

# Cell implantation therapies for Parkinson's disease using neural stem, transgenic or xenogeneic donor cells

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## Abstract

A new therapeutic neurological and neurosurgical methodology involves cell implantation into the living brain in order to replace intrinsic neuronal systems, that do not spontaneously regenerate after injury, such as the dopaminergic (DA) system affected in Parkinson's disease (PD) and aging. Current clinical data indicate proof of principle for this cell implantation therapy for PD. Furthermore, the disease process does not appear to negatively affect the transplanted cells, although the patient's endogenous DA system degeneration continues. However, the optimal cells for replacement, such as highly specialized human fetal dopaminergic cells capable of repairing an entire degenerated nigrostriatal system, cannot be reliably obtained or generated in sufficient numbers for a standardized medically effective intervention. Xenogeneic and transgenic cell sources of analogous DA cells have shown great utility in animal models and some promise in early pilot studies in PD patients. The cell implantation treatment discipline, using cell fate committed fetal allo- or xenogeneic dopamine neurons and glia, is currently complemented by research on potential stem cell derived DA neurons. Understanding the cell biological principles and developing methodology necessary to generate functional DA progenitors is currently our focus for obtaining DA cells in sufficient quantities for the unmet cell transplantation need for patients with PD and related disorders. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Dopamine; Fetal cell; Parkinson's disease; Regeneration; Stem cells; Transplantation

## 1. Introduction

The relatively new concept of replacing large numbers of degenerated neurons by implanting new cells into the adult brain has created a complementary therapeutic strategy to that of traditional pharmacological therapies for Parkinson's disease (PD). The specificity of cellular degeneration which occurs in PD (DA neurons of the SN), and the relatively major synaptic target region of these degenerating DA cells (the caudate, putamen and SN), have made PD the most accessible therapeutic application for neural cell implantation methodology.

Early clinical transplantation studies involved autologous transplantation of catecholamine-containing adrenal medulla cells [1,2]. The absence of objective reductions of PD signs, the low adrenal medulla graft survival and the reported morbidity of patients reinforced the scientific rationale for using fetal neural donor cells instead. Cell implantation for PD using fetal DA cells is likely to improve

greatly by scientific and technical advances. The development of brain cell transplantation with embryonic neurons and glia is innovative both from a technical and biological standpoint and will require much work to optimize. The scaling up of this method from rodents to primates has proved very challenging; particularly in obtaining an acceptable, abundant and reliable cell. In the initial series of clinical pilot transplant experiments performed in Europe, the first two PD patients did not show a meaningful recovery. Parallel technical and cell dose enhancements produced dramatically better results in the next two patients receiving unilateral fetal VM suspensions. MPTP-exposed patients received VM DA cell suspensions bilaterally into the striatum and this caused motor improvement in association with increased fluordopa uptake [3]. Recent data from the studies of Lindvall and colleagues indicate DA cell survival in patients for almost a decade after surgery, with meaningful clinical improvement [4]. The transplantation of non-dissociated human VM tissue pieces has also provided benefits to many patients [5,6]. In this series of transplantation studies carried out by Olanow and colleagues in the US, autopsy from two bilaterally transplanted (6.5–9 week human fetal

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VM) patients who died 18–19 months after surgery showed over 200,000 surviving DA neurons, which reinnervated about 50% of the right putamen and 25% of the left putamen [7]. Electron microscopy revealed axo-dendritic and occasional axo-axonic synapses between graft and host, and analysis of TH mRNA revealed higher expression within the fetal neurons than within the residual host nigral cells [7]. Autopsy of another patient in this surgical group showed over 130,000 surviving DA neurons, reinnervating almost 80% of the putamen [8]. Notably, both patients had shown major improvements in motor function and increases in fluorodopa uptake in the putamen on PET scanning.

An alternative source of fetal donor cells for clinical cell implantation therapy for neurodegenerative disease is xenogeneic. The remarkable homogeneity in cellular (neurons and glia) basic structure and function suggested that even discordant mammalian species (rodent into non-human primates) could effectively replace local synaptic function after cell loss in the adult brain [9,10]. Such across-species cell transfer (xenotransplantation) allows a more standardized acquisition of larger quantities of appropriate fetal tissue than from human abortions. The immunological reaction of complement activated rejection and T-cell mediated responses leading to rejection of xenografts can in many ways be inhibited by immune suppression [11]. Transplantation studies in animals have shown survival, function, and afferent/efferent connections of xenogeneic cells when transplanted into animal hosts [12,13] (and see reviews [14,15]). In the first pilot-clinical trial, the transplantation of E27 porcine VM into the caudate and putamen on one side of the brain of twelve immunosuppressed PD patients produced some clinical improvements [16]. The overall results indicated that the scaling up problems, also seen with human fetal cells, were significant, further compounded by more vigorous immunological responses in primate and human hosts compared to laboratory rodents. One patient from this study died seven months after surgery from a pulmonary embolism; histological analyses using species-specific markers showed porcine neuron projection axons and forming synapses in the host brain. All three identified transplant sites contained DA neurons (a total of 630 DA neurons), and non-DA neurons expressing pig-specific neurofilament [17]. Pig glial cell, including astrocytes also survived in the patient's brain. Microglial and T-cell markers showed low reactivity in and around the pig cell graft perimeter.

## **2. The scientific foundation of cell implantation therapy for Parkinson's disease**

Basic research involving cell implantation has made it abundantly clear that biological cell replacements strategies can provide the basis for reconstruction and repair of damaged or dysfunctional neuronal connections of the damaged or disease afflicted adult brain [18]. Functional effects of intrastriatal grafts of fetal DA cells have been

illustrated in a range of animal behavioral tests [19–21]. The behavioral effects observed are dependent on the survival of DA neurons within the striatum, since grafting of other tissue produces no behavioral effects [22,23], and removal of transplanted tissue [24] or immune rejection of transplanted neurons [25] reverses transplant-induced behavioral recovery in animal studies. Embryonic day (E) 12–17 fetal rat tissue [26], pig E 27–29 [13] and 6.5–9 weeks old human fetal tissue [5] ventral mesencephalic (VM) donor tissue neuronal exhibit survival and functional effects when transplanted into the adult dopamine depleted striatum. The minimum number of surviving transplanted DA neurons required for behavioral effect in rodent animal models is approximately 100–200 [12]. Using current micro-dissection techniques and cell preparation, only 10% of the transplanted VM cells are phenotypically DA, and only 1–20% of these DA neurons survive implantation depending on trophic and immunological factors [7,17,27–32]. Therefore as many as 10–15 fetal VM per patient may be required for sufficient survival and adequate DA synapse replacement [33].

Factors that are important for maturation and connectivity of DA neurons during normal ontogeny likely also influence development and integration of grafted embryonic tissue when placed in an adult host brain. Current methodology for fetal cell implantation in animal models and patients includes the transfer of numerous types of fetal neuronal and glial progenitor cells. Thus, the implanted neurons are transferred into the host brain with their own contemporaneous glial and angiogenic factor releasing cells, thereby providing a milieu that may contribute to the observed normal cell autonomous development of transplanted fetal VM cells. Adding appropriate trophic factors to fetal cell preparations can enhance survival and growth of implanted DA neurons into animal models of PD [28,34–39]. The ability of fetal neurons to be placed into an ectopic region of an adult brain, survive, and extend neurites within this region is remarkable. The functional effects of VM transplants into DA-depleted striatum is often correlated with degree of striatal reinnervation [26,30]. However there is some limitation in the ability of the transplanted neurons to extend neurites in the adult brain. Even though the graft-induced elevations in tissue DA concentrations are substantial [40], values taken distant from the graft suggest that reinnervation of the whole striatum does not occur. The hypothesis for this sharp decline in density of DA fiber outgrowth is that age-dependent characteristics within the host brain alter outgrowth, since extensive outgrowth can be achieved when transplanted into immature (neonatal) host brain. Expression levels and patterns of adhesion molecules expressed by mature host brain are thought to be the culprits of this reinnervation-inhibitory effect. Allogeneic cell implantation into immature host brain shows robust neuronal and glial migration away from the transplant site and a high degree of integration and target-directed neurite outgrowth [18]. In contrast, fetal neural cells transplanted

into mature brains show neuronal reaggregation around the implant site and less extensive axonal outgrowth into host brain, suggesting an age-dependent increase in inhibitory or decrease in growth-promoting processes. Clearly, both promoting and repulsive host factor and substrate activities influence axonal guidance and extension of transplanted developing neurons [18].

Data arguing against any absolute outgrowth-inhibitory properties of adult brain come from studies showing long-distance and target-specific axonal growth from human embryonic transplants into adult rat brain [41], as well as from porcine embryonic transplants into adult rat brain [13]. The species-specific markers used in our studies of fetal porcine transplants into adult immunosuppressed rat brain allowed comparison of donor glial fiber and donor axonal growth in different host brain regions, demonstrating their distinct trophic characteristics. Target zones in adult host gray matter were selectively innervated by embryonic donor axons normally destined to form synapses there, whereas donor glial fibers grew irrespective of any target orientation within white matter tracts [13]. Xenogenic pig axons branched profusely in gray matter target region and only rarely penetrated or crossed white matter tracts. DA fibers from transplants placed into the SN were found coursing up toward the striatum through myelinated fiber bundles, then branching into host gray matter. Notably, we found that the non-DA VM cells also grew toward distant gray matter target zones, such as mediodorsal and ventral anterior thalamus. These data suggest that directional cues for axons, whether diffusible or substrate-bound, are provided by adult host target regions. Since porcine neural development continues four to five times longer than mouse, these axons may grow and make synapses for a longer time (with slower maturation) than that seen in rat-to-rat studies. These general differences are borne out in the time-course comparisons of functional recovery in rodent porcine-transplant recipients (8 weeks post-transplantation) as compared with allografts (4 weeks post-transplantation) [18].

### 3. Anatomical and cell type specification of dopamine neurons

The current understanding of the maturation and phenotypic specializations of DA neurons located in the adult substantia nigra parallels the observations made of the development of committed fetal dopamine neurons placed as grafts into the adult CNS (Fig. 1). The molecular signaling necessary for the final morphological specializations and connectivity of the nigro-striatal DA system must therefore be largely intrinsic to the developing DA neurons; or alternatively, present in significant detail in the adult brain for this process to be completed in a normal way. On the post-synaptic host side; different regions of the striatum are associated with specific behaviors in rat: the dorsal striatum receives primary afferents from the motor areas of

neocortex, and has been shown to be preferentially involved in rotational recovery after DA neuron transplantation [42]. In the intact rat, the subpopulation of nigral DA neurons from A9 SN which co-express AHD project their axons to the dorsal-lateral and rostral regions of the striatum. When transplanted into adult DA-denervated rat striatum, these AHD/TH neurons innervated this region of the DA-depleted striatum [18,43], showing a preferential reinnervation of the dorsolateral striatum corresponding to the normal projection pattern of AHD/TH neurons. Specific innervation by subsets of transplanted DA neurons was also demonstrated by Schultzberg, showing reinnervation of the DA-depleted striatum by the population of grafted A9 VM neurons lacking cholecystokinin (CCK) [44]. The CCK<sup>+</sup> fibers were found in a narrow zone immediately adjoining the graft. These data suggest the presence of mechanisms, which selectively favor the ingrowth of fibers from the appropriate DA neuronal subset. Thus enrichment of the DA-neuron subpopulation which specifically expresses AHD may allow more appropriate reinnervation of striatum after transplantation, and influence the degree of functional recovery in PD [18] (Fig. 1).

### 4. Repair of synaptic function and regulated dopamine release after implantation of new dopamine neurons

The most important factor in obtaining complete and sustained functional effects may be the presence of new synapses for biochemically and physiologically appropriate DA release in the host striatum. Embryonic DA neurons produce new connections with the mature host striatal neurons. Synaptic connections between transplanted VM cells and host cells, as well as afferents from host neurons to transplanted cells, have been extensively documented [45,46]. Functional analyses indicate that pharmacological delivery into the striatum may not be as effective in ameliorating the motor symptom of PD, as regulated, synaptic release obtained with transplanted DA neurons [33]; When DA is directly administered into the ventricle of PD patients, serious psychosis can develop [47]. Even from a cell biological standpoint, the rationale for normal range DA release is illustrated by differential display experiments that show abnormal upregulation of over 10 genes within the striatum after abnormal DA exposure *in vivo* [48]. Complications associated with unregulated DA levels are obvious when observing effects of long-term L-dopa administration: as PD progresses, and the DA neuron degeneration continues, the unregulated formation of DA within the striatum and abnormal down-stream activity in the basal ganglia can lead to motor abnormalities such as dyskinesias. Physiologically appropriate DA functions can be achieved by DA neurons or, alternatively, cells which express the complete set of feedback elements required to regulate release and uptake of DA. Several studies have shown normalized metabolic activity throughout the basal ganglia after

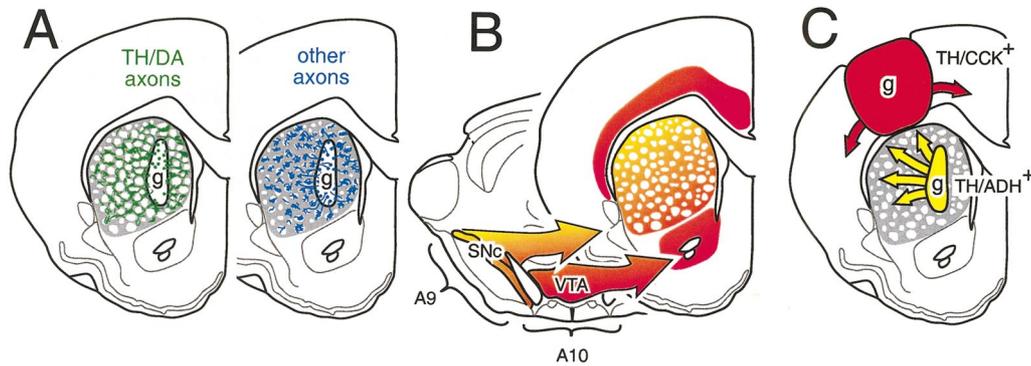


Fig. 1. Target-specific innervation by grafted fetal cells. (A) Target zones in adult host gray matter are selectively innervated by embryonic pig donor DA axons normally destined to form synapses there, whereas non-DA donor fibers grow into host myelinated bundles. (B) In the intact rat, the subpopulation of nigral DA neurons from A9 SNc, which co-express AHD, project their axons to the gray matter of dorsal-lateral regions of the striatum. The ventral tegmental area (VTA) neurons from A10 co-express CCK, and project to ventromedial striatum, nucleus accumbens, neocortex and limbic regions. (C) When the enriched population of TH/AHD neurons obtained from a medial (versus lateral) VM dissection is transplanted into DA-lesioned adult rat striatum, these neurons preferentially reinnervate their normal dorsolateral striatal target, shown to be involved in rotational recovery after DA neuron transplantation. TH/CCK neurons from VM show different patterns of outgrowth when placed into cortex. (Reprinted with permission from Trends in Neurosciences 1997; 20:477–482. © Elsevier.).

transplantation. Using cytochrome oxidase histochemistry as an indicator of neuronal metabolism in the 6-OHDA-lesioned rat; the lesion-induced increases in activity of the entopeduncular nucleus and SN reticulata were reversed by intrastriatal VM grafts, whereas the lesion-induced increases in globus pallidus and subthalamic nucleus were not affected by grafting [49]. Similarly, in MPTP-treated monkey receiving VM transplants, DA cell implants increased the metabolic activity of the implanted striatum, particularly in the region of grafts containing greater numbers of DA neurons [50]. Positron emission tomography (PET) and carbon-11-labeled 2B-carbomethoxy-3B-(4-fluorophenyl)tropane (11C-CFT) have been utilized as markers for striatal presynaptic DA transporters in a unilateral lesion model in rat. In the lesioned striatum, the binding ratio was reduced by 15 to 35% of the intact side. After DA neuronal transplantation, behavioral recovery occurred only after the 11C-CFT binding ratio had increased from 75 to 85% of the intact side, revealing a threshold for functional recovery in the lesioned nigro-striatal system after neural transplantation [23]. Auto-regulation of DA release and metabolism by intrastriatal grafts has been shown by *in vivo* microdialysis. Infusion of a non-selective DA agonist (apomorphine) reduced DA concentrations in the grafted striatum [12,51], indicating auto-regulation of DA levels by transplanted cells. Evidence for the formation of functional synapses and appropriate DA regulation by transplanted fetal DA neurons comes from the observation that dyskinesias, expressed either as contraversive circling after repeated L-dopa injections in rodents [52] or L-dopa-induced dyskinesias in non-human primates are reduced after transplantation. These data indicate that DA levels within the transplanted striatum will be regulated in a functional manner by the transplanted DA neurons.

## 5. Potential use of stem cells for obtaining donor cell for transplantation

Most living systems undergo continuous growth. There are many examples of cell division and differentiation for maintaining cell populations in adult human bodies; for example, the bone marrow that recruits stem cells capable of dividing into most of the cells necessary for blood and immune systems throughout life. Part of entire adult organs can be regenerated, such as the liver. Cells in the lining of the gut are shed on a daily basis with replacements growing in from layers below. In the skin, the basal cell layers of the dermis provide a plentiful source of growth; that also signifies a continuous growth process. These specialized cells can divide to maintain or increase growth of organ systems in the adult body. The recent fascination with the most pluripotent of such cell; the so-called stem cells, illustrate a renewed interest and deepening molecular understanding of developmental biology. While for the last 60 years most text-books of embryology has detailed most biological sequences in the development of mammals, it is not until recently that a molecular and mechanistic data of cellular signaling pathways involved in cell fate and development of organ systems has been obtained. In addition, recent cloning experiments have illustrated that even mammalian adult cell nuclei (containing DNA) has the material for establishing all cells of a whole organism after transfer to a fertilized egg-cell. The fertilized oocyte goes through a few rounds of cell division and then the resulting cluster of cells (in the range of 250 cells; see Fig. 2) is capable of imbedding itself in the wall of the uterus in mammals. At this stage, each of the cells in the inner cell mass cluster is usually capable of forming any part, or the whole of the entire body plan. This type of cell is therefore

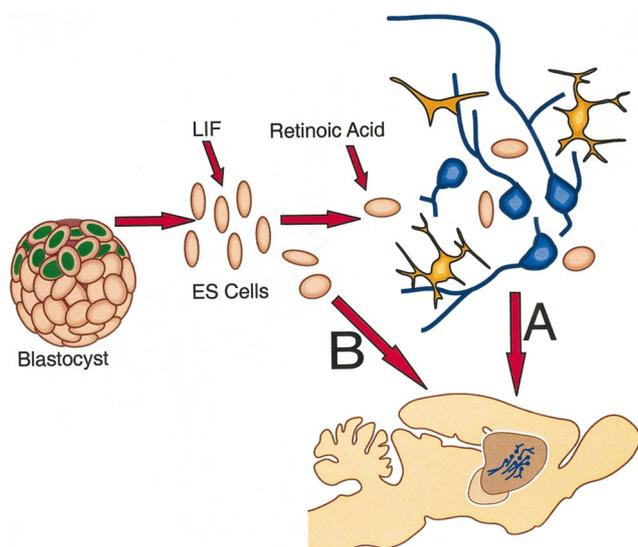


Fig. 2. Basic steps for ES cell procedures including in vitro expansion, chemical or spontaneous induction into neurons after implantation into the adult brain. Totipotent embryonic stem cells derived from the inner cell mass of blastocyst are propagated in culture in the presence of leukemia inhibitory factor (LIF). Prior to transplantation, LIF is removed. The cells are treated with retinoic acid (A) or are transplanted directly (B) into adult brain. Regardless of pre-treatment with retinoic acid, the transplanted ES cells differentiate to form cells with neuron-like morphology and phenotypic expression of neuronal markers.

denoted stem cell, or in this case, embryonic stem cells. From this initial group of stem cells, all other cells that form the living body are generated. The developmental sequential orchestration of the growth of the body into its specialized parts and unique form and function follows a strict pattern and sequence in the embryo and neonate. Nevertheless, as previously mentioned, in the adult organism, many cells with the body remain capable of division and growth into specialized cell systems. Recently, such divisible (yet non-malignant or carcinogenic) cells have gained increased attention. The idea that such multipotent cells present in the blood stream, or even in the brain, are still capable of multiple cellular fates has intrigued biologists and the public. In particular in the brain, in addition to the well-known fact that olfactory epithelium and a few other brain regions (including the dentate gyrus of the hippocampus) there may also be dividing cells capable of other types of growth or repair. Such continuous cell division may be necessary for maintenance and adaptive function of many cellular systems.

In experimentation, stem cell-like behavior has been observed from embryonic stem cells, growth factor-expanded neural progenitors, immortalized cell lines and embryonal carcinoma cells. Growth factor-expanded cells have been implanted into the adult brain, with survival of small cell clusters [53–55]. Immortalized cell lines have shown capacity to differentiate into several neuronal cell types when transplanted (for review, see Ref. [56]). The implantation of immortalized cells into neonatal brain

resulted in differentiation into neurons and glia with apparent region-specific morphology [57–60]. Notably, when transplanted into adult brain such immortalized cells (generated from embryonic striatum or hippocampus) are usually fated to form glia [61]. Brain implants of embryonic carcinoma cell lines have been shown to survive and grow as neurons when treated with retinoic acid [62–65]. We transplanted mouse D3 and E3 normal ES cells into adult mouse striatum and adult 6-OHDA-lesioned striatum, which spontaneously developed into neurons and other cells (Fig. 2). Many TH<sup>+</sup> neurons were found, while dopamine- $\beta$ -hydroxylase<sup>+</sup> cells were infrequent. Non-neuronal regions sometimes were immunoreactive for glial fibrillary acidic protein. Many neurons, including DA and 5-HT catecholaminergic cells, grew axons into the host brain. The axonal growth into gray matter was not abnormal, but did not resemble the five caliber fiber innervation seen in normal DA growth in the striatum [13,66]. ES derived serotonergic neurons grew in a less restricted pattern than TH<sup>+</sup> neurons. Mouse D3 and E3 ES cells placed into mouse kidney capsule grew into similar neuronal phenotypes as those placed in the adult brain. These data suggest that neuralization is a possible default pathway, and occurs spontaneously if pre-gastrula cells are prevented from getting patterned signals from other embryonic cell layers [67]. This is not entirely surprising, given that the early gastrula ectodermal animal cap, normally destined to become epidermal tissue, will form neural tissue if disrupted [68]. There are known inducing factors discovered for epidermal differentiation during gastrulation, such as BMP4 [69]. Homozygous knock-out mice lacking functional BMP receptor (BMPRI) will not survive past gastrulation [70], a time when epidermis would normally form. Inhibitors of BMP4 or activin, such as noggin, follistatin, and chordin, from the Spemann organizer region, can cause ectopic formation of neural tissue. Taken together, these findings indicate that disruption of these epidermis-inducing signals causes neural differentiation. Given that our experiment involved dissociated and expanded ES cells, this may be equivalent of such disruption. Nonetheless, it remains to be determined if other growth factors present in brain and kidney capsule can induce TH<sup>+</sup> neurons. The absence of kidney formation in GDNF-knockout mice suggests that GDNF may play a role in both kidney and brain development [71]. While these ES cells form neurons of TH<sup>+</sup> (putative DA) phenotypes that extend axons, into the adult host striatum, such neurons may also be able to create the kind of behavioral recovery seen with implantation of normal phenotypic fetal DA neurons.

In conclusion, there is a large unmet need for obtaining a donor cell source for clinical cell implantation to PD patients. While human fetal DA donor cells work in principle, as shown in human pilot studies, this cell source is not available or workable in a standard clinical environment. Analogous fetal DA donor cells from other animal species are potential alternatives to human fetal tissue. For example, the pig or rodent meso-striatal DA system also contains cell

groups A8, A9, and A10 that differentiate into the homologous cell groups seen in humans and function after transplantation to the mature brain. Alternatively, functionally appropriate DA neurons could be derived from progenitor or stem cell populations. Moreover, genetic engineering and immortalization technology could be applied to progenitor and stem cells, in order to obtain sufficient numbers of DA neurons of appropriate design for cell transplantation to a large number of PD patients.

*Statement on animal experiments:* All animal experiments have been carried out in accordance with the National Institutes of Health Guide for the care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1978). All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to in vivo techniques, if available.

### Acknowledgements

This work was supported by NIH grant P50 NS39793-01 (OI, KSK), DOD grant DAMD 17-99-1-9482 (OI) and the Century Foundation of Sarasota Memorial Hospital.

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