JOHN JONES SURGICAL SOCIETY • 1997 •

NEWSLETTER

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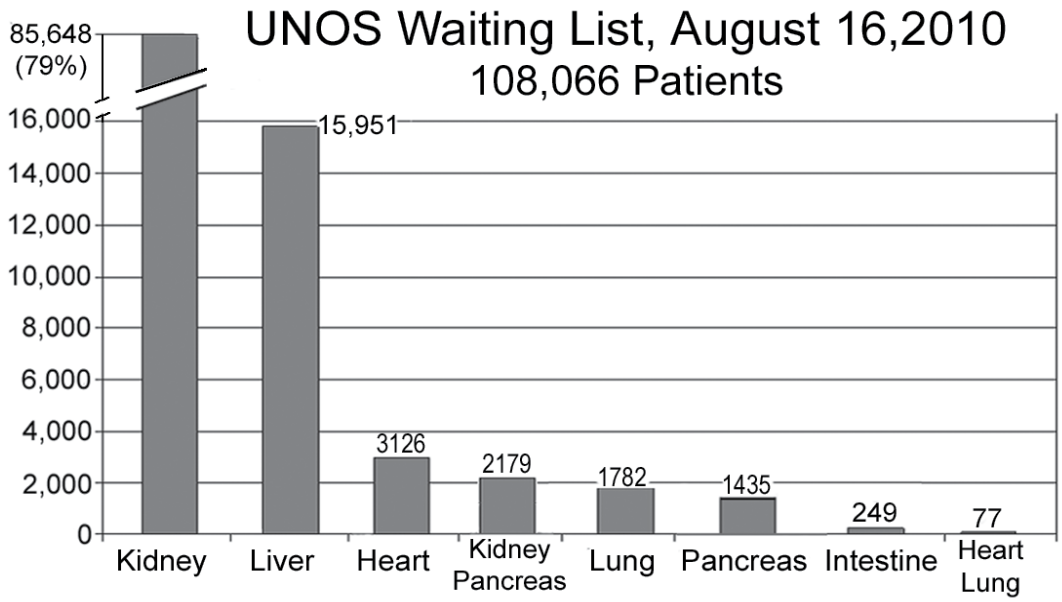


CUMC 2007-2009 Transplant Activity Profile*

Activity	Kidney	Liver	Heart	Lung	Pancreas
Baseline list at year start	694	274	174	136	24
Deceased donor transplant	123	124	93	57	11
Living donor transplant	138	17	—	0	—
Transplant rate from list	33%	50%	51%	57%	35%
Mortality rate while on list	9%	9%	9%	15%	0%
New listings	411	217	144	68	23
Wait list at year finish	735	305	204	53	36

2007-June 2008 Percent 1-Year Survival

	No	%	No	%	No	%	No	%	No	%
Adult grafts	610	91	279	86	169	84	123	89	6	100
Adult patients	517	96	262	88	159	84	116	91	5	100
Pediatric grafts	13	100	38	86	51	91	3	100	0	—
Pediatric patients	11	100	34	97	47	90	2	100	0	—

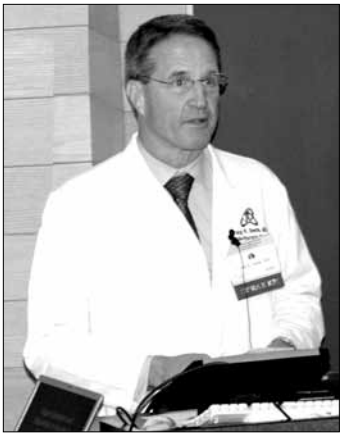
Summary Data

Total 2009 living donor transplants	155 (89% Kidney)
Total 2009 deceased donor transplants	408 (30% Kidney, 30% Liver)
2007-June 2008 adult 1-year patient survival range	84% Heart to 100% Pancreas
2007-June 2008 pediatric 1-year patient survival range	90% Heart to 100% Kidney or lung

**Health Resource and Service Administration's Scientific Registry of Transplant Recipients (SRTR)*

Ed Note.

The figure shows the US waiting list for whole organs which will only be partially fulfilled by some 8,000 deceased donors, along with 6,600 living donors, who will provide 28,000 to 29,000 organs in 2010. The Medical Center's role in this process is summarized in the table, and the articles that follow my note expand on this incredible short fall and its potential solutions.


Craig Smith introducing Judy's Video

Mark Hardy's day-long symposium, "Transplantation – A Glimpse at Past, Present, and Future" constituted the John Jones Surgical Society's 10th Annual Spring Meeting. Craig Smith opened the symposium with Judy Reemtsma's video of her husband Keith's pioneering work at Tulane, Utah, and Columbia just 1 month shy of 10 years since he passed away. Mark's amazing aggregation of experts is the *sine qua non* inspiration for this "Transplantation Issue."


Judy Reemtsma and Mark during AM break.


Presidents Steve Libutti and Ken Forde

The JJSS business meeting occupied only 30 minutes but was almost as notable as Mark's symposium. Ken Forde and Eric Rose founded the Society in 1997, and Ken was its first and only President until 16:40 on May 21, 2010, when he gleefully turned the office over to Steve Libutti. Steve looks equally happy and undoubtedly comforted by Jose Guillem's simultaneous election to the position of President-Elect.

This event has some obvious parallels, such as King John's capitulation in ceding The Magna Carta at Runnymede in 1215, and Karl the First of Austria's renouncement in 1918, ending the Hapsburg Monarchy that had ruled territories of varying size continuously since 1526. No youngster can match the perspective of a nonagenarian when dealing with events of these historic proportions: Fortunately two of Ken's many good friends and admirers fall into this category and have given us their thoughts along with regrets for being unable to be on the scene.

"JJSS would not be alive today were it not for the attentions of Ken at its birth, and the consistency of his care during its growth. The importance of his presence among his surgical colleagues from near and far has guaranteed the survival of our Society, and beyond survival, its growing influence on our surgical community at Columbia. Cheers to Ken, from an ancient member."

Fred Herter

"As well as being the JJSS co-founder, with Eric Rose, and being its guiding light, as so well expressed by Fred Herter, Ken has played extremely important roles in the Department of Surgery, the Medical School, and the University, in addition to being very helpful to me personally, as well as being a good friend. Congratulations to Ken for the many jobs he has performed, all exceedingly well done, and my very best wishes to both Ken and his charming and equally impressive wife Kay."

Fred Jaretzki


Fred Herter and Fred Jaretzki


Robert Brown, Adam Greisemer, Hugo Sondermeijer, Spencer Amory, Jean Emond, Lloyd Ratner, and Ken Forde, all variablly enraptured.


Dave Tilson looks interested in whatever Ken Steinglass is selling; Foster Conklin is listening but dubious.

Henry Spotnitz is the new treasurer and Spencer Amory, Andre Campbell, Herbert Mandel, and Roman Nowygrod were elected as new members of the Steering Committee. The day concluded, as it has for the past several years, with a lovely dinner at the New York Athletic Club.

Jim Chandler

Sanctity and the Societal Value of Organ Donation

Michael R. Marvin, Kenneth M. Prager, Max V. Wohlauer, and James G. Chandler

Chief Transplant
Division U. Louisville

Chair CUMC Ethics
Committee

Surgical R-4
University of Colorado

Editor JJSS
Newsletter

Individual sanctity is an essential component of humanness with deep roots in culture, religion, and law. Death deletes all vitality and the very essence that characterizes a living human being, but the sanctity of an individual transcends death, irrespective of how the latter is defined. It persists in the minds of those who knew, or know of the decedent to slowly dissipate over generations and, in some instances, almost never. Think of a friend's memorial service, purposefully delayed to allow the honoree's good qualities to emerge from the veil of grief; recall the reverence with which you read a favorite teacher's obituary, and note that three-years of controversy preceded the merging of Washington and Lincoln's commemorations into a single "President's Day" in 1971.

Society's valuation of whole organ donation began cautiously with the successful transplantation of one adult monozygotic twin's kidney into his twin brother on December 23, 1954, after first confirming their genetic match by exchanging skin grafts. The Peter Bent Brigham team believed that the ethical issues were minimized by advance airing in public fora, the brothers' mutual affection, and the expectation of success implied by the skin grafts. But, as noted by Joseph E. Murray,[1] in his 1990 Nobel address: "For the first time in medical history, a normal, healthy person was to be subjected to a major surgical operation not for his own benefit." Were the circumstances such that the donor could have said no without becoming a family pariah and burdened with guilt? Sure, but the driving force was concern for his brother. The outcome was good: the recipient lived for 8 years free of dialysis, and the donor outlived his brother, but Murray would not achieve success with a cadaveric renal allograft until 1962.

Nobel laureate Joe Murray in 1990

Whole organ allotransplantation opened to hearts in 1967-68, with dismal results. As of October 23, 1968 only two of the world's 65 heart allograft recipients were surviving out to 5 months.[2] Transplanting hearts was especially provocative and overly reported. Some viewed excising a donor heart as tantamount to ripping out the soul, and with these results, one could argue, "For what purpose?" Most early proponents abandoned the procedure, or paused to regroup, as Starzl[3] had done with livers in 1963, but in the US, Norman Shumway pressed on at Stanford. His former associate, Richard Lower had moved to the Medical College of Virginia (MCV) in 1965 to extend David Hume's busy renal transplant program to include hearts. There were new technical hurdles that still had to be overcome, but the real culprits were immunosuppressant's marginal therapeutic index, rejection monitoring without biopsy guidance, ignorance of the need to treat cytomegalovirus, and donor warm ischemic time with its ultimate companion reperfusion injury.[4]

Brain Death and Tucker vs. Lower

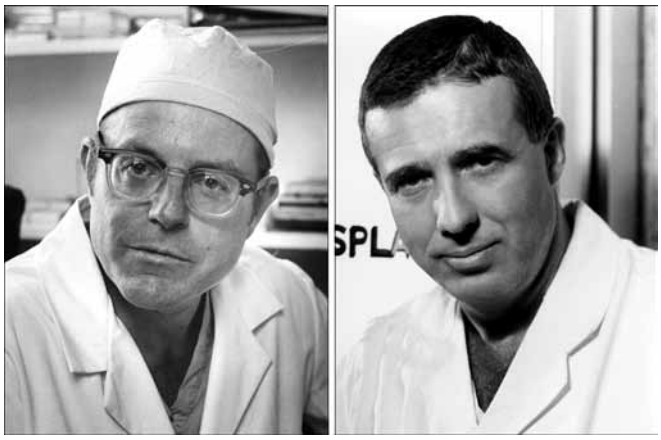
Belgian Guy Alexandre, who had been a research fellow in Murray's laboratory, harvested and implanted a kidney from a beating-heart, severely head-injured, comatose donor at Louvain's Saint Pierre Hospital on June 3, 1963, after convincing his chief, Jean Morelle, of the irreversibility of the coma.[5] Harvesting kidneys from a donor who could be demonstrated to have suffered irreversible loss of brain function was an attractive approach to minimizing warm ischemia time, beginning with pump assisted in-situ circulatory cooling. Harvard instituted an ad hoc review panel to study the issue, which published its conclusions in 1968, stating that irreversible coma was defined by complete unresponsiveness; no spontaneous movement, including breathing; and a flat electroencephalogram in the absence of confounding factors such as drugs or hypothermia.[6] These criteria were similar to Alexandre's, which had been presented at a 1966 CIBA symposium as having been applied to nine kidney donors whose *coma dépassé* (beyond coma) always began with severe head injuries.

