SOMITE

# Somite Therapeutics

## The current landscape and future promise of cell replacement therapy

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### I. Stem cells and cell replacement therapy

This is an extraordinary time when the repertoire of approaches for addressing human disease and injury has been greatly expanded by modern biomedical science. The promise of stem cell-based therapies in particular has brought new hope to many patients, with novel interventions to a wide range of disorders seemingly within reach.

In spite of the enormous excitement around this approach, it must be noted that cell replacement therapies and harnessing stem cells to address disease have been talked about for a long time. What are the difficulties that have held the field back? What are the specific challenges that have limited the development of this approach in certain settings? And what opportunities has that created that Somite.AI is poised to exploit? To start to answer these questions requires understanding the history of the clinical use of tissue and cell transplant therapies.

The first therapeutic use of stem cells was in the guise of bone marrow transplant. Bone marrow transfusions date back to 1939 when bone marrow was used in an attempt to increase leukocyte and platelet counts in a patient with aplastic anemia. At that stage the bone marrow was just seen as a source of developing blood cells. By 20 years later, it was realized that bone marrow could convey something with longer lasting potential, and in 1958, a bone marrow transplant was used to save 5 workers who had been critically exposed to radiation in an accident in Yugoslavia. This was followed by the pioneering work of Ernest McCulloch and James Till in the 1960's who identified the blood-forming hematopoietic stem cell (HSC), showed that a single HSC could repopulate all the cell lineages of the blood, and predicted that HSCs were self-renewing, a property quickly confirmed by Louis Siminovitch. The enormous subsequent success of bone marrow transplant is largely attributable to the fact that HSCs are self-renewing as well as relatively easily accessible.

Another tissue that shares these properties of self-renewal and accessibility is the skin. In 1975, Howard Green identified adult skin stem cells (SSCs) and showed that a single SSC could give rise to a layer of skin in vitro. By 1979 he had developed the system to the point where lab grown skin could be used to treat burn victims, the first instance where human cells were cultured in a laboratory for therapeutic use. The results were at times dramatic, for example saving the lives of two children from Wyoming, who had each been burned over 95% of their body, by rescuing cells from small unburnt patches.

In spite of these successes more than 45 years ago with blood and skin, achieving similar results in restoring other tissues has not been forthcoming. Stem cells for other adult tissues have proved difficult to identify, purify, maintain and/or amplify in vitro, as exemplified by the search for a source of cells for muscle cell therapy, discussed in depth below. In this context, attention turned to pluripotent stem cells, equivalent to the earliest cells of the embryo, which are capable of ultimately giving rise to all the cell types in the adult. Embryonic Stem Cells (ESCs) were first isolated by Martin Evans in 1981 through culturing mouse blastocysts (embryos isolated after a few cell divisions post-fertilization). It took 17 more years before Jamie Thomson's lab generated human embryonic stem cells (hESCs) (Thomson et al 1998). The time lag between producing mouse ESCs and human hESCs was due to their having quite distinct morphologies and requiring very different culture conditions. This opened the door to a variety of potential cell therapies, and clinical trials were initiated using hESC-derived cells for a range of conditions including spinal cord injury, macular degeneration, Parkinson's disease and diabetes (reviewed in Ilic and Ogilvie 2017).

The use of hESCs presents significant ethical issues in as much as they are derived from early human embryos, destroying them in the process. This concern was eliminated by the breakthrough discovery by the lab of Shinaya Yamanaka in 2006, of a set of factors (Oct3/4, Sox2, Klf4 and cMyc) capable of inducing pluripotency in somatic cells (Takahashi and Yamanaka, 2006). These so-called "induced pluripotent cells" (iPSCs) can generate all the cell lineages of the embryo, just like ESCs.

A year later, two groups building on the by then extensive knowledge of hESCs, generated human iPSCs (Takahashi et al 2007, Yu et al 2007).

