SUPPLEMENTARY MATERIAL

*Cases*

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| **Case 1. The Rutgers Study (Xu et al., 2019)** |
| Xu et al. at Rutgers University (along with researchers from the University of Nebraska, UT Health Science Center at Houston, and Kent State University) made various DS and neurotypical (control) cerebral organoids from human induced pluripotent stem cells (hiPSCs).9 Furthermore, they transplanted these brain organoids into mice and performed behavioral studies. In particular, the study aimed to uncover “the role of human OLIG genes in regulating interneuron production” with a hope that novel therapies could follow. The findings of the 2019 study “suggest OLIG2 as an excellent potential target for developing personalized prenatal therapy for DS.”9  After generating, genetically altering, and analyzing the cerebral organoids, they were transplanted into mice. Three behavioral tests were performed in random order: an open field test to measure basal global activity, elevated plus mase test to measure anxiety, and a novel object recognition test to measure learning and memory. The results of these behavioral tests only show a measurable reduction in novel object recognition in the DS chimeric mice, “suggesting that DS chimeric mice had impaired recognition memory.”6 All mice were euthanized after the behavioral tests. |

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| **Case 2. The Kyoto Study (Kitahara et al., 2020)** |
| The Kyoto study established two cerebral organoid groups based on the stages of callosal projection neurons (CPNs) and how well they represented the cerebral cortical development stages. Organoids 6-weeks after initial differentiation (6w-organoids) represented the developmental stage before CPN production, while organoids 10-weeks after initial differentiation (10w-organoids) represented the late developmental stage with a production of CPNs. Mouse pup populations were then split into two groups, half received 6w-organoids and the other half received 10w-organoids. Two craniotomy windows were opened, a small cavity of 1 mm was made into both sections of the cortex, and the cerebral organoids were placed into the cavities after being cut to dimension. 6-week-old mice underwent two surgeries. The first surgery opened the craniotomy windows, performed the lesioning, and closed the craniotomy window. After one week, the mice then received the 10w-organoids. The other six 6-week-old mice received the 10w-organoids immediately after lesioning.12  Researchers also transplanted the hESC-derived brain organoids (10w-organoids) into 3-year-old “purpose-bred male cynomolgus monkeys (*Macca fasclcularis*)” (n = 4). Citing ethical concerns, the Kyoto researchers only transplanted the 10-week organoids into the primary motor cortex (“bilateral precentral gyrus”) of macaque brains. According to the researchers, transplanting the 6-week organoids increases the risk of cellular overgrowth, which would result in cranial compression. Furthermore, researchers tried to avoid affecting the higher brain functions by restricting the transplantation sites to the primary motor cortex. Researchers also noted that advancing this form of research would require addressing ethical questions, specifically whether animals can be humanized and if cerebral organoids *in vitro* can have consciousness.12 All four macaques were euthanized at 12-weeks post-transplantation (12 wpt) and their brains were further examined. Euthanasia was performed via transcardial perfusion while under deep anesthesia with pentobarbital. No behavioral studies were performed with either the mice or macaques. |

*Tables*

**T1.** Table of definitions for the Six Principles. All definitions come from Beauchamp and DeGrazia.8

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| **Animal Ethics Principle** | **Definition** |
| No Alternative Method | *“Use of animal subjects must be the sole ethically acceptable way to address a research problem whose solution offers the prospect of a social benefit. (A claim that no ethically acceptable alternative method is available should be demonstrated on carefully reasoned grounds in protocols.)”* |
| Expected Net Benefit | *“The prospect of social benefit from research study must outweigh its expected costs and risks to human beings. (The basis for a net benefit and the projected costs and risks should be demonstrated on carefully reasoned grounds in protocols.)”* |
| Sufficient Value to Justify Harm | *“The prospect of a net benefit for human society from a research study must be sufficiently valuable to justify expected harms to animal subjects. (The basis for judgements of sufficient value to justify harm should be stated in protocols.)”* |
| No Unnecessary Harm | *“Animal subjects must not be harmed unless a particular harm is necessary for and morally justified by scientific purposes.”* |
| Basic Needs | *“Animal subjects’ basic needs must be met in the conduct of studies unless failure to meet specific basic needs is necessary for and morally justified by scientific purposes.”* |
| Upper Limits to Harm | *“Animal subjects must not be caused to endure severe suffering for a lengthy period of time. In rare, extraordinary cases, exceptions may be warranted if the research is necessary for and morally justified by critically important social and scientific purposes.”* |