William Tucker went to the MCV hospital, which was just down the street from his Richmond shoe repair shop, on the evening of May 25, 1968 to inquire about his injured brother. He was told that his brother had died in the afternoon, without mentioning his having been the world's 16th heart transplant donor.[7] The family did not learn about this, until William and another brother went to the morgue to claim the body and were asked: "Did you know that they took your brother's heart?"[8]

Bruce Tucker was a 56 year old man who fell while intoxicated, sustaining a basal skull fracture and was brought unaccompanied to MCV around 6:00 PM on May 24, 1968. He underwent a craniotomy to relieve a subdural hematoma, and a tracheostomy for mechanical ventilation, which failed to halt his deteriorating neurological status.[9] Lower and Hume had been training their team for 3 years and were actively seeking a potential heart donor for a patient already in the hospital. When they learned about Tucker, they asked others, including the police, to search for his family to discuss donation of

his heart and kidneys, but no contact was ever made. Apparently, no one thought to look through his personal effects, which included a wallet containing his brother's current business card. Early in the afternoon of May 25th, a neurologist concluded that Tucker's brain was dead, and on that basis, the surgeons moved the patient to the operating room to prepare him for removal of his heart and kidneys. The respirator was disconnected. Spontaneous respiration was not observed, and after 5 minutes, he was reported to be dead to the medical examiner, who agreed that the harvest could proceed. He was liberally interpreting a Virginia law regarding unclaimed bodies that required a two-day waiting period. Bruce Tucker had been in the hospital for just 23 hours, and it is not known if his heart ceased to beat after 5 apneic minutes or if it did and restarted when ventilation was resumed. Dick Lower was a remarkably sensitive and unassuming person, but in this situation he neither recognized nor respected Bruce Tucker's sanctity. Bruce was black in a society that was still desegregating but not the abandoned derelict he was presumed to be. The recipient of this 9th US heart transplant was white and survived for just seven days before becoming the first heart recipient to die from acute rejection.

This was an ethical debacle that assured a law suit, if not a murder charge, and could have provoked massive public disapproval. In fact, Professor Jura Wada at Sapporo Medical University in Hokkaido was charged with murder for not doing more to revive a brain death donor whose heart was used in Japan's first heart transplant, performed on August 8, 1968. The murder charge was eventually dropped for insufficient evidence, but Japan would not allow another heart transplant for 31 years. Whereas, in the US, Lower continued to perform heart transplants and had the pleasure of seeing a later 1968 recipient live for an additional 6.5 years. Bruce Tucker's treatment may have influenced 1968's original drafting of the Uniform Anatomical Gift Act, which mandated obtaining consent and precluded a donor's physician from participating in removal of a part.[10] Virginia deleted the two-day waiting period from its unclaimed body statute in the same year, and in 1970, Kansas became the first State to enact a law recognizing irreversible coma as one criterion for being legally dead.



Richard R. Lower *(1929-2008)* David M. Hume *(1917-1973)*

Courtesy of Ms. Jodi L. Koste, Archivist, Tompkins-McCaw Library for the Health Sciences, Virginia Commonwealth University.

The inevitable trial commenced on May 25, 1972. William Tucker, represented by future Virginia governor, Doug Wilder, sued Lower and MCV, alleging that Lower had hastened Bruce Tucker's death by shutting off the ventilator for the purpose of obtaining his heart and kidneys. The issue of consent was moot because the statute of limitations expired before the suit was filed. Judge Compton's initial instruction to the jury charged them to base their verdict on Virginia's current law that defined death as "total stoppage of the circulation of the blood and a cessation of animal and vital functions …" Members of Harvard's ad hoc panel testified for the defense, apparently influencing the judge to reconsider his earlier instruction and finally charge the jury to consider "among other things the time of complete and irreversible loss of all function of the brain" in determining the time of death. Whereas, the judge's "change of heart" assured Lower's exoneration, it did not lead to a redefinition of death as proclaimed in the contemporary press.

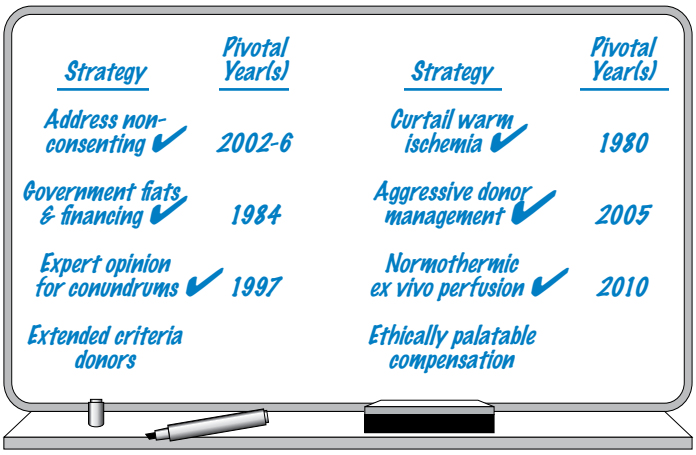
Neither Harvard's ad hoc committee, nor Tucker vs. Lower, could turn medically defined brain death into a legal criterion of being dead.[11] The committee had no authority or jurisdiction to do so. Lower and Hume must not have believed that the neurologist's declaration that Tucker's brain was "dead" meant that Tucker was dead. Why else would they have exposed a donor heart to 5 minutes of apnea? The lay jury did not have all the medical facts, including uncertainty about the pre-excision status of Tucker's heart, and simply concluded that no wrongful death had occurred. Two months later a Task Force on Death and Dying of the Institute of Society, Ethics, and the Life Sciences published their appraisal of the Harvard criteria in the Journal of the American Medical Association.[12] They observed that "These proposals have been widely discussed both by physicians and by the public. While they have gained acceptance in some quarters, they have stimulated considerable controversy and criticism and have given rise to some public disquiet." They then sought to mollify the latter by adding "The new criteria are meant to be necessary for only that small percentage of cases where there is irreversible coma… and where the traditional signs of death are obscured because of the intervention of resuscitative machinery."

The Dead Donor Rule and Society's Need for Donated Organs

The Dead Donor principle states that vital organs should be taken only from dead patients, and that retrieval of vital organs for transplantation should not cause the death of the donor.[13] Brain death became compatible when it was codified by the Uniform Determination of Death Act (UDDA), drafted in 1980 by the National Conference of Commissioners on Uniform State Laws. The UDDA states that: "An individual who has sustained either (1) irreversible cessation of circulatory and respiratory functions, or (2) irreversible cessation of all functions of the entire brain, including the brain stem is dead. A determination of death must be made in accordance with accepted medical standards." It was quickly endorsed by the American Bar and Medical Associations, and adopted by 45 States. The others relied on precedent-setting court cases but also cited the UDDA.

By 1980, iterative improvements in donor management, ex vivo preservation, immunosuppression efficacy and toxicity, and acute rejection diagnosis, along with the computer based United Network for Organ Sharing (UNOS) had advanced whole organ transplantation to the status of a predictable and widely applicable therapy. This resulted in a burgeoning demand, and an ever widening gap between society's need for organs and their availability. Ethicists, who had properly focused on protecting the rights and sanctity of donors faced new threats from aggressive efforts to increase the number of available organs and were simultaneously obliged to ponder means whereby this ethically defensible goal could be achieved with the least discomfort.

White Board Proposals for Increasing Number and Quality of Donor Organs



Why They Say No

Neal Garrison[14] of the University of Louisville was the first to advocate decoupling discussions of being brain dead and organ donation in an optimistically titled article published in 1991. He and his colleagues found that bringing up the two issues together, ostensibly to create some good out of a bad situation, resulted in only18% of 62 families consenting to donation; whereas, proposing donation after the family had time to assimilate the implications of brain death, resulted in a significantly greater 57% (53/93) consent conversion rate. The Kentucky Organ Donor Affiliates Organ Procurement Organization (OPO) instituted hospital based Family Support Liaison programs in most of the same hospitals in 2004, which was associated with a conversion increase from 42 to 72% over the succeeding 4 years.[15] A similar program introduced at Los Angeles County Hospital in 2001 resulted in a significant consent rate increase and a 17% increment in donated organs.[16]

The principles of obtaining consent are teachable but require careful listening, patience, and empathy. Physicians certainly have the latter quality, but patients and families are happiest when their doctor is focused on the best possible outcome for the patient, especially when the situation is dire. Moreover, some surgeons' personal perspectives may interfere with their being convincing proposers of organ donation. A New York University and Albert Einstein College of Medicine survey of 30 surgical attendings, 41 surgical residents, and 35 medical students revealed a 61% overall willingness to be organ donors with proportionally more older and experienced respondents expressing refusal.[17] Among all responders, only 49% had declared themselves as an organ donor on their driver's license. Both institutions have busy transplant centers, and 13% of those who would not permit removal of their own organs indicated that their refusal stemmed from observing or being involved in a procurement procedure. Physician reluctance is also the best explanation for the results of a study conducted in a semi-rural UK National Health Service Trust in the greater Manchester area.[18] Instituting mandatory referral of all potential organ donors to an existing, in-hospital, 24/7 Bereavement and Donation Service in 2007 increased the number of potential organ donor referrals from a fairly constant 3 per year to 31 referrals in the mandate's first 11 months.

