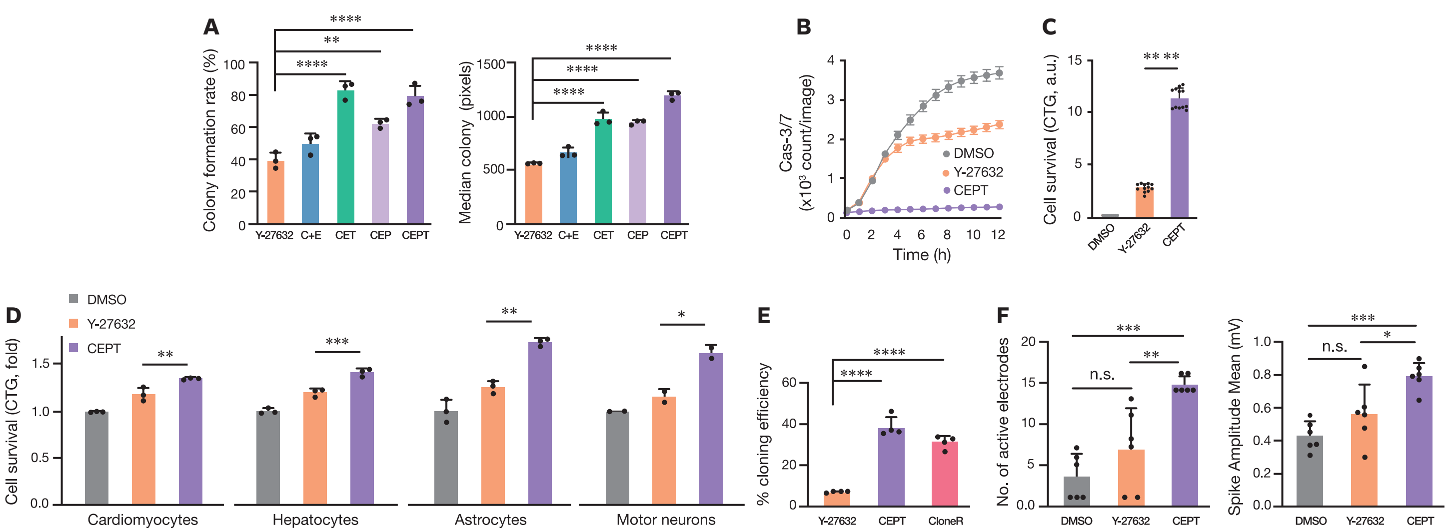

Figure 4. CEPT promotes survival, clonal growth, and enhanced cryopreservation and functionality of human ESCs and iPSC-derived cell types. A, Effect of small molecule combinations on hESC colony number and size. **B**, Apoptosis and **C**, cell survival in cryopreserved hESCs and **D**, iPSC-derived cells. **E**, CEPT promotes single-cell cloning in hESCs. **F**, Electrophysiological characterization of iPSC-derived cardiomyocytes 5 days post-thawing. (Adapted from Chen et al. 2021, Nat Methods).

PERSPECTIVES – Bottlenecks and next steps

The availability of human iPSCs that can recapitulate the donor's phenotype in vitro has transformed biomedical research, with applications in patient-specific disease modeling, high-throughput drug discovery, safety pharmacology, and regenerative medicine. Despite offering unprecedented opportunities, limitations to realizing their full clinical potential persist. One of the most significant challenges to the clinical translation of iPSCs is cost: recent estimates[34] suggest that generating, characterizing, and banking one research-grade iPSC line can cost between $10,000-25,000, while production of GMP-grade iPSC-based therapy suitable for implantation can be much more costly, averaging $800,000 per patient. These costs are even higher for autologous applications that require rigorous process and quality controls, and although it is arguably one of the most advantageous aspects of iPSC-based approaches, the development of autologous therapy can be cost-prohibitive, with most companies opting to produce lines of allogeneic, "off-the-shelf" iPSCs that can be used to treat all patients with a particular disease.

Beyond process complexity, the high costs associated with iPSC manufacture stem primarily from low cell survival, which limits our ability to efficiently scale up iPSC production to clinically relevant volumes without compromising quality or other considerations such as tumorigenicity, heterogeneity, and immunogenicity.[5] The lack of standardization in culture techniques is another major hurdle, and heavy investment in the optimization and automation of iPSC reprogramming and expansion is an emerging key industry trend.

The characterization of Y-27632 and its effects on cell survival and viability marked a turning point in stem cell research. Y-27632 supplementation has now been standard practice in stem cell studies for more than a decade, providing a wealth of knowledge as to its benefits and mechanism of action in the enhancement of cell survival. As cell therapy manufacturers increasingly turn to Y-27632 for efficient development of iPSC-based modalities, FUJIFILM Wako Chemicals U.S.A. Corporation's newly launched, GMP-grade Y-27632 can support scalable workflows for long-term, GMP-compliant iPSC culture and functional downstream differentiation.

For research applications where single-cell dissociation is a requirement, the novel CEPT cocktail may provide additional benefits. Excitement over its cytoprotective capabilities has triggered use in a variety of applications including automated iPSC culture,[30] optimization of embryoid body and organoid models,[33] directed neural differentiation,[31,32] and placental development and function studies.[35] More studies are underway (and needed) to fully assess its utility in translational research.

CEPT's superior potency and target specificity offers a powerful tool to support efficient iPSC culture with wide-ranging implications for disease modeling, drug development, tissue engineering, and regenerative medicine. FUJIFILM Wako Chemicals U.S.A. Corporation's complete CEPT cocktail consists of a mixture of all four components, providing superior cytoprotective effects for safe and efficient iPSC reprogramming, long-term cell culture, single-cell cloning and gene editing, embryoid body and organoid formation, and cryopreservation and cell banking.

At Fujifilm, we strive for continuous innovation that can be leveraged in the advancement of biomedical research and applications. Our comprehensive solutions for upstream and downstream iPSC-based product development are designed to propel iPSC technologies from pre-clinical to commercial manufacture, helping fully realize the clinical potential of iPSC-based interventions and offering promise to patients in need.

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