The advent of hESCs and hIPSCs has led to an explosion of efforts to develop protocols for directing their differentiation into various cell types of potential clinical utility. It turned out, however, that certain cell types were easier to generate than others. For example, ESCs/IPSCs readily differentiate into neuronal progenitors in vitro. Hence, this approach has been intensely explored as a source of therapeutics for addressing neural injury and degeneration, and neural-based eye disease. Although no stem cell-based therapies are yet available in the market, as of the start of 2022, there were 90 registered clinical trials using hESCs or hIPSCs (summarized in Ilic and Ogilvie, 2022). These include trials to treat ocular disease, neural degeneration, spinal cord

injury as well as cardiovascular disease, diabetes, and cartilage defects. Moreover, many of the early results of these trials have been extremely encouraging.

While stem cell-based cell therapies are thus on the cusp of becoming an important therapeutic modality, it must be noted that as of this date, there are still no cell replacement therapies in the market. A number of factors have held up the rapid implementation of this approach. These issues can be divided into two categories, those that are generic which have to be controlled in any use of stem cell-derived therapeutic products, and those specific to particular indications and/or cell lineages.

There are two major concerns that need to be confronted for implementing any stem cell-based cell replacement therapy: tumorigenicity and immunogenicity. While a strength of using stem cells is the ability to proliferate indefinitely, providing a nearly unlimited supply of transplantable cells, this same property raises the risk of tumor formation after transplantation.

The first step towards mitigating against this is to develop highly efficient procedures for directing differentiation. Often the protocols take the stem cells through a series of steps under different culture conditions to achieve this. While the length of time and multiple conditions involved could be viewed as negatives from a manufacturing standpoint, they have an enormous advantage in assuring that no ESCs or iPSCs remain in an undifferentiated state by the end of the process. Thus, in the very first iPSC-derived clinical cell transplants study, providing iPSC-generated retinal pigment epithelial cells to macular degeneration patients, the transplanted cells showed a greater than 95% purity and no tumorigenicity, either in preclinical testing or in transplanted patients (Mandai et al 2017). In cases where the differentiation protocol itself does not result in enough purity, differentiated cells can be selected with lineage-specific antibodies, as has been done in preparation of corneal epithelial cells (Hayashi et al, 2017), dopaminergic neurons (Kikuchi et al 2017) and cardiomyocytes (Sougawa et al 2018). If this is not sufficient, a “suicide gene” can be introduced as a safeguard that can be triggered if tumorigenic complications arise (Kojima et al 2019).

Taken together the issue of tumorigenicity is one that needs to be closely assessed in preclinical models and carefully monitored in any human studies. For example, in a preclinical safety study of human ESC-derived dopaminergic neurons for use in treating Parkinson’s Disease, 70 rats were transplanted at the maximum feasible dose, along with animals of controls given vehicle or undifferentiated ES cells. Animals were assessed, at various time points up to 39 weeks, for mortality, body weight, organ weight, food consumption, behavioral and motor functionality, and histopathology (Kirkeby et al 2023). Notably, no tumor formation was observed in the experimented animals, although massive teratomas were observed following ESC transplant.

As this study indicates, the issue with potential tumorigenicity, is manageable and can be carefully addressed at the preclinical phase. This has led to the approval of the current 90 stem cell-based cell replacement trials noted above. It is moreover

important to note that in all these ongoing human trials there has not yet been a single report of a tumor arising from the transplanted stem cell-derived cells.

The second general problem confronting stem cell-derived transplantation is immunogenicity. In principle, the invention of iPSC technology provided an unprecedented opportunity to actually give patients cell replacement therapy using their own, genetically identical cells, a so-called "autologous transplant." Animal models show that this approach can work. For example, autologous clinical-grade patches of iPSC-derived retinal pigment epithelial cells engraft without immunoreaction in both rodents and pigs (Sharma et al 2019). However, taking such an approach to humans is enormously problematic at a practical level. In the first place, the financial cost of creating, testing, gaining approval and manufacturing a new iPSC line for each patient is prohibitive. Moreover, the time investment involved in creating a new line for each patient would also be an unrealistic investment. This is especially true in cases where the iPSCs and their derivatives are needed acutely, such as in generating cardiomyocytes to treat heart failure.