Families' concerns about whole organ donation include a basic core that should be anticipated and brought out at points in the conversation when a family seems less forthcoming. Ambiguity about brain death is the basis for many of their issues.[19] They worry that consenting might result in withholding a treatment, which could conceivably give the patient a slim chance to recover, that the patient will feel additional pain from diminished medication to preserve circulation, or as part of the procurement procedure, and that they cannot be with the patient when he dies. There are also cost concerns about additional hospital charges and that preparation of the body for viewing will be more costly, or not even possible and religious concerns about delaying burial.

In 1978, Clive Callender,[20] an early transplanter and now former Surgical Chairman at Howard University convened a group of 40 individuals to address why minorities and blacks in particular, were reluctant to become organ donors. Their findings encompassed unawareness, misperceptions, distrust, fears of prematurely being declared dead, and disregard for a preference for black organs going to black recipients. The result was the 1982 birthing of a District of Columbia Organ Donor Program which stressed "face to face presentations by culturally sensitive and ethnically similar community messengers who were health care providers, transplant recipients, persons awaiting transplants, donors and donor family members." This educational outreach by transplantation stake holders increased the number of DC residents signing donor cards from 25 to 750 per month and raised the percentage of African American organ donations from 3% to nearly 12% within one decade. In 1955, Callender leveraged this success into a national Minority Organ Tissue Transplant Education Program, known as "MOTTEP." NIH funding allowed MOTTEPP to create specific programs for Latino-Hispanics, Asia and Pacific Islanders, and Native Americans in community-based MOTTEPs that stretch from Honolulu to the US Virgin Islands, achieving near parity in minority organ donation.

Minority Donation & US Population Ethnicity

Ethnicity	Population %	Donation Rate %
White	71.7	73
African American	12.7	12.8
Hispanic	10.9	11
Asian	3.8	2.0

Sources: UNOS data, June 2010 and 2000 US census data

Envision one of Callender's "culturally sensitive and ethnically similar" transplant stake holders in a family consultation room. You're Irish; the man sitting across from you talks like you do, uses the same phraseology, and looks a little like your older brother, who is being well cared for in the ICU with what you now recognize as an essentially hopeless head injury. As the conversation progresses you learn that the man across from you loves his Guinness almost as much as your brother did and is able to be with you because someone else in a similar situation said, "Yes, he would see it that way too."

Is It Time To Take The Gloves Off?

Although often initially viewed as contentious intrusions, the US government's role in advancing organ donor management has been remarkably enlightened, beginning with its 1984 mandating of non-profit OPOs and establishment of The Organ Procurement and Transplantation Network (OPTN).[21] The latter is a fabulous resource and a model for public-private partnership. The Department of Health and Human Services' Health Resources and Services Administration (HRSA) underwrites its cost and contracts with non-profit UNOS to direct its programs. The total number of US deceased donors peaked at 8,085 in 2007, and declined to 7,990 in 2008, the first ever deceased donor decline in OPTN's history.[22] This occurred despite an increase in the number of DCDs and was aggravated by

the widely publicized November 2007 transmission of HIV and hepatitis C from one organ donor to four recipients. There were 8,021 deceased donors in 2009, still lagging behind 2007. OPTN lists more than 108,000 persons waiting for organs as of August 16, 2010: 79% are waiting for kidneys and about 1.4% of those waiting for all organs have died on the list in the first 6 months of this year. Are these data sufficiently compelling to warrant our government shifting its stance from facilitating organ donation to legislating intervention?

Presumed consent means that a citizen has to opt out while living by registering his refusal in a government maintained database. Individuals would carry a card documenting their registration, but OPOs would be legally required to check the electronic database. Austria, Belgium, Singapore, and Spain, among others have this system.[22] Before and after data are available for the first three. In Austria, where the law was implemented in 1982, a 7-year, 4.6 donors per million of population per year (DMY) rate rose 119%, to 10.1 donors per million (DM) in its fourth year. The 5th year included expanding to full time transplant coordinators and together they produced 27.2 DM – a 490% increment over the pre-enactment baseline. Opt out legislation in Belgium and Singapore in the '80s and early '90s initially just applied to kidneys: in 3 years, Belgium's kidney donation rate had doubled to 41.3 DM, and Singapore's increased 6.7 fold from 4.7 to 31.3 DM. Transplantation had become a growth industry in those years, which is what prompted enactment of the laws. These retrospective observations overestimate the law's influence by not being adjusted for projected rate increments had the laws not been enacted.

Maryland's R. Adams Cowley Shock Trauma Center compared their year 2000 traumatic brain injury (TBI) admissions with those of the Lorenz Böhler Trauma Hospital in opt-out Vienna providing a tiny but unblemished data set.[24]

Shock Trauma Center & Lorenz Böhler Year 2000 TBI Admissions

	STS	LBTH
TBI	761	276
Glasgow coma scale 3-8	258 (34%)	52 (19%)
Brain-death & medically suitable	39 (15%)	7 (13%)
Organ donors	18 (46%)	7 (100%)
Organs transplanted per donor	3.8	4.0

Presumed consent is an encroachment on autonomy that could alienate a substantial proportion of the public. Its introduction in Sweden in 1966 was associated with a decrease in organ donation from a mean of 13.4 DMY in 5 preceding years to an average of 12.5 DMY for the next 5 years.[25] But, the donor rate had already dropped to 11.7 DM in the year before the law was implemented. Both mandated and presumed consent can have hard and soft variants with the latter allowing the family to override a patient's elected intention to donate or failure to opt out, which might make the legislation more palatable. Noteworthy or not, Sweden's opt out is the soft variant and a family veto provision is actually a further encroachment on donor autonomy.

A political debate based on presumed consent's existing confusing and uncontrolled data would be polarizing and inconclusive. A carefully designed study of two populations with similar established donation patterns and one enacting an opt out law should be a required prerequisite, but it too might be subverted by the Hawthorne effect* which would likely increase donations in the state without a new law.

*Simply studying a process improves its human performance component.

Mandated consent is a halfway step that preserves autonomy and would be compatible with current driver licensing. By a certain age all adult persons would have to consider organ donation and enter their decisions into an electronic database.[26] Your first license after the law's implementation would not be issued without your indicating yes or no and a birth-certificate component of the database would seek out persons who were of age but had not sought a driver's license or gone elsewhere to register.

The New Jersey Hero Act, passed on July 22, 2008 will demand in 2013 that all NJ drivers indicate a decision to be a donor, or acknowledge being adequately informed without consenting to be a donor.[27] Its interim measures ensure access and an informed citizenry well before then. The NJ Motor Vehicle Commission now maintains a "Donate Life NJ Registry" accessible to non-drivers, as well as drivers, through an online portal. NJ public schools include information about organ and tissue donation in the Core Curriculum Content Standards for Comprehensive Health and Physical Education for grades 9-12. Public institutions of higher education must now provide the same information, either through student health services or as part of the curriculum. All Medical and Nursing schools in NJ must include instruction in organ and tissue donation and recovery as a condition of receiving a diploma and offer such training for continuing education credit. Beginning in 2011, physicians licensed prior to the act will be "encouraged" to complete an online, credit-based course, and previously licensed nurses will be required to take an online, one credit hour course to be relicensed. Enlightened, yes - is it intrusive, ask New Jersey nurses in 2011, or better yet, defer judgment to early 2016 when OPTN will have 3 years of comparative data.

Brain Death's Discomforting Hegemony

Brain death's broader acceptance is pragmatic, especially valued for the recovering thoracic organs, and served as the predominant source of transplant organs for the decade of the 1980s. Neurologist, James Bernat and his Dartmouth colleagues even criticized UDDA's portrayal of irreversible cessation of circulatory and respiratory functions and of all functions of the entire brain, including the brain stem, as equivalent descriptors of being dead. In their minds, "Permanent cessation of spontaneous cardiopulmonary functioning works as a test of death only in the absence of artificial cardiopulmonary support because only there does it produce the true standard of death - the irreversible cessation of all brain functions."[28] Others have had difficulty looking past some discomforting facts. The brain is not always completely dead. Viable areas with neurologic functions unrelated to clinical brain death (B-D) criteria remain such as those, regulating the secretion of hypothalamic hormones, accounting for a less than 50% incidence of clinical diabetes insipidus. Patients with irreversible coma look disconcertingly normal, as if they have adjusted to the ventilator and are asleep. Mechanical ventilation and nutritional support can allow such individuals to metabolize enteric feedings, excrete waste, and survive for months, for example, to give birth to nearer-term babies by caesarean section.

Robert Truog, an anesthesiologist-ethicist at Boston Children's Hospital and Franklin Miller[29] of NIH's Bioethics Department concluded that "… although it may be perfectly ethical to remove vital organs for transplantation from patients who satisfy the diagnostic criteria of brain death, the reason that it is ethical cannot be that we are convinced that they are really dead." They viewed valid consenting by the patient or a surrogate for withdrawal of life support, or for organ donation, as being separate but similarly proper ethical

bases for the consented action, be it disconnection from the ventilator, or removal of a vital organ. Bernat[30] attempted to diffuse these concerns by separating detectable but "random and purposeless cellular physiologic activity" from the brain's irreplaceable systemic integrated functioning, but some countries and the world's three monotheistic religions would wrestle with their reservations about brain death's parity with circulatory demise for years.

Denmark

A Danish Council on Ethics was formed in 1987 to study the issue and reported in 1988 that a majority thought that retaining the cardiac death criterion was perfectly consistent with organ donation.[31] They recommended that the time of death be the cessation of heart function, but with the aid of a respirator it should be possible to artificially defer the "death process" for up to a maximum of 48 hours for removing organs for transplantation.

This meant that relatives had to take leave of the donor while the heart was still beating for the patient to be moved to the operating room where the respirator was turned off. After the patient's heart stopped, the respirator was reconnected and an attempt was made to restart the heart or continue manual massage until the kidneys had been removed. In 1989, the Council abrogated the Dead Donor rule, agreeing to the taking of organs once brain function had ceased "during the death process," but that the time of death was when the heart later stopped. The Council then promoted an unprecedented public debate by widely distributing its deliberations. Public opinion was 80% in favor of the Council's minority, who recommended declaring death when B-D criteria were satisfied, leading the Danish Parliament to endorse the public's sentiment in 1990.

Japan

Japan had difficulty overcoming repercussions from the Jura Wada case, delaying passage of an Organ Transplant law until 1999. The prevailing attitude towards brain death remained tentative and was reflected in statutory treatment of brain death differently from cardiac death where family members or the patient could consent to donation.[32] Brain-death donation required advanced written consent by the potential donor, and family members could override the donor's intention, resulting in just 81 brain-death donors over 12 years. The legislature revised the law in 2009 to accept family consenting for brain-death donation if the potential donor had not intentionally opted out, but should he have indicated prior willingness to donate, the family still has veto power when irreversible coma is the death determining criterion.

Judaism

Judaism is generally supportive of brain death but has had to subordinate "The principal of ain dochin nefesh mipnei nefesh – that one life may not be set aside to assure another life – applies with full force even where the life to be terminated is of short duration and seems to be lacking meaning or purpose" and to reinterpret a responsibility into empowerment: "… God has imposed on man the awesome responsibility of defining the moment of death, the point after which the needs of the dead may, and indeed must, be subordinated to those of the currently living.[33] In the earliest years of heart transplantation, heart donation was forbidden and viewed as a double murder - that of the donor and that of the recipient. The Chief Rabbinate of Israel officially accepted brain-death as being dead in 1986, and the Rabbinical Council of America did the same in 1991.

Islam

"In Islam, the killing of a terminally ill person, whether through voluntary active euthanasia or physician assisted suicide, is judged an act of disobedience against God."[34] However, intent and consideration for a patient's well being can create a situation wherein the act of disobedience is acceptable: "Pain relief treatment or withholding or withdrawing of life-support treatment, in which there is an intention of allowing a person to die when there is no doubt that their disease is causing untreatable suffering, are permissible." Jordan's Council of Islamic Jurisprudence (majma` al-fiqh al-islami) incorporated "complete cessation of all functions of the brain, when expert physicians ascertain that the cessation is irreversible and the brain is in the state of degeneration" into the guidelines provided by the Shari`a to determine death.[35] The change concludes with: "In this condition it is permissible to discontinue the life supportive system from the patient even when some of the patient's organ's like the heart are kept functional by artificial means. *God knows the best!*"

Catholicism

The Catholic Church is unique in having a succession of single, infallible, earthly leaders to limit wavering, yet its views, publically aired in 2008, typify the ruffling affect of inherent contradiction. Lucetta Scaraffia,[36] a Professor of Contemporary History at Rome's La Sapienza University, authored the front page article of the September 3, 2008 L'Osservatore Romano, recounting the Vatican's position on brain death and describing dissention with its original posture. The Catholic Church endorsed Harvard's ad hoc committee's concept of irreversible coma in a 1985 Pontifical Academy of Sciences statement that was reinforced in speeches by John Paul II in 1989 and again, in addressing the Transplantation Society's World Congress on August 29, 2000. All legitimized organ removal from donors defined as dead after irreversible coma has been verified, even if still breathing† and the heart is beating. But when the Pontifical Academy of Sciences met in January 2005 to discuss the question of the "signs of death," the assembled philosophers, jurists, and neurologists from various countries, agreed that brain death is not the death of a human being, and that the criterion of brain death, not being scientifically credible, should be abandoned.

Benedict XVI,[37] who had not previously spoken on the subject as Pope, granted a Nov 7, 2008 audience to participants in Rome's international congress on "A Gift for Life: Considerations on Organ Donation." After noting that "Organ donation is a unique testimony to charity …" and acknowledging "the long waiting list of those whose only hope for survival is linked to the small number of non-useful donations." The Pope made the following statement that seemed gently or at least partially to refute Scaraffia's article:" In any case, it is useful to remember that the various vital organs can only be extracted "ex cadavere" [from a dead body], which posses it's [sic] own dignity and should be respected. Over recent years science has made further progress in ascertaining the death of a patient. It is good, then, that the achieved results receive the consensus of the entire scientific community in favor of looking for solutions that give everyone certainty. In an environment such as this, the minimum suspicion of arbitrariness is not allowed, and where total certainty has not been reached, the principle of caution should prevail."

Donation after Cardiac Death (DCD)

Non-beating heart donors were the primary source of cadaveric renal allografts for almost 20 years and the source of the heart

†Clinical diagnosis of higher brain death or the vegetative state

for Christian Barnard's second, and the world's first successful heart transplant.[38] Organs sourced from beating-heart, B-D criteria donors were associated with better outcomes but accounted for only a fraction of in-hospital deaths. Controlled DCD began with the University of Pittsburgh Medical Center's 1992 promulgation of a "Policy for the Management of Terminally Ill Patients Who May Become Organ Donors After Death." and subsequently modified to "… After Removal of Life Support."[39] These patients were ventilator and often pressor dependent. The patient or family did not wish to continue supportive care and would readily consent to its withdrawal.

The potential for organ donation depends on separate consenting and likelihood that support withdrawal will shortly result in cessation of effective circulation. The donor's physicians discontinue the support, document the patient's course, provide comfort care, administer heparin, and in most instances, insert femoral cannulae under local anesthesia to be used for in-situ cooling. As originally described, they pronounce the patient dead after no pulse can be appreciated for a 2-minute interval thought to be sufficient to preclude cardiac autoresuscitation. The transplant team is then called to assume management of the decedent. This has to be a tightly protocoled sequence of compassionate care and hand off that considers the patient, the family, and the quality of the organs that will be going to recipients.

The patient and family are best served by the end of life care being given by physicians and staff with whom they are familiar and by allowing them to be present until death is declared, even in an operating room, if that is their choice.[40] The focus is on the patient's comfort, compliance with his wishes and those of his family, and on helping them to understand why certain drugs or treatments are included to ensure the quality of their gift to others. Selecting patients for whom withdrawal of life support should result in death in less than an hour, or at most two, is in the best interests of all parties and is somewhat predictable based on the number and criticality of life support measures and the patient's infirmity.[41,42]

Predictors of Death within One Hour of Life Support Withdrawal[‡]

Pre Withdrawal	**Likely**	**Unlikely**
Age 0-30+1, 51±3	3	1-2
BMI <25=1, >30=3	3	1-2
Heart rate <60=3, 60-100=1, >100=2	3	1-2
Systolic BP <85=3, 85-105=2, >105=1	2-3	1
FiO2 <0.5=1, 0.5-0.8=2, >0.8=3	3	1-2
Tracheostomy=1, intubation=3	3	1-3
Number of vasopressors 0, 1, 2, 3	2-3	0-1
Withdrawal + 10 min		
O2 Sat % >90=1, <79=3	3	1-2
Apnea=9	0 or 9	0
Rate>12=1,<12=3; Tidal volume >200=1, <200=3	6	2
Inspiratory force >-20=1, <-20=3	3	1
Total Score (>30 ≈ 0.5 probability of death within 60 min)	31-42	10-18

[‡]Unverified blending of two data sets

Health professionals and families were discomforted by the imprecision of basing the end of a life on not feeling a pulse for 2 min. In 1997, The Institute of Medicine (IOM) suggested that "accepted medical detection standards include electrocardiographic changes consistent with absent heart function, [along with] zero pulse pressure [as monitored] through an arterial catheter."[43] These parameters were quickly entered into most hospital protocols. Electrocardiographic silence is not required, because the criterion determining death is the absence of effective circulation. The IOM also suggested that 5 minutes, rather than 2 minutes, be adopted as an arbitrary, but reasonable standard, in line with the Society of Critical Care Medicine's recommendation of not less than 2 or more than 5 minutes. Hospitals were slower in accepting this recommendation but more than 90% of OPOs now use 5 minutes.

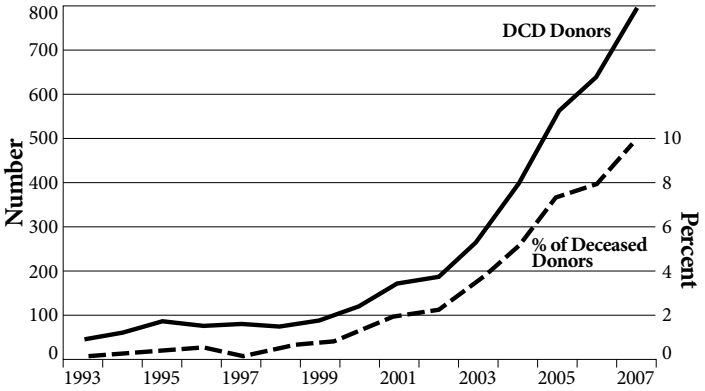
"Irreversible," The Elephant in the Room

Irreversibility is not an absolute phenomenon. Irreversible is a conditional adjective that needs constraints specifying the situation surrounding the nouns that are being labeled. Its unconstrained use introduces ambiguity into Harvard's definition of profound coma and the UDDA's stipulation of cessation of circulatory and respiratory functions and of all functions of the entire brain, including the brain stem as the two legitimate routes to being declared dead. The irreversible nature of the loss of all functions of the entire brain, including the brain stem is not based on experimental data, and should never be tested in human patients because the halfway state that might be achievable is not good for the patient or society. The DCD process shines a bright light on the elephant. Two or 5 minutes of no or ineffective circulation from ventricular fibrillation or stand still is typically reversible in an operating room setting. It is irreversible in DCD, partially because the heart is hypoxic and maybe dilated, but mainly because the care givers have determined, with the family's consent that the patient should die. Busy procurement coordinators will tell you that they have experienced at least one resumption of a shallow pressure tracing when they began measures to curtail warm ischemia, which is addressed by asking the donor's physicians to return and do another 5-minute countdown. This should not be surprising. The data pertaining to 2 or 5 minutes being sufficient to avoid autoresuscitation are anecdotal, and spontaneous resumption of cardiac activity has been reported to occur as long as 33 minutes after abandoning CPR.[44]

The Denver Children's Hospital Pediatric Heart Transplant Team[45] conducted a cautious, IRB approved exploration of the potential to recover a DCD infant heart. This involved three infant donors over a three-year period from 2004 to 2007 in which the team also performed 17 infant heart transplants sourced from brain-death, beating-heart donors. The protocol required that death occurr within 30 minutes after extubation for the patient to be a candidate donor and that the critical care attending should wait 3-minutes after the cessation of effective circulation before declaring the infant as being dead. The first recipient had lived for at least 6 months, but the hospital's Ethics Committee, being more concerned about the recipient getting a good heart than preserving an ireversible fallacy, recommended that the observation for the next two infants be shortened to 1.25 minutes. All three recipients survived for 6 months which was the primary endpoint and no late deaths occurred over the observation period of 3.5 years.

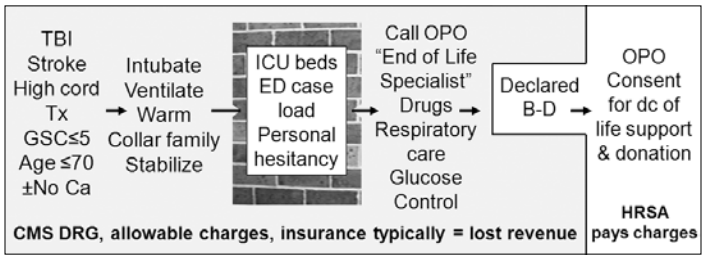
Bringing the elephant out into the bright sunlight evoked responses from four ethicists. Truog and Miller's way out has already been aired. Professor Robert Veatch[46] of Georgetown's Kennedy In-

stitute of Ethics, whose analysis clarified Tucker vs. Lower's ramifications, observed that "If a heart is restarted, the person from whom it was taken cannot have been dead according to cardiac criteria." He pondered the dilemma of having to abandon the Dead Donor Rule or liberalize B-D criteria and worried about criminal prosecution, without reaching a conclusion. Bernat[47] was the least disturbed, declaring his confidence in the 75-second interval's assurance of irreversibiity in the donor and suggesting that restarting the donor's heart in another individual might be irrelevant to the irreversibility of the donor's cardiac death. This was an interesting quandary but is really a non-issue. Primary graft failure is the most common cause of short-term mortality after heart transplantation.[48] Its relationship to ischemia time is not linear, but primary graft failure has a lethality of 85%, so non-heart beating donors are not US heart donors.



The DCD debate continues with some advocating procurement of kidneys from unsuccessfully resuscitated patients, termed Uncontrolled DCD, as opposed to Controlled (intentional) DCD.[49] Others are arguing that DCD in either form contravenes both the Dead Donor principle and the UDDA.[50,51] This leads them to the unhappy conclusion that the organ supply-demand gap trumps reverence for the Dead Donor principle and UDDA's unconstrained use of irreversibile as a contingency to circulation cessation. Invasive innovations directed at preserving organ function aggravate this ethical dilemma, particularly when they acknowledge the charade by excluding the brain and heart from access to their interventions.[52] Public acceptance of DCD is surprisingly good as judged by the proportional increase of DCD donors. Registration to be a donor is not a consent to withdraw life support. Rarely is a suitable potential DCD donor able to give valid consent for withdrawing his own life support – amyotrophic lateral sclerosis can be one example – making DCD essentially a family affair that likely reflects Truog-Miller's consent-driven ethicality.

Stop Lost Organs and Revenue



A university trauma service reviewed 135 TBI patients, who died in the hospital during 2004, looking for missed brain death organ retrieval opportunitites.[53] DCD was not an option. The mean age was 38.5 years, and the mean ICU stay was 2.5 days. Less than 24-hour deaths, withdrawal of care before progression to brain death, and medical unsuitability or death before donation in the 52 patients meeting B-D criteria eliminated 70% of the TBI population. Donation was declined by 15 familes, leaving just 25 donors or 18.5% of original pool. The authors focused on failures to get consent and limited donation of thoracic organs in non-chest injury patients. The cartoon and their data suggest that more could be gained by boosting care-giver ardor for organ retrieval and OPO intercession. Early identification of potential donors and verification of their potential by aggressive resuscitation and OPO involvement might have prevented many of the early deaths and determinations of futility before brain death in those short ICU stays, as well as a improving consent conversion. An all out effort to resuscitate and stabilize presumably non-surviveable TBI patients will yield more transplantable organs and diminish hospital revenue loss from uncompensated services through HRSA reimbursement. Its spotty application, especially with variable OPO involvement, would be disruptive, difficult for families, and increase the hospital's burden of uncompensated services.

HRSA launched an Organ Donation Breakthrough Collaborative in 2003 to study retrieval process at high performing institutions and disseminate best practices to what eventually became 950 of the Nation's largest hospitals. In 2004, the Collaborative set a 75% conversion rate as a National Goal, and in 2005. they added achieving a yield rate of 3.75 organs transplanted per donor (OTPD) and DCDs comprising at least 10% of an institution's deceased donors.[54]

Pharmacologic and Hormonal Mitigation of Brain Injury's Systemic Effects

Cerebral hemorrhage, trauma, or an anoxic event results in brain edema which impedes perfusion within the fixed confines of the skull. Ischemia of the hypothalamus induces the Cushing reflex (widening pulse pressure and bradycardia) and hemodynamic instability. Brain inflammation disrupts the blood-brain barrier leaking inflammatory cytokines to cause similar changes in other organs.[55]

A retrospective study compared the transplanted organs accruing from 700 brain death donors who received a methylprednisolone bolus and infusions of vasopressin and either triiodothyronine or L-thyroxine to those recovered from thousands of registry brain death donors who had received none, one, or two of the three hormones. The triple hormone group yielded significantly higher 3.8 OTPD rate vs. 3.1 for the controls, and a multivariate analysis, including demographic factors, showed significant incremental organ transplant probabilities ranging from 2.8% for lungs to 7.3% for kidneys.[56] The huge none-one-or-two control group makes small differences statistically significant but introduces a plethora of unknowable management variables that weaken the data.

There is a historic precedent for the cocktail approach: Novitzky and his colleagues[57] at Cape Town's Groote Schuur hospital mixed 2μg of Triiodothyronine, 100mg of cortisol, and 10-30 IU of insulin to be given as often as hourly to 26 potential brain death donors from the time of consent until recovery of the heart. They observed significant improvements in donor MAP and base deficit, as well as a related halving of the dopamine dose.

Methylprednisolone has been prospectively studied in 100 B-D criteria liver donors randomized to receive or not receive a 250 mg bolus of methylprednisolone at the time of consent and subsequent infusion of 100 mg/h until organ recovery.[58] Methylprednisolone resulted in significant down regulation of inflammatory signaling factors and significantly less ischemia/reperfusion injury, as evidenced by lower AST and ALT levels over the first 10

post-transplantation days, and lower total serum bilirubin levels at 10 days and out to 6 months.

Seventeen years ago, Orlowski and Spees[59] reported a 95%, 30-month survival for 21 heart grafts obtained from L-thyroxine treated brain death donors compared with an 83% 30-month survival for grafts taken from donors not receiving the hormone. L-thyroxine or its synthetic analog T4 act synergistically with vasopressors in brain injured patients, increasing their efficacy at lower doses to the point of sometimes being able to discontinue them.[60] The same study has shown that despite T4's selective use in more unstable donors, it is associated with a significantly higher OTPD rate of 3.9 vs. 3.2 from donors in whom T4 was not needed.

With respect to insulin, brain death's sympathicomimetic inflammatory milieu increases gluconeogenesis and peripheral insulin resistance while impairing its release from the pancreas. Free-water loss from depressed or absent vasopressin secretion requires substantial dextrose water infusion to combat hypernatremia and doubles the imperative for closely monitored intravenous insulin administration to prevent glucosuria compounding diabetes insipidus' free water clearance. There is no direct evidence associating hyperglycemia with poor transplant outcomes, but Blasi-Ibanez et al.[61] at the University of California, in San Francisco, showed that pre-recovery hyperglycemia was common among 458 deceased kidney donors: 72% had final glucose levels >200 mg/dl and 39% had levels ≥250 mg/dl. Admission creatinine and glomerular filtration rates were the primary determinates of poor, pre-recovery renal function, but it was also associated with hyperglycemia and wide swings in glucose levels. A study is in progress using a computer based insulin delivery system to target donor glucose levels between 100 and 140mg/dL.[62] The baseline delivery protocol has been sufficient for nearly 75% of organ donors. The others have shown remarkable insulin resistance, frequently requiring 30-40U boluses and infusion rates in the order of 40-50U/h.

Protocolized Donor Management

UNOS Region 11, encompassing Kentucky, Tenessee, Virginia, and the Carolinas, developed a Donor Management Goal (DMG) panel of clinical variables aimed at meeting HRSA's target for organs transplanted per donor.[63] A Phase 1 trial instituted in November 2008, suggested that the arbitrary values were a bit too strict so we will focus on Phase 2 which ran from May to December of 2009. There were 467 donors, and 82% of recovered organs were transplanted. Overall there were 3.34 OTPD.

DMG Achievement and Organ Transplant Probability

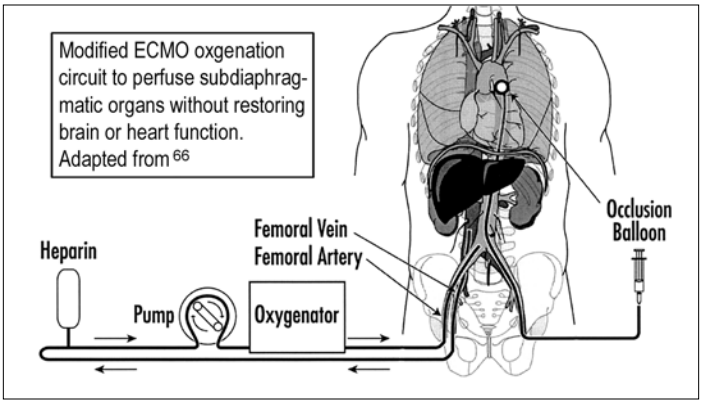
Final DMG target	% achieved	Significant impact if met
60-120mmHg MAP	≥90	None
4-12mmHg CVP	73	>15-fold increase for lung.
>80 PAO2 on FIO2≤40%	69	
7.30-7.45pH	≥90	None
Na ≤160meq/L	≥90	Negative for heart
Glucose ≤200mg/dL	83	Positive for pancreas
Single, low dose pressor	87	Positive for heart
4 hrs of Urine output 0.5-7mL/kg/h	≥90	None
All goals met	66	Positive for heart & lung

When all 8 DMGs were achieved, the transplant yield was 3.45 OTPD vs. 2.59 when they were not. Univariate analysis indicated that restricted pressor use, followed by PAO_2, and CVP were the primary predictors that a donor's recovered organs would be transplanted. A multivariate analysis related DMG achievement to specific organs and emphasized their particular relevance to the lungs and heart.

Los Angeles County Hospital and the University of Southern California Medical Center have managed potential donors according to a protocol introduced in 1998 that requires floating a catheter into the pulmonary artery to monitor aggressive fluid resuscitation.[64] If volume replacement does not yield a MAP ≥70mmHg which is true in nearly all instances, they begin a vasopressor, moving up to a maximum 10μg/kg/min. If that does not achieve a ≥70mmHg MAP, they are quick to turn to their 50% dextrose, 2g methylprednisolone, 20U insulin, 20μgT4 cocktail to be followed by continuous infusion of T4 at10μg/h. The protocol stresses vigilance and quick intervening to treat an expected near 50% incidence of diabetes insipidus with desmopressin (vasopressin if pressor action is desired), an approximately 10% occurrence of neurogenic pulmonary edema with high-frequency percussive ventilation, a >50% incidence of coagulopathy and thrombocytopenia with FFP, cryoprecipitate, or platelets, and occasional SIADH (inappropriate antiduretic hormone secretion) with hypertonic saline or fluid restriction. Adoption of this protocol was associated with an 82% increase in the number of donors, a 71% increase in organs recovered, and an 87% decrease in potential donors lost from hemodynamic instability.[65]

Normothermic, ex vivo Perfusion and Repair

Michigan Health System investigators used an extracorporeal membrane oxygenator (ECMO) normothermic circuit, isolated from the heart and brain, to perfuse sub-diaphragmatic organs in 15 DCD donors from whom 30 kidneys, 7 livers, and a pancreas were recovered.[66] The 2005 report is unforthcoming about outcomes except in



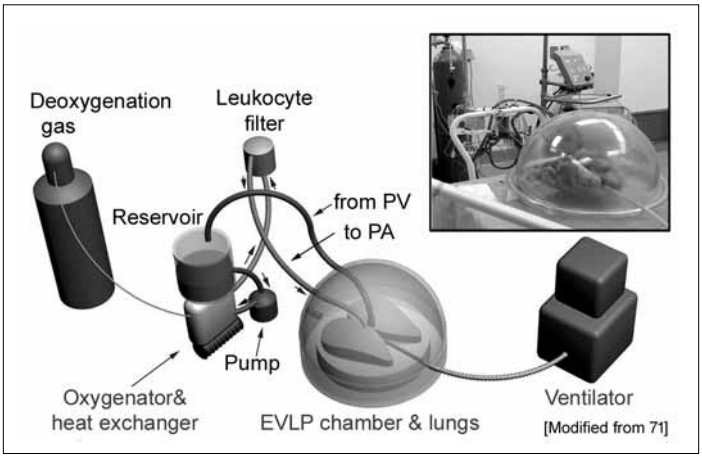
noting that 22/24 kidneys that were transplanted functioned immediately. No other series have been reported and the Michigan investigators have retreated to the laboratory.[67] Normothermic perfusion, however, is alive and well in several incarnations but not quite into the clinic. Cold perfusion has been the mainstay of organ preservation but is known to damage mitochondria through ATP (Adenosine-5'-triphosphate) depletion and alter plasma membrane lipids, cell structure, and microtubules, resulting in time-related cell lysis. This limits its use to 24 hours or less and when this limit is approached, both short- and long-term organ function are adversely affected.[68]

Brockmann and his colleagues[69] at Oxfords Nuffield Department of Surgery compared the survival of pigs transplanted with livers recovered from heart beating donors, or after 40 minutes of warm ischemia that were perfused for 20 hours with warm oxygenated blood or conventional cold University of Wisconsin solution.

The warm preservation livers were inserted into a pre-primed heat exchanger-membrane-oxygenator circuit with dual inflows to perfuse the hepatic artery and portal vein. The perfusate was autologous blood, laced with prostacyclin, heparin, soluble parenteral nutrients, lipid emulsion, and multivitamins. The heat exchanger was set at the pig's normal body temperature of 39°C. Recipient survival with heart beating sourced livers was 86% with normothermic perfusion and 27% with cold preservation. Perfusion of 40-minute-warm-ischemia livers with 39°C, nutrient-laden blood resulted in 83% recipient survival; whereas, recipients implanted with cold perfused identical warm-ischemia livers all died. Both differences were significant and had correspondingly significant differences in liver enzyme levels. Normothermic perfusion and probably some of the additives appear to have mitigated warm ischemia injury.

Stig Steen[70] of opt out Sweden's Lund University harvested contused lungs from a brain death traffic accident victim whose PAO_2 was 67mmHg with a FIO_2 of 70% for research after the lungs were turned down by every Nordic transplant center. He reconditioned the less injured left lung by beginning a slow <20mmHg pulmonary artery perfusion of Steen solution§ at 25°C and gradually increasing the perfusate temperature to 37°C. Ventilation included maintaining end-expiratory pressure at 5cmH_20 and a brief doubling to resolve a persistent medial basal segment atelectasis. Mean pulmonary artery pressures decreased and measurements of pulmonary vein perfusate PO_2 gradually improved. When the numbers stabilized, perfusion was discontinued, clamping the pulmonary artery cannula and trachea, leaving the lung semi-inflated to be immersed in Steen solution, which was kept at 8°C and ECMO oxygenated. The lung was implanted in a 70-year old man with a 20% of predicted FEV, 17 hours after it had been recovered. The recipient was quite active with 74% of predicted FEV at 3 months but died of sepsis at 11 months secondary to superimposing chemotherapy for an Epstein-Barr positive, large cell lymphoma in the transplanted lung on immunosuppression.

Cypel and others[71] at the University of Toronto used intra-airway delivery of an adenovirus vector encoded for the human interlelukin-10 gene. Il-10 is an anti-inflammatory cytokine that inactivates antigen-presenting cells, inhibiting pro-inflammatory cytokine secretion. They maintained clinically unsuitable lungs from 10 human donors, randomly selected to be transfected or not, in a normothermic ex vivo lung perfusion (EVLP) system for 12 hours.



The EVLP deoxygenates the acellular Steen solution perfusate returning from the lungs by exposing it to a gas mixture of 86% N_2, 8% CO_2, and 6% O_2 and then passes it through a leukocyte filter before sending it to the pulmonary artery to be re-oxygenated by the ventilated lungs. The adenovirus was diluted in water and sprayed into each segmental bronchus.

Transfected lungs showed significant improvement in perfusate oxygenation and pulmonary vascular resistance, a shift from pro-inflammatory to anti-inflammatory cytokine expression, and recovery of the alveolar-blood barrier's integrity compared to the control lungs. These observations suggest that ex vivo Il-10 gene therapy might salvage a portion the 80% of donor lungs that are injured by brain death's pro-inflammatory cytokine flood. The EVLP facilitates lung biopsies to aid in determining transplant suitability.

The heart has a higher metabolism rate than lungs or liver which make it especially vulnerable to cold storage ATP depletion and consequent cardiomyocyte death. It is going to be the toughest nut to crack, but one company already has a portable normothermic perfusion system that allows the heart to beat with adjustable loading, which can be set to optimize coronary perfusion.[72,73]

Epilogue

There will come a day when the giving of viable organs will be regarded as an expansion of individual sanctity, which it is. A multiplier is applied and dispersed into the minds of people who have good reason to remember and respect a person they never knew. Obituaries will laud the deceased as a willing donor, and children will grow up in families that view organ donation as an obligation that comes with their surname and the gift of life at birth.

Acknowledgement

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Invited Commentary

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Mathias Biebl Johann Pratschke

Organ transplantation only became accepted as a standard treatment for end stage organ failure when long-term graft survival was achieved by improved immunosuppressive strategies, together with the development of uniform definitions of brain death, enabling standardized organ retrieval in numbers sufficient to justify funding transplant centers. Improved clinical outcomes following solid organ transplantation engendered an enormous and ever continuing rise in patients entering waiting lists for transplantation, especially for kidneys. This mismatch results in many patients dying while waiting for transplantation. An analysis of the patients on the liver waiting list in the United States from 1999 to 2008 demonstrated a constant (although slightly declining) annual number of liver transplants. Despite all efforts, this is paralleled by an also almost constant dropout rate from death or becoming too sick for transplantation, affecting between 186.8 and 160.5 patients per 1000 patient-years at risk.[1] As a result, every transplant professional is ethically obliged to try to utilize every possible organ.

While there are considerable worldwide differences in the percentages of live donor transplants, mainly influenced by ethical and legal issues, the vast majority of all solid organ transplantations rely on organs from deceased donors. Broad acceptance of the medically precise definition of brain-death by the 1980 Uniform Determination of Death Act (UDDA) resulted in almost exclusive reliance on brain dead donors as the source of organs. Non-heart beating organ donation was reintroduced in the early 1990s driven by the urge to expand the potential donor pool to out of hospital cardiac arrests, unsuccessful in-hospital resuscitations, futile care-withdrawals, and severe cerebral trauma with persistent brain stem function.

The most favored form of donation after cardiac death (DCD) is controlled withdrawal of intensive care until the onset of cardiac arrest. In this situation, the transplant team's awareness of organ warm-ischemia vulnerability and their obligation to not deliberately transplant a severely damaged organ is pitted against the shortest ethically sustainable "no-touch" time between cardiac arrest and the start of organ perfusion. This discomforting dilemma is not a good exit strategy for the imbalance of organ supply and demand. The Belgian experience with DCD kidney retrieval showed that it did not substantially increase the total organ pool and rather resulted in a proportional shift from brain-dead to DCD donors by not allowing a potential donor to progress to brain death determining criteria.[2]

Different legal systems regarding organ donation have been implemented across the world to ensure ethical conduct in dealing with deceased donor organ recovery. One differentiating feature is how a government assumes the will of its people to become organ donors. Both "opt in" and "opt out" are ethically valid, diametric concepts that are operative within the Eurotransplant region, which encompasses Belgium, the Netherlands, Luxembourg, Germany, Austria, Slovenia, and Croatia. Some Eurotransplant countries require prior active consent of an individual to allow consideration of organ retrieval after death. Others, including Austria and Belgium, have implemented an opt-out approach as a national endorsement of transplantation's societal value, wherein an individual may actively decline to be a donor. While this legal setting is sometimes irrationally portrayed as encouraging unethical preying upon severely brain damaged trauma victims, the reality is far different. When a patient is declared brain dead, the decision whether organ donation will be considered is left with the patient's relatives, and their rejection to organ donation is always respected. Although Austrian law would allow for organ retrieval without involvement of the donor's relatives, the negative publicity of even a single case where the family's wishes were ignored would far outweigh the benefit of the organs retrieved. OPOs in opt-in countries probe a potential donor's

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thoughts about donation by asking the relatives, which is more similar than different from our approach in an opt-out system.

The Eurotransplant report for 2009 lists 25.4 donors per million inhabitants in Austria compared to only 14.6 donors per million in Germany where the culture and health care system are similar to ours.[3] It is important to recognize that this huge difference in organ availability also impacts the quality of the available organs. Our public perception of organ donation as a natural part of Austrian citizenship rather than private gifts to anonymous persons is a positive influence on family members' decisions and a substantial factor in our higher donation rates. In an opt-in system, healthy persons harbor a concept of their bodily integrity being violated, without its balancing societal value, and simply turn their minds away from this unsavory issue.

Donor management, which is the term commonly used to describe the ICU staff's efforts to stabilize organ function as the patient approaches brain death criteria, is ethically troubling because the patient is not yet a donor. In almost all societies the moment of death and the treatment of the earthly remains are characterized by dignity and silence. The loss of central physiologic regulation as brain stem function ceases goes unnoticed and does not perturb the silence. In reality, a violent "cytokine storm" is being unleashed that causes profound hemodynamic instability and, if untreated, eventual cardiac arrest. Fortunately, this is amenable to pharmacologic intervention. Many centers treat this expectantly with a preset cocktail of steroids, insulin, and thyroid hormone, as experimental studies have demonstrated tremendous up-regulation of pro-inflammatory cytokines in deceased donors in comparison to living donors. These cytokines aggravate the organ's subsequent ischemia-reperfusion injury which can be successfully mitigated by administering steroids.[4,5] Consensus is lacking about anti-inflammatory therapy of brain-dead organ donors, but we have routinely administered 1g of methylprednisolone prior to cold organ perfusion during the retrieval procedure, and have now changed donor preconditioning to repeated steroid pulses from the time death is declared until organ retrieval.

Hypothermic machine perfusion rather than static cold storage was once empiric, but now has a firm scientific basis for kidneys.[6] In Austria, our compact geography usually equates to brief cold ischemia times, so static cold storage has been the standard. We now use hypothermic machine perfusion for anticipated longer cold ischemia times when the donor hospital and transplant center are unusually far apart and for grafts that were subjected to prolonged warm ischemia, typically in DCD situations, improving both early organ function and prolonged survival.

Our transplant professionals deal every day with the tension between preserving the dignity and sanctity of the deceased donor and the life extending value of each successfully transplanted organ, always mindful of their obligation to use maximum diligence and every available tool to obtain the best possible organ quality and function in the recipient.

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Joe Buda, Paul Gerst, Jean Emond, with Jonathan and David Kinne

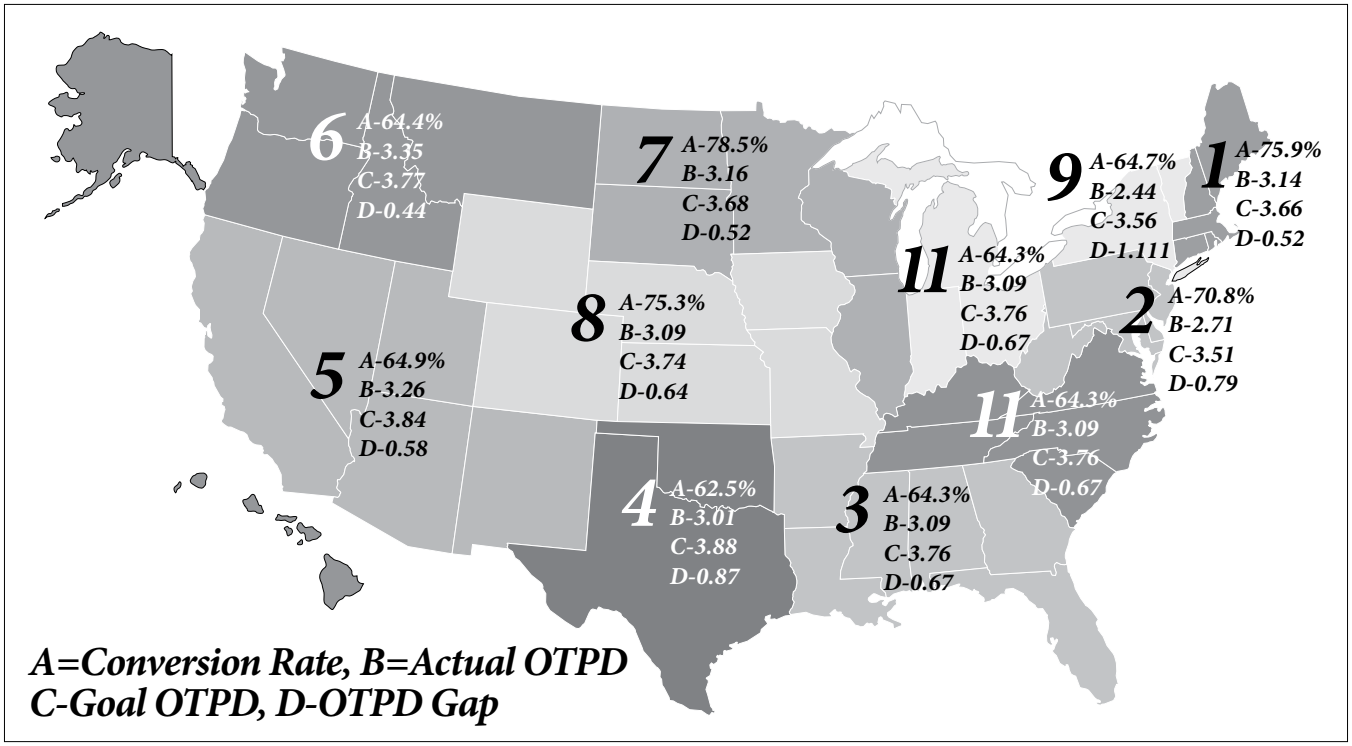
Nor Uriel, Ulrich Jorde, and Alexander Iribarne

John Schullinger has had enough break, but J.B. Price has Ken, Trisha, and Marianne Wolff in stitches.

Spencer Amory, Larry Jordan, and Val Jeevanandam

Regional Disparity in Access to Organ Transplantation– National and Regional Strategies for Bridging the Gaps

Michael J. Goldstein



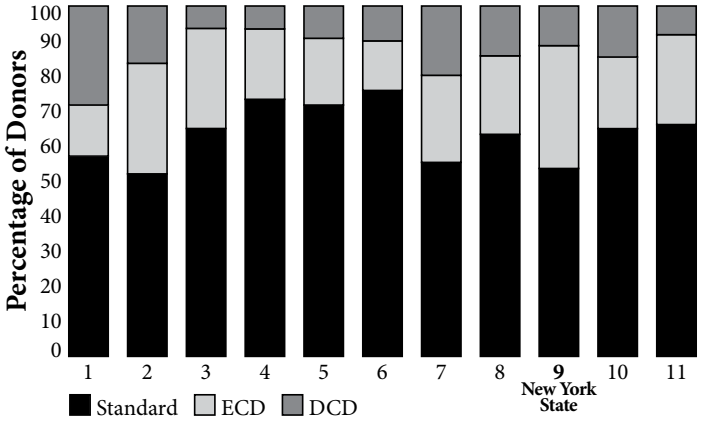
Donor efficiency by region: 9's 64.7% conversion rate and 2.44 OTPD

Michael J. Goldstein

The United Network for Organ Sharing (UNOS) employs 58 non-profit OPOs to manage the consent and organ recovery processes in a defined geographic area. This OPO-based model evolved through pioneers, practice-based improvement, and government oversight. The UNOS system for organ donation and sharing is one of the most successful in the world with 26.3 deceased donors per million people, but it does foster inequities. Access to transplantation has assumed more importance than organ quality. The national waiting list for life-saving organs has reached 108,000, which causes OPOs and transplant centers to focus on maximizing utilization of deceased donor organs. Organs recovered in an OPO donor service area (DSA) are allocated locally before they are allocated to other areas in the nation. The DSA donor demographics and, on a larger scale those of the UNOS region, vary significantly and define the balance between supply and demand, so organ availability and quality is highly disparate.[1]

Three key metrics define organ efficiency: consent conversion rate, organs transplanted per donor (OTPD), and percent donation after cardiac death (DCD). OTPD is a measure of donor health, organ quality, and transplant center utilization. A Health Resources and Services Administration (HRSA) task force developed these metrics to build a national collaborative throughout the country based on best practice strategies in 2004.

Deceased donors are classified into standard criteria (SCD), expanded criteria donors (ECD) and donation after cardiac death (DCD) donors. New Jersey, Pennsylvania, and West Virginia's Region 2 has the smallest percentage of SCDs with New York's Region 9 having the second smallest and the largest percentage of ECDs. ECDs, along with DCDs, yield fewer transplantable organs per donor, are typically unsuitable for thoracic organs and more likely to have delayed function than SCD organs.[2]



Deceased donors by type and region

Interregional organ sharing could reduce access disparity. Some OPOs are net exporters of organs due to small waiting lists, lack of transplant centers, or less aggressive transplant teams. It is imperative to get organ offers to centers that are most likely to utilize marginal or higher risk organs to limit discards. UNOS created DonorNet in 2007 to provide nationwide donor data to facilitate cross region distribution.[3] This notification system has actually hampered the ability to get undesirable organs to those willing to utilize them by replacing a simple phone call to an aggressive program with an

inefficient national game of "whisper down the list," leading to discards for prolonged cold ischemia. UNOS is evaluating new expedited placement strategies to fix this problem.

UNOS has spotlighted broader sharing to reduce waiting list deaths for years. The Department of Health and Human Services proposed its "Final Rule" to promote national equity over local necessity in 1998. Despite this, the OPO system of organ allocation remains focused on loco-regional demand to the detriment of disadvantaged neighboring DSAs. Advancements in the technology and medicine of organ preservation will facilitate wider sharing and more equitable distribution if, and when, policy makers get serious about parity.

Bridging the gaps between donation supply and local organ demand by growing transplant center waitlists is a national goal. The Alliance for Donation and Transplantation and HRSA's Donation and Transplantation Community of Practice are two forces committed to this challenge. Together these societies have co-produced the National Learning Congresses for donation and transplantation for the last 6 years. The goals have been to increase annual transplants performed to 35,000 by reaching four milestones: an average of 3.75 OTPD, 75% donor conversion rate, 10% DCD organ donation, and 20% growth in transplant center capacity.[4]

We are seeing some favorable trends in organ donation to help this effort. Both deceased and living donor numbers have increased in 2009 after a dip in 2008 – deceased by 0.4% and living by 6.3%. DCD donor numbers continue to increase and now comprise 11% of deceased donors. There were 21,853 deceased donor transplants performed in 2009, a 0.5% increase from 2008, and a 22.6% rise in all transplants performed comparing the periods before and after HRSA's National Donation and Transplantation Breakthrough Collaborative.

These changes have impacted the waiting list. There is a general "flattening" of the waiting list growth since 2007 – most significant for kidney listing. There were significant decreases in thoracic organ listing in the middle of the last decade, most likely representing a conservative listing strategy and poor access to good quality donor organs. We are now seeing a reversal with slight increases for heart and lung listings over recent years. Most importantly, death on the waitlist, which varies between 2-10% per year by organ type, has peaked and is falling for all organs!

Increasing donor designation on state donation registries is an important strategy for bridging regional gaps. Donate Life America is committed to publicizing donation awareness and working with state DMV agencies to increase actionable donor designation. Although far from its goal, national donor designation has risen among organ donors from 17% in 2007 to 28% in 2009. New York State has been among the least successful in this effort, with only 13% of its population on the consent registry, and dead last in the country with only 11% of DMV licensed drivers.

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JJSS Banquet Pictures

Arthur Lee, Kay and Kenneth Forde

Julius Jacobson quoting Osler about "Work... which makes the dull student bright, the bright student brilliant, and the brilliant student steady."

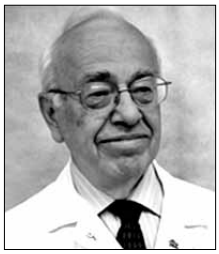
Normally, they give you a watch, but when you've been President for sooo long.....

Gary Tannenbaum, Michael Marvin, Lloyd and Andrea Ratner, Ellen and Carey Dolgin

Bioengineered Scaffolding for Cell Transplantation and Engraftment

Hugo P. Sondermeijer and Mark A. Hardy


Hugo Sondermeijer


Mark Hardy

Biomaterial carriers and tissue engineering offer great promise for enhancing survival and engraftment of transplanted cells. Many different materials have been investigated. Most of them have been known in the medical field well before tissue engineering was established. For instance, collagen has been used as a biological filler material for cosmetic surgery, and polymerized fibrin is used for repairing meningeal tears after head trauma, as well as for sealing bronchial fistulas and stopping bleeding from lacerated spleens and livers. Both of these materials are used as matrices to transplant cells to damaged tissues. Other materials have been engineered to mimic the extracellular matrix through functional customization. Biomaterials can be synthetic as well as naturally occurring. Ideally, they are biocompatible, non-immunogenic, sufficiently porous to accommodate newly formed blood vessels, and have predictable resorption rates. Polylactic acid (PLA) is a synthetic polyester that degrades into lactic acid in the body, which is easily removed. Naturally occurring polymers such as collagen, which can be recombinant or derived from animal products, and alginate from seaweed, have proved to be suitable for cell transplantation, but may induce unwanted immunologic reactions.[1] This problem with alginates, for example, can be overcome by proper chemical purification.

The goal of tissue engineering is to surmount the limitations of unstructured cell- and biomaterial-based therapies.[2] Growing relevant cells into a desired three-dimensional (3-D) organ or tissue in vitro is a commonly used tissue engineering maneuver. Without specific direction, cells randomly migrate across a plane, exhibiting contact inhibition, to form a two-dimensional, single-cell layer. They can be coached into acquiring an additional dimension by seeding them onto 3-D porous matrix scaffolds to attach and assume its structure through colonization.

Biomaterial- and cell-transplantation for treatment of type I diabetes mellitus

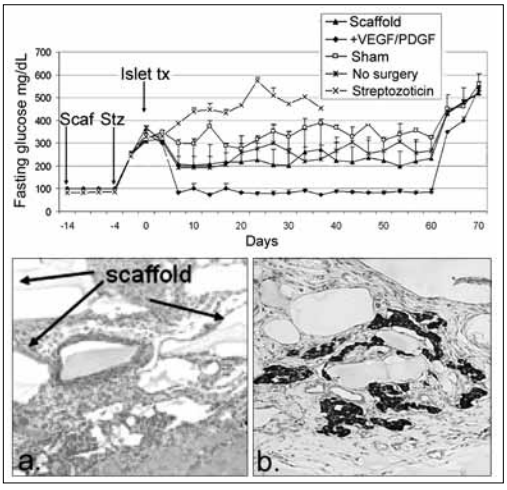
Clinical cell-transplantation trials for tissue regeneration have met with mixed results. In 2000, Shapiro and his colleagues[3] at the University of Alberta, in Edmonton, reported remarkable success with a steroid-free protocol for pancreatic islet transplantation with a median follow up of 12 months, which we have been unable to emulate. We have had just two successful clinical islet transplants, and their efficacy lasted only 6 months.[4]

Fourteen years ago Juang et al.[5] at the Joslin Diabetes Center, in Boston, injected syngeneic mouse islets into subcutaneously implanted polyvinyl alcohol (PVA) or polyglycolic acid (PGA) scaffolds that had been in place for 7 days to become pre-vascularized. Four of the 5 streptozotocin diabetic mice with PGA islet injected scaffolds became normoglycemic for 3 months and had numerous well vascularized islets within their scaffolds when sacrificed. Mice with PVA scaffolds remained hyperglycemic because the PVA scaffolds were not receptive to vascularization and contained only a few viable islets.

Shapiro's Alberta group and others, including ourselves, are continuing to explore transplantation of 3-D islet cell scaffolds into extra-hepatic locations, evaluating several materials and implant sites. Dufour[6] transplanted islets enmeshed in poly (glycolide-L-lactide) copolymer fibers (Vicryl™) in combination with collagen/laminin gel (Matrigel™) into the epididymal fat pad of diabetic mice. These islets functioned as long and as well as bare islets that were implanted beneath the-kidney capsule, known for years by experimentalists to be well vascularized and accommodating. Kin and others[7] from the same group have implanted islets in an FDA approved, absorbable, composite biodegradable scaffold (Ethisorb™ Durapatch) into omental pouches constructed in diabetic beagle dogs. Two-thirds of the test animals maintained normoglycemia without exogenous insulin for up to 2 months; whereas, none of the animals with pouches implanted with bare islets ever became insulin independent.

Berman and her coworkers[8] at the University of Miami's Diabetes Research Institute, used the Ethisorb™ Durapatch to support allogeneic islets in an omental pouch in diabetic cynomolgous monkeys, demonstrating the potential of this scaffolding in a more clinically relevant animal. However, significantly more islets were needed in the pouch scaffold to achieve euglycemia than the numbers of bare islets injected into the portal vein in a positive control group.

RGD (Arg-Gly-Asp) is an amino acid sequence that promotes cell adhesion and is present throughout the extracellular matrix. It can be chemically linked to alginate or other biomaterials to enhance their adhesive properties, making them more accommodating to blood vessel penetration and, hence, cell survival. We used 3-D, RGD peptide-modified alginate scaffolds with and without the addition of VEGF and PDGF vascular growth factors to generate pre-vascularized intramuscular abdominal wall sites in rats that were made diabetic with streptozotocin 10 days later.[9] On the 14th day, allogeneic islets were injected into the scaffolds with appropriate sham and no surgery controls. The addition of VEGF and PDGF doubled the successful transplantation rate from 3/6 to 6/6 and reverted the animals to euglyciemia for 60 days.



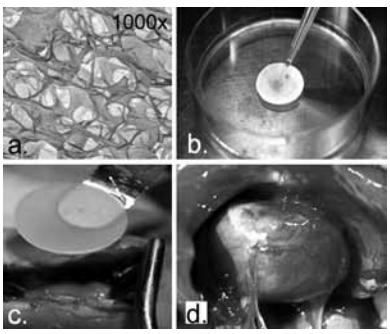
a. VEGF/PDGF scaffold showing fibrovascular penetration;
b. Anti-insulin stained viable islets at 60 days

Mao et al.[10] at Peking University's Reproductive Medical Center in Beijing, have developed islet-like cells from human embryonic stem cells to reverse hyperglycemia after transplantation into diabetic SCID (Severe Combined ImmunoDeficiency) mice using poly (lactic-co-glycolic acid) scaffolds as a possible solution to the limited availability of human islet cells. These cell-scaffolds reversed hyperglycemia for 7 weeks, which was equivalent to the results with bare islets beneath the kidney capsule. Kodama and his colleagues[11] at