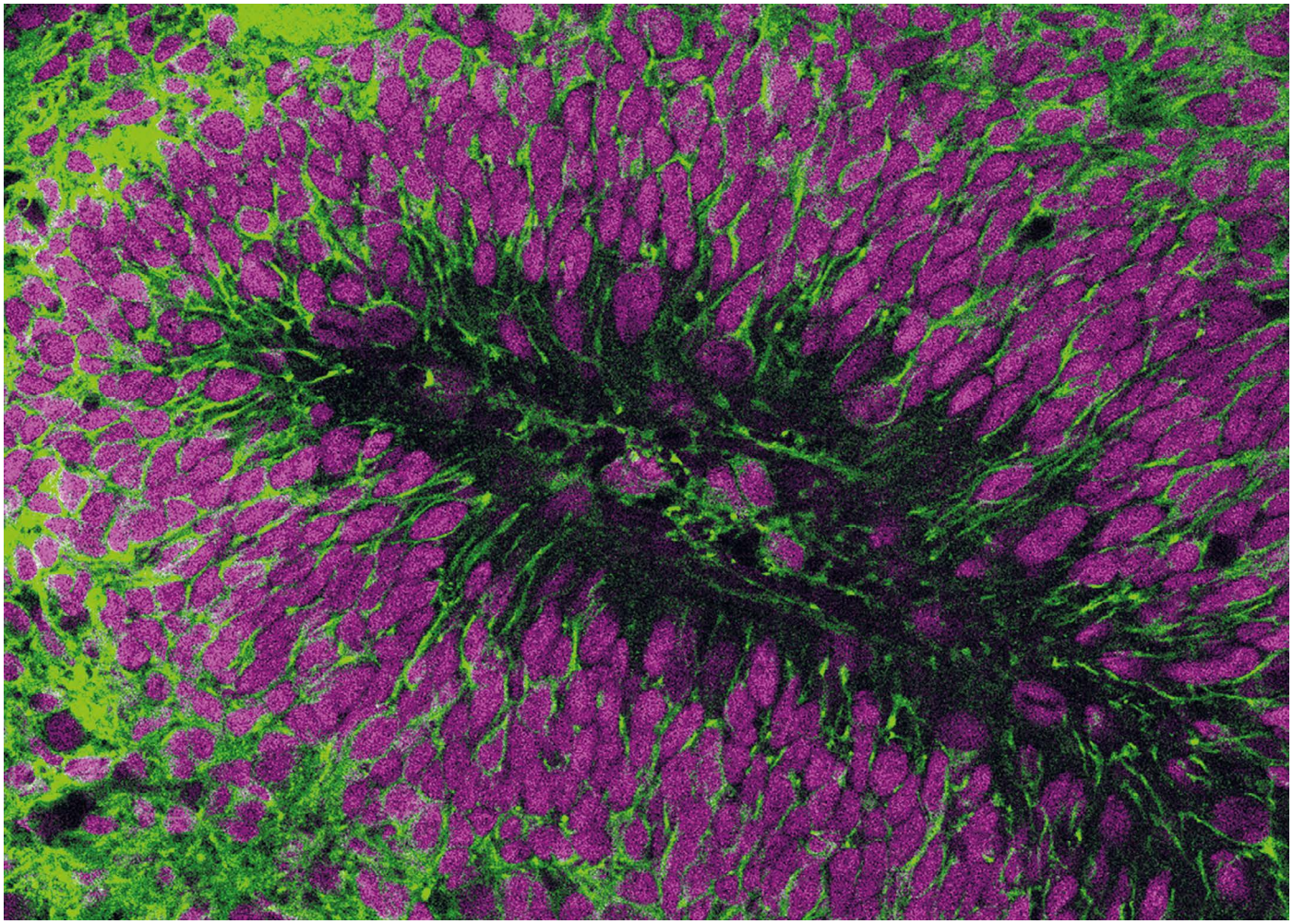


RAQUEL REAL, MANUEL PETER, RICK LIVESEY AND VINCENZO DE PAOLA

Human brain cells and their nuclei two months after being transplanted into a mouse brain.