In the absence of autologous cells, stem cell-based therapies are a novel form of allografts which currently, like organ transplants, would require patients to undergo life-long immunosuppression. While the field of clinical immunology has come a long way in the last decades, with much improved combinatorial drug protocols, patients on immunosuppression still face serious side effects and susceptibility to infections. Some tissues do appear to be immune-privileged, such as the brain, spinal cord, retina and cartilage (Carson et al 2006, Taylor 2016). Patients transplanted for indications involving these tissues can easily be weaned from immunosuppression. For example, Parkinson's patients have been successfully weaned from immunosuppression after allogeneic fetal neural stem-cells, with their grafts retained for more than 20 years after immunosuppression was terminated (Li et al 2016).

For most indications, however, long-term immunosuppression remains a deterrent, limiting use to only the most severe disease settings (for example if stem cell-based muscle replacement therapy were an option, it might be embraced even with immunosuppression by end-stage patients with muscular dystrophy, but not by patients dealing with incontinence from sphincter muscle weakness). There are currently at least two approaches one can consider to overcome the immunogenicity issues in allogeneic settings, and thereby broaden the utility of stem cell-based approaches.

One way to reduce tissue rejection is through HLA haplotype matching. This is a current standard of care approach for hematopoietic stem cell (i.e. bone marrow) transplant. There are world-wide registries with millions of registered potential donors, allowing immunologically compatible donors to be matched for the vast majority of patients. However, to have that scale of diversity in prepared iPSCs, to provide thousands of perfect HLA matches, is not feasible. The best option for HLA matching

thus seems to be to focus on the most common HLA haplotypes. For example, judicious selection of just 10 iPSC lines, chosen to be homozygous for the most common HLA types identified in 10,000 British individuals would provide a complete match for 37.7% and a beneficial partial match for 67.4% of the UK population (Taylor et al 2005). HLA matching has been tried with at least some success in cell therapy. For example, HLA matching decreased immune rejection of cardiomyocyte transplants, although some immunosuppression was still needed for the initial engraftment (Kawamura et al 2016).

An alternative approach is to construct iPSC or ESC lines where gene editing is used to try to make the cells invisible to the host immune system, an approach called "cloaking." In principle, all Class I MHC, including HLA-A, HLA-B, and HLA-C can be inactivated by removing their common subunit beta2 microglobulin. Similarly, Class II MHC, HLA-DP, HLA-DR and HLA-DQ can all be silenced by deleting their common trans-activator CIITA. This approach is complicated because loss of all Class I MHC leads to cell lysis by natural killer T-cells (Ichise et al 2017). Ways to potentially overcome this might include introducing a chimeric molecule fusing parts of the non-classical MHC Class I protein HLA-E to beta2 microglobulin (Gornalusse et al 2017), or leaving one intact copy of HLA-C (the least critical for immune rejection) (Xu et al 2019). Overall, one would have to consider cloaking as extremely promising, but not yet reduced to practice. Nonetheless, with multiple companies (Sana, Clade TX, Pluristyx…) and academic researchers working on it, and the enormous motivation of opening the door to transplantation free of concern for rejection, this is likely to be a usable approach in the not too distant future.

Given that the problem of tumorgenicity seems manageable, and that paths are open for dealing with immunogenicity, the current excitement around stem cell-based cell replacement therapy is more than justified. Why, then, are clinical trials exploiting this approach so narrowly focused (most involving various neural cell types to address neural degenerations, spinal cord injury or ocular disease)? The answer lies in the fact that pluripotent stem cells have a propensity to differentiate into neural lineages. Coaxing them to form other cell types has been far harder than anticipated. For example, it took more than two decades of intense efforts to derive a protocol for generating functional pancreatic insulin-producing islet cells from iPSCs (Pagliuca et al 2014). Part of this problem has been that in many if not most cases, stem cell differentiation protocols have been developed empirically through trial-and-error studies. This is in contrast with the approach taken by Somite.AI (discussed in more detail below), which utilizes a unique combination of harnessing knowledge of the differentiation pathways that generate cell lineages in the embryo, together with powerful machine learning tools to accelerate and refine differentiation protocols. This approach has given Somite.AI access to a range of clinically relevant cell types that have not previously been available. As an illustrative example, we focus on the

generation of muscle stem cells (satellite cells), for use in therapeutic settings such as treating muscular dystrophies.