BLENDING BRAINS

Transplanting human cells into animal brains brings insights into development and disease, along with new ethical questions. **By Kendall Powell**

In a darkened room in a laboratory in London, a group of students and researchers watch a clump of human brain cells settle into their new home: a living mouse brain. On a computer monitor next to a microscope, the human cells light up in flashes of simultaneous activity. Over time, the cells sprout new connections a few centimetres long, and form networks with each other. It's captivating viewing for his students, says Vincenzo De Paola, who runs the lab at Imperial College London. "It's all they want to do. I can't tear them away," he says.

They have front-row seats to an unusual show. De Paola's group is one of just a handful of labs able to study human neural cells at work in a live, developing brain — a system that is otherwise largely off limits for both ethical and technical reasons. "We cannot study these processes as they unfold in a fetal human brain," he says. "Instead, we wanted to watch human cortical neurons mature and form active networks in a live animal."

De Paola's system is a specialized type of neural chimaera — an area of research that has expanded immensely in the past five years, sparking a debate about the ethics of blending human and animal brain tissue. Proponents

say that such systems are necessary to manipulate live human neurons and are already yielding important insights into health and disease. For example, using neural chimaeras, scientists have found differences in how neurons develop and behave in Down's syndrome and Alzheimer's disease.

But others warn that such chimaeras represent an ethical grey zone, because of the potential to blur the line between humans and other animals, or to recapitulate human-like perception or cognition in an animal. Some researchers say these kinds of chimaeras should only be used if no other cell or animal model is appropriate. "Is this a really good model for answering a scientific question or are we pushing boundaries for the sake of it?" asks Naomi Moris, a developmental biologist at the Francis Crick Institute in London. Ethicists are asking at what point a collection of human neurons in another animal's brain embodies something that deserves a unique moral status.

Although research using chimaeras — entities made up of cells from different organisms or species — has been going on for decades, these neural chimaeras broach new ethical territory. A 2021 special report on neural chimaera research by the US National Academies of Science, Engineering and Medicine (see go.nature.com/3pii9q5) flagged issues such as the possibility of endowing animals with new cognitive abilities or human disease symptoms that could be distressing. The committee advised that although current regulation of stem-cell and animal research was adequate, the field should be kept under close surveillance. The committee also encouraged the use of pilot studies and close monitoring of animals to identify any new or unusual behaviours.

There will be plenty for regulators to keep an eye on. Researchers are starting to consider going beyond transplanting a few isolated cells to creating chimeric animals with human brain regions. Studies that transplanted human brain stem cells into monkeys' brains helped to launch a 2018 clinical trial testing whether human brain stem-cell transplants can treat Parkinson's disease. In 2019, Japan reversed a ban on government funding for research using human–animal chimeric embryos. In many countries, including the United States and the United Kingdom, research that mixes human brain cells or tissue with another animal's brain is legally allowed, and can be government-funded with an extra layer of review. The United States prohibits government funding for human–animal chimeric embryo research.

Observers expect the field to be fast-moving. "We know it will be quickly evolving," says Insoo Hyun, director of research ethics at Harvard Medical School in Boston, Massachusetts.

A research mainstay

The history of biology abounds with chimaeras. Starting in the early 1900s, embryologists cut and pasted together pieces of embryo from different animal species — merging a chicken with a quail, for instance, to work out where developmental signals originated, says Ali Brivanlou, a developmental biologist at Rockefeller University in New York City.

Researchers have also been introducing human elements such as organs, cells or genes into other animals for decades. Often, the rationale is to better understand how biological systems are working, says Brivanlou, or to find treatments for disease. Cancer researchers routinely transplant human tumours into mice, and since the late 1980s scientists have created mice with human immune systems[1].

Another motivation is more utilitarian: to find ways to grow human-compatible organs in animals to alleviate the shortage of organs for transplantation. In the past few months, researchers have transplanted genetically modified pig kidneys and a pig heart into humans.

But transplants of human neurons that survive long-term have only been created in the past decade. In 2013, Pierre Vanderhaeghen, a neuroscientist then at the Université Libre de Bruxelles, and his colleagues perfected the delicate process[2] of growing human neurons from stem cells to the point that they would flourish — but not grow uncontrolled — in the mouse brain when transplanted.