## II. Cell therapy and restoration of muscle function

Duchenne Muscular Dystrophy (DMD) is the most common (1 in 5000 male births) and one of the most severe forms of muscular dystrophy; characterized by progressive weakness of limb and trunk muscles and the diaphragm (Emery, 2002). Most patients die by their 30s from cardiac or respiratory failure. DMD, and the milder Becker Muscular Dystrophy (BMD) are both caused by mutations in the structural muscle protein dystrophin (Hoffman et al 1987). The loss of dystrophin in myofibers causes a failure in membrane integrity (Schmalbruch, 1975), among other cellular defects, ultimately leading to cycles of degeneration and attempted regeneration accompanied by inflammation and fibrosis. A key to a disease modifying treatment would therefore appear to be restoring production of dystrophin in the affected muscle cells. Approaches explored to accomplish this include gene therapy using viral or non-viral vectors to deliver a version of the dystrophin gene to myofibers (Bengtsson et al 2016). Alternatively, attempts have been made to develop methods to restore activity of mutated dystrophin genes by altering transcription/splicing (exon skipping) or translation (stop codon suppression) (Verhaart and Aartsma-Rus 2019), or by correcting molecular defects by gene editing (Nelson et al 2017). However, in spite of wide-spread excitement for their potential, gene therapeutic approaches have thus far been ineffective in altering the course of the disease due to problems in delivery, efficiency, and other issues (reviewed in Elangkovan and Dickson 2021).

Cell-based therapies offer an attractive alternative to gene therapy, especially in the context of muscle disease. Because skeletal muscle is a syncytium, i.e. a multinucleated fusion of multiple myoblasts, it has long been envisioned that healthy myogenic cells could be fused into diseased muscle fibers to restore function. Indeed, in a sense muscle cell therapy can be viewed as a gene delivery system for introducing good copies of the dystrophin gene to the DMD muscles. But to be effective in the long run, the cell therapy needs to go one step further and populate the stem cell niche, to provide long term stability to the tissue with further rounds of repair and renewal.

A cell type with the potential to do this is the muscle stem cell, or satellite cell. These cells are actively maintained in a quiescent state associated with mature muscle, but quickly begin to divide following muscle injury, giving rise to proliferating myoblasts that differentiate and repair the damaged tissue, as well as undergoing self-renewal to maintain the stem cell population (Scharner and Zammit, 2011; Cheung and Rando, 2013; Zammit et al 2004).

Using model systems, satellite cells have been explored extensively in a transplantation context, and have proven to be exceedingly effective at repopulating the stem cell niche and providing an ongoing source of functional myoblasts (Collins et al 2005;

Marg et al 2014; Xu et al 2015). These cells have extraordinary proliferative potential. Indeed, a single adult mouse satellite cell can give rise to over 50,000 myoblasts in just four weeks (Sacco et al 2008).

Thus, satellite cells are, in principle, ideal candidates for cell-based therapy in treating DMD. In practice, however, the major problem has been in finding an adequate source of such cells. The issue is that while satellite cells can be isolated from muscle biopsies, they are a relatively rare population relative to the muscle fiber nuclei and myoblasts. Thus, to obtain sufficient quantities for therapeutic use, the satellite cells need to be expanded in vitro. Unfortunately, satellite cells rapidly lose their regenerative potential when grown ex vivo (Charville et al 2015, Montarras et al 2005).

A solution to the problem came from the generation of satellite cells from iPSCs, using the breakthrough technology upon which Somite.AI is founded (Chal et al 2015, Chal et al 2016) (discussed more fully below). Not only does this method allow differentiation of vast numbers of satellite cells at a high purity, but in addition – because they are produced by the same differentiation trajectories as are followed in the embryo – they are created in conjunction with the support cells that maintain them in their niche during fetal development. Thus, unlike satellite cells isolated from mature muscle, these stem cell-derived satellite cells do not lose their potency while growing in vitro.

The ability to produce satellite cells in hand, the next question becomes how to deliver them to the defective muscle tissue. Systemic delivery is appealing, as it could in principle target all the muscle fibers in the body. However, systemic approaches have proven extremely challenging. Filter organs efficiently remove cells delivered intravenously before they can reach the muscles. While this can be circumvented by arterial injections (Sampaolesi et al, 2003), to be therapeutic, satellite cells have to make their way through the vascular wall (Gerli et al 2019) and home to the muscle tissue (Torrente et al 2003). Molecules and pathways are being explored to address each of these steps. But even if there is success in overcoming these barriers, it remains questionable whether it is technically and physiologically feasible to deliver a high enough dose of injected satellite cells to restore function to muscle throughout the body of DMD patients.

The alternative to systemic delivery is to directly target specific muscles by localized injections. This approach, effectively tattooing the target muscle with multiple injections closely spaced according to a grid pattern, has been pioneered by Jacques Tremblay and colleagues. Carried out prior to the breakthrough advances allowing production of functional satellite cells, a series of clinical trials were established using allogenic myoblasts expanded in culture (Partridge 2002; Mendell et al 1995; Gussoni et al 1992; Karpati et al 1993). Myoblasts are at a later stage of differentiation than satellite cells. Although donor myoblast-derived dystrophin was transiently produced in these studies, there was inconclusive functional improvement and eventual loss of the transplanted cell lineage due to a lack of self-renewal. Nonetheless, effective protocols

were established for initial cell delivery and appropriate immunosuppression protocols were established (Skuk et al 2006).

One particularly informative “N of one” study involved a pair of identical twin girls carrying a DMD mutation. Through random X-inactivation, one twin was severely symptomatic (the X chromosome carrying the wild type allele of DMD being inactivated throughout most of her muscle tissue), while a monozygotic twin with the opposite X-inactivation pattern was asymptomatic. Myoblasts from the healthy twin were transplanted into muscle of the affected twin. Because immunosuppression was not required, the long-term effects of the therapy could be assessed. While long term efficacy could not be asserted, for the reasons discussed above, there was some evidence for a higher volume increase on the grafted side and long-term survival of dystrophin-providing cells. Moreover, long term safety of the procedure could be confirmed 20 years after this treatment (Hogrel et al 2013)

It must be noted that these direct injection protocols have focused on a very limited set of small muscles such as those of the hand, and as such do not represent a potential curative treatment. There is nonetheless a compelling need for such a therapy, and indeed if successful it would be transformative for the DMD patients. The muscles of the hand are among the last to be lost during the progressive course of DMD. Thus, in end-stage patients, wheelchair bound, without use of their voice, their ability to grip and manipulate a joystick or to tap out messages using a keyboard is their last tether allowing them to move their body, feed themselves and communicate with the outside world. When the hand muscles finally atrophy, they are completely cut off. And with improved life-prolonging therapies, more and more DMD patients are surviving well into their thirties and suffering this heartbreaking existence. Moreover, successful restoration of grip strength in these individuals would serve as a powerful proof-of-principle, encouraging targeting of (for example) larger arm muscles. Additionally, such successful use of satellite cells in cell replacement therapy would give powerful support to further development of their use in other clinical indications, from restoring sphincter strength in cases of incontinence to treating volumetric muscle loss following catastrophic injury.

The use of satellite cells in cell replacement therapy is just one example of the wave of stem cell-based therapies poised to have a transformative impact on a wide range of patients. As discussed above, most of the vanguard of such therapies have focused on neural indications, simply because iPSC and ESC lines have proven particularly easy to differentiate in that direction. To fulfill the enormous promise of the approach to have a much wider clinical impact requires new approaches to direct stem cell differentiation. This is where Somite.AI comes in.

## III. Somite.AI and the future

The revolutionary approach taken by Somite.AI, to produce a range of specialized cell types for cell replacement therapy, is grounded on three critical insights. First, there is

a unique cell type -the somite cell- found within the developing embryo that has the capacity to give rise to a large number of therapeutically relevant cell types (muscle cells, skeletal cells, tendon cells, brown fat cells, etc.), and is in fact poised to do so. Since the somite cells can be relatively easily nudged to differentiate into each of these lineages, being able to produce a large quantity of somite cells at high purity would provide an extraordinary platform for addressing many different clinical indications. Second, developmental biology has matured as a science to the point where we now have detailed information on how cell types arise throughout embryogenesis. Of particular note, we now know the identity of key genes ("markers") uniquely expressed in key cell types, such as somite cells, their derivatives, and intermediate cell types on the path to becoming these cells. These genes, or reporters based on them, can be used to unambiguously identify cells during their differentiation and can form the basis of assays for optimization. Of equal importance, we now know in intricate detail, many of the protein signals that direct these different cell differentiation pathways in the embryo. This knowledge gives Somite.AI an enormous advantage as the same signals can be used to recapitulate embryonic events in vitro, in a step wise progression, to generate the somite cell platform and to exploit it to produce cell types required for therapies. Third, the revolution in Artificial Intelligence can be brought to bear to attain a deeper understanding of these embryonic events and to utilize them to refine our knowledge of cell transitions in the embryo and to optimize the use of this information to more efficiently and precisely direct cell behaviors in vitro. In particular, obtaining and analyzing data on the set of genes active in individual cells within the embryo, with both spatial and temporal context provides a "digital twin" of the embryo – a digital contextualized representation of the developed processes that, among other things, generates all the cell types in the body. The game-changing advantage of exploiting a digital twin of the embryo is explored in depth in a second, accompanying white paper. Here we address, in a bit more depth, the nature of the somite as a platform, and the ability to access it through developmental biology.

One way to conceptualize the derivation of the cell lineages in the embryo, ultimately producing all the cell types of the adult, is as a branching tree. As the base of its trunk is the pluripotent stem cell (equivalent in the in vitro ESC and iPSC cell types). This stem cell can give rise to all the cell types higher up in the tree (branches, twigs and leaves). With each branching event there is a further restriction of competency, such that more restricted progenitor cell types can only produce a subset of possible cell types. Thus somewhere up the tree is a neural cell branch. At its fork from the trunk is a neural progenitor that can give rise to all types of neurons, but no longer to other cell types. A further branch might represent a neural retinal progenitor, capable of yielding the neural contribution to the retina, but not the brain. Ultimately, individual leaves would represent specific retinal cell types such as rods or cones. In this context, at a powerful branch point near the base of the embryonic tree, lies the somite progenitor cell. Above it are branches to a broad set of distinct cell types (ranging from skeleton to

brown fat) that would seem to have little to do with each other, except for the critical fact that they all branch off the somite cell.

The transitions at the base of the tree, between the pluripotent stem cell and the somite cell, are well understood. The pluripotent stem cells ("epiblast cells" in the embryo) marked by expression of genes such as Nanog, yield three separate lineages, the future ectodermal, mesodermal, and endodermal cell types. The mesodermal progenitors, marked by expression of brachyury, generate several subsequent lineages including presomitic precursors expressing Msgn1 and Tbx6. These early steps are orchestrated by signals produced by surrounding tissues in the embryo, most notably members of the Wnt and Fgf pathways, and both positive and negative effectors of the Bmp pathway. These same signals, when applied in the correct sequence and relative concentration, can direct formation of Tbx6/Msgn1-expressing presomitic precursors from iPSC, or ESCs, in vitro (reviewed in Pourquie et al 2018).

Once the presomitic cells are established as a mesenchymal cell population within the embryo, they have to be transformed morphologically into a series of round epithelial structures, the "somites" forming two parallel columns running anterior to posterior (head to tail) on either side of the neural tubes. These future segments of the embryo (for example producing the vertebrae of the spine) are produced sequentially from presomitic mesoderm. The mechanism by which this occurs involves the oscillatory expression of many genes, with clock-like precision, including members of the Fgf, Wnt and Notch pathways. The pattern of gene expression generated by these oscillators takes the form of a wavefront progressing from posterior to anterior. As the wavefront reaches presomitic cells in a permissive state, they undergo a mesenchymal to epithelial transition and thereby pinch off a block of anterior presomitic mesoderm to form a new somite boundary (reviewed in Pourquie 2018). The landmark discovery of the first known oscillatory genes in the presomitic mesoderm, and mechanistic insights that discovery provided into the sequential generation of somites, conducted by Somite.AI co-founder Olivier Pourquie (Palmeirim et al 1997) is considered one of the most important breakthroughs in modern developmental biology. More recently, in an elegant series of experiments that set the stage for generating the somite-based platform upon which Somite.AI is based, the Pourquie lab showed that the conversion of presomitic mesoderm to somites can be recapitulated in vitro, through the same oscillatory gene pathway, starting with either mouse (Chal et al 2015) or human (Diaz-Cuadros et al 2020) pluripotent stem cells.

The reason that the ability to access somite cells is such a powerful platform is that they can directly differentiate into so many critical cell types. It has long been known that the embryonic somite ultimately splits into three morphologically distinct components, giving rise to skeletal tissues, muscles and dermis, and endothelial cells (reviewed in Christ and Ordahl, 1995). More recently, it was discovered that brown adipose tissue is actually a sister lineage of muscle cells (i.e. has a common precursor) and that it, too, originates in the somite (Seale et al 2008; Sebo et al 2018). In a similar

way, it was found by Somite.AI Cofounder Cliff Tabin that the progenitors of the tendons and ligaments also originate in the somite as a sister lineage to the skeletal precursors (Brent et al 2003). Finally, while it would perhaps seem obvious that the precursors that form the muscle in the embryo would arise from the same cells as the satellite cells that maintain the muscle in the adult, this was, at one point controversial, with claims also being made for satellite cells originating in the bone marrow or blood vessels (De Angelis et al 1999; LaBarge and Blau, 2002). Definitive demonstration of a somitic organ for the critical satellite cells was provided by fate mapping studies carried out in the laboratory of Somite.AI Cofounder Cliff Tabin (Schienda et al 2006).

Importantly, when the somite cells are first specified, and the epithelial somite forms in the embryo, the individual somite cells are not committed to becoming any special cell type (Aoyama and Asamoto 1988). However, cells in different quadrants of the somite are quickly exposed to distinct signals emanating from surrounding tissues, such as Wnt from the overlying dorsal ectoderm and dorsal neural tube, Shh and Bmp antagonists from the notochord and ventral neural tube and Bmps from the lateral plate mesoderm (reviewed in Christ and Scaal 2008). It is the combination and differential concentration of these factors that specify the somite cells to different cell fates. And, as we have seen in the case of producing muscle cells, these fate decisions can be recapitulated in vitro with somite cells derived from ESC, or iPSCs.

Thus, detailed knowledge of these cell types, cellular transitions, and morphogenic cues that guide them in the embryo allow the logical development of a road map for similarly manipulating cells in vitro. Not only is this approach more powerful than empirically deriving protocols by trial-and-error, the protocols that are generated in this manner are likely to be more efficacious and yield higher purity of desired cell types because they are based on unlocking the inherent potential of embryonic cells, using the cues they evolved to use as guides along their normal developmental trajectories.

Gaining an even deeper knowledge of these critical embryonic developmental pathway, utilizing an AI empowered digital twin, will allow development of protocols modulating cell differentiation pathways with ever-greater precision. Thus, bringing together proprietary ability to generate the somite cell platform, deep knowledge of embryonic development, and cutting-edge AI technology, Somite.AI is in a truly unique position to develop cell products to lead the wave of bringing cell replacement therapies to alleviate human suffering.

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