"We wanted to know how those neurons trained in the dish would act in the battlefield of the brain."

Scientists use two types of human stem cell to make neurons for chimaeras: either embryonic stem cells (ES cells), which are originally derived from embryos, or induced pluripotent stem cells (iPS cells), which are derived from adult cells that are reprogrammed to an embryonic-like state. Both types have the potential to become any tissue in the body and can be directed to grow into neurons. "Cells derived from human pluripotent stem cells are a lot more plastic than other cells transplanted in the past," which could result in better integration of the human cells, says Hyun.

In 2016, the US National Institutes of Health proposed lifting its funding moratorium on research using animal embryos containing human cells. Comments from the public, largely expressing opposition, flooded into the agency. (The funding ban remains in place.) But a 2020 survey of 430 people in the United States found that 59% supported human–pig chimeric embryo research designed to produce human tissues in pigs[3].

Pigs with human kidney or liver tissue are one thing; neural tissue might not be so acceptable. "It is the brain that people associate with moral status," says Hyun. Although researchers say none of the research comes close to producing human-like cognition in an animal, this association is making them reconsider — at what point does an animal's brain become too human-like for society's comfort?

Mingling brain cells

In the past five years, researchers have developed several ways to make neural chimaeras. They vary in complexity from transplanting single human neurons or a chunk of cultured brain tissue, to combining embryos from two species to try to produce chimeric brain tissue from scratch.

The most minimal way to get a rare glimpse of human neurons at play is to transplant just a few cells at a time. Vanderhaeghen's group, now at the Flemish Institute of Biotechnology (VIB)–Catholic University of Leuven (KU Leuven) Center for Brain & Disease Research in Belgium, has been doing this with pyramidal neurons — the most abundant type in the human cortex — grown in a dish from ES cells. They wondered how the cells might wire up over longer timescales in a living animal. "We wanted to know how those neurons trained in the dish would act in the battlefield of the brain," says Vanderhaeghen.

His group, working with Vincent Bonin's team at VIB Neuro-Electronics Research Flanders in Leuven, transplanted a soup of human neurons that integrated as single cells, rather than as a clump, into the cortex of a newborn mouse[4].

The human neurons took their customary time to mature, between 6 and 12 months compared with 5 weeks for their mouse neuron neighbours. Even in the environment of the mouse brain, they stuck to their lengthy timeline, says Vanderhaeghen. "This suggested that this prolonged developmental timing is encoded intrinsically, in the neurons themselves."

The team found that the human neurons developed normally, integrated into and functioned within the mouse's visual circuit, responding just as mouse cells did to visual stimuli, such as moving black and white bars. That the human neurons settled into a foreign brain and worked normally was surprising — and it hints that cell transplants might be used to repair damaged brain circuits in the future.

"We expected some connectivity, but we were quite stunned at how specific the responses were," says Bonin. "There are a million ways this could have failed."

The team has also transplanted healthy human neurons into the brains of mice with a genetic predisposition to Alzheimer's disease. The work[5] showed that the human neurons degenerate in the diseased brain, whereas the mouse neurons remained alive. This not only confirmed that human neurons are particularly vulnerable to Alzheimer's disease, but also gave researchers a way to watch what happens to

human neurons in a living diseased brain.

De Paola, who also runs a group at Duke–NUS Medical School in Singapore, studies how human neurons connect with one another and how this is disrupted in developmental disorders. His group grafted pyramidal neurons made from human iPS cells into the somatosensory cortex of adult mice[6].

In contrast to Vanderhaeghen's transfers, these transplants grew into dense micrografts of human tissue in the mouse brain and survived until the experiment ended after five months. "We were surprised at how much growth there was, it was a massive network," says De Paola. "Well, 'massive' is relative – it was about the size of a large lentil."

The grafted cells kept mostly to themselves – more than 90% of the connections were human to human – but they did send out projections to other parts of the mouse cortex, and received a few projections, blood vessels and immune cells from the mouse brain, he says. Those supports allowed the chunk of tissue to keep developing for five months, playing out the behaviour normally expected in a developing human fetal brain – pruning neuronal branches and connections, and starting to fire in coordinated waves.

De Paola's team did the same transplant experiment using neurons made from cells from people with Down's syndrome[6]. They found that these neurons formed less dynamic networks, with lower neural activity – but it's unclear what relationship, if any, exists between the two features. The team is exploring that next. "We can do that experiment in this model. Obviously, we cannot do it in a human adult or fetal brain," De Paola says.

Could these groups' transplants somehow change the mouse's visual or sensory perception to a more human version? Neither team has tested cognition or behaviour in the transplanted mice, but all report that the mice generally behaved like their non-transplanted peers. Both De Paola and Vanderhaeghen are sceptical that the limited numbers of human neurons and connections could change a mouse's outlook. "I don't think stimulating even a few thousand human cells would drive human behaviour or perception," says Vanderhaeghen. But he and De Paola think that those working in the field should try to determine at what point that might change.

Long-lived organoids

A major advance in the study of human brain tissue in the laboratory has been the rise of brain organoids, self-organizing structures formed when brain stem cells are grown in 3D culture.

Brain organoids have become increasingly intricate since they were first created in 2013 by Madeline Lancaster and Jürgen Knoblich[7]. Some researchers have even stitched multiple organoids together into 'assembloids'.

"For the first time, we are able to monitor living human brain tissue in a disease context."

Organoids are complex enough to be a good way to ask many questions about the human brain, but even assembloids are still far from the complexity of the real thing, says Sergiu Pasca, a neuroscientist at Stanford University in California. That's because they lack sensory input, blood vessels, immune and support cells, and don't receive feedback, he says. Plus, once the structures grow beyond 3–4 millimetres in size, the cells in the middle die owing to lack of nutrients from the cell-culture broth. It can be hard to support their growth beyond a couple of months.

To overcome these limitations, neuroscientists have begun to transplant organoids into an animal's brain to more closely model the complexity of human brain circuits and how they go awry in disease.

Neuroscientist Rusty Gage's group at the Salk Institute for Biological Studies in La Jolla has succeeded in transplanting human organoids into mouse brains and keeping them alive for up to 11 months, nearly the mouse's entire lifespan[8]. Using this system, they have unpublished results showing that the human neurons mature from an embryonic-like condition to a more complex state akin to neurons in an infant, and eventually show characteristics of adult neurons. The human brain tissue integrated into the mouse brain, grew blood vessels, matured and responded to stimuli, and even formed sparse, but working, connections with mouse neurons.

Abed Mansour, who established the organoid transplants as a postdoctoral fellow working with Gage, says the system has advantages for studying what happens to neurons in neurodegenerative disorders such as Alzheimer's disease. Human neurons in organoid transplants send long projections into the host brain. "This might become an excellent system to ask how this process differs between healthy human neurons and disease-affected neurons," says Mansour, who now leads his own group at the Institute for Medical Research at the Hebrew University of Jerusalem.

Gage's group now plans to transplant brain organoids made from the cells of people with Alzheimer's disease into healthy mouse brains and, conversely, healthy human organoids into mouse brains that mimic Alzheimer's symptoms. The aim is to tease apart which cell types – the neurons themselves, or other brain cells such as astrocytes – contribute to the inflammation seen in the disease.

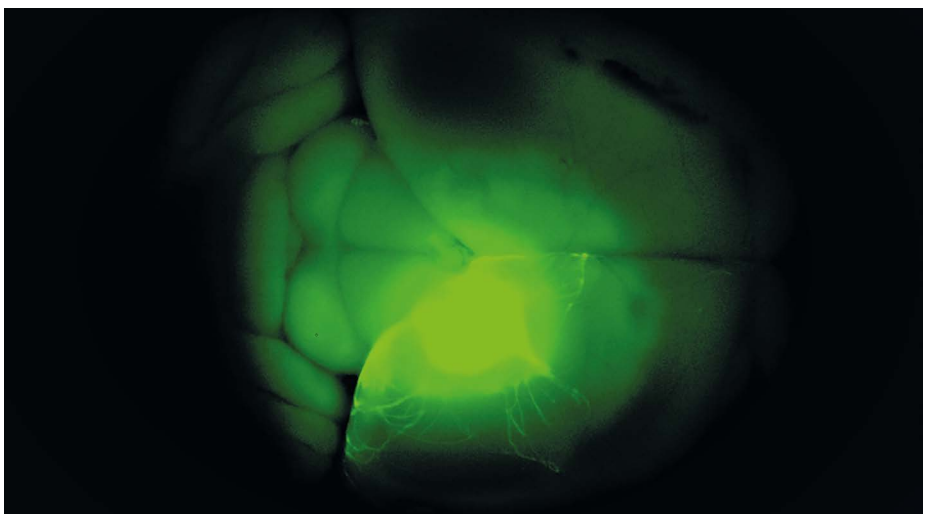
"For the first time, we are able to monitor living human brain tissue in a disease context," Gage says. One day, he says, this research could lead to personalized organoid transplants that replace diseased or injured brain tissue.

For Lancaster, now a developmental biologist at the MRC Laboratory of Molecular Biology in Cambridge, UK, organoid transplantation has its place, but she urges researchers to examine closely the animal experiments they are doing and make sure they are justified. "We need to be careful as researchers – this is such a hot field with a lot of papers being published," she says.

As for the ethical status of organoids, when in the dish they are essentially considered to be a fancy 3D cell culture. Lancaster, Gage and others do not consider them to be capable of human perception, sensation or cognition. And Gage says that the transplanted organoids do not integrate well enough to confer any meaningful 'human-ness' either.

Chimeric embryos

Another way to study human brain development in a living organism is to add human



ABED MANSOUR

Scientists transplanted a human organoid (bright green) into a mouse brain to study how neurons behave in the context of health and disease.

ingredients or component parts into the earliest stages of another animal's developing embryo. Several groups have tried to make human–animal chimeric embryos to study organ development, with a view to one day making organs for transplant.

One approach is to add human stem cells to animal embryos within a few days of fertilization, when they are still just tiny balls of dividing cells. Scientists have tried this with rodents, livestock and, in a controversial 2021 study, with monkeys[9], which are much more closely related to humans. However, these chimeric embryos either do not develop beyond very early stages or the human cells die off rapidly. Scientists think that the cells from such different animals are simply too distinct to coexist and communicate as intimately as embryonic cells must to develop.

There is another way to generate transplantable organs — and maybe one day, brain tissue that could be used to study and treat disease. In a method called blastocyst complementation, scientists use a slightly later-stage embryo, called a blastocyst, and introduce mutations that prevent it from generating a particular organ, such as the pancreas. They then take stem cells that can produce that organ from another animal, and inject them into the blastocyst.

Stem-cell biologist Hiromitsu Nakauchi's research group, which is split between Stanford University and Tokyo Medical and Dental University, has used blastocyst complementation to grow a mouse pancreas in a rat[10]. "In a sense, we are using embryos as a bioreactor because they should know how to generate an organ," Nakauchi says.

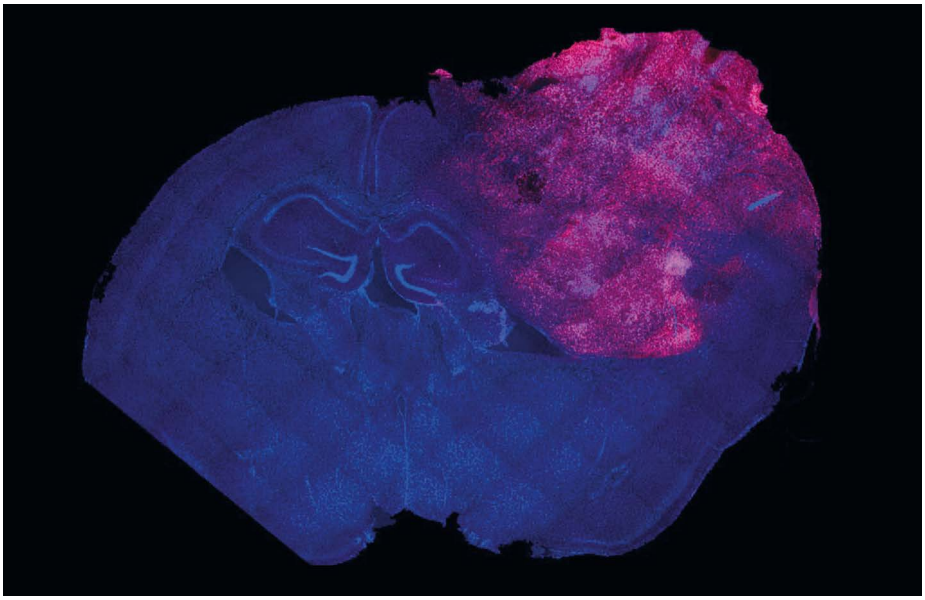
Whether animal blastocysts can develop normally with a human pancreas, kidney or brain region is an open question. When human cells are mixed into an embryo from the very beginning, the organism often fails to develop normally, but creating a specific niche for one organ or tissue type could make it easier for human cells to contribute to the organism's development, says Bjoern Schwer, a molecular biologist at the University of California, San Francisco. Blastocyst complementation hasn't been tried with human neural stem cells yet, but Schwer's group and others are thinking about preliminary experiments to do so. His first question is whether a piece of non-human primate brain could be grown in a rodent.

Schwer's group has already used blastocyst complementation to make mouse–mouse chimeric brains. In a 2018 collaboration with Frederick Alt's group at Harvard Medical School, the researchers used the technique to grow the entire forebrain region from one mouse strain in the embryo of another strain[11].

"It's a pretty modular system, that we can use in different ways to get rid of and replace different brain regions," says Schwer. For instance, replacing part of a mouse brain with the corresponding non-human primate brain region, instead of human brain cells, could give researchers an easier and more ethically palatable way to study the *in vivo* development of a monkey brain region that closely resembles its human counterpart. Schwer has obtained approval from his university to use macaque and marmoset ES cells to try to produce a small piece of primate forebrain in a mouse brain.

He is also contemplating what it might take, both ethically and technically, to use blastocyst complementation to grow a piece of human brain tissue in a developing mouse's brain. Such an experiment could test how certain mutations drive human brain-tumour growth, for instance, and perhaps find ways to shut them down.

"We haven't gone there yet, but that is sort of the hope," says Schwer. Much like the other neural chimaera researchers, he doesn't think a small piece of human brain in a mouse's brain would lead to human-like cognition.



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Human brain cells made from stem cells (purple) grow in a section of the mouse brain (blue).

Crossing a cognitive line

But it is exactly that possibility that concerns ethicists and the public. "The neural combinations touch on what it is that makes us essentially humans — our minds, our memories, our sense of self," says Alta Charo, a bioethicist based in Washington DC and professor emerita at the University of Wisconsin-Madison. The public, she says, finds the idea of a human mind trapped in an animal's body, or a creature with a semi-human brain, disturbing.

She and other ethicists think there is a large gap in the public's understanding of why this research is being undertaken. Neural-chimaera researchers should be sharing their work with the public more often, she says, for instance following Pasca's example of giving TED-style talks. As research progresses, Charo notes that researchers will have to consider what proportion of human brain tissue might begin to approach cognition in a dish or evoke human characteristics in a mouse — and when that might add emotional distress or pain for the animal.

Another concern is the unpredictable behaviour of human embryonic cells placed into an animal embryo, and whether they could grow out of control. "What's challenging is the uncertainty of what proportion might take over an embryo," says Moris. "We are trusting the embryo to 'do its thing' but it might not be what we expect."

Of course, neural-chimaera research requires material from human donors and also brings up questions about consent, and how to properly inform people that their cells could be reprogrammed into neurons and given new life in a dish, a mouse or an embryo.

Schwer feels that anyone who gives cells for iPS cell studies should be consulted before their cells are used to make neural tissues. "I would want to know, wouldn't you?"

For his part, Brivanlou is optimistic that the future benefits of the work might change the equation. "The minute you cure a disease with this — cure a kid with Huntington's disease or fix your grandmother's Alzheimer's disease — everyone is in agreement. The journey to get there, however, is bumpy, and that's where we are right now."

Kendall Powell is a freelance journalist in Boulder, Colorado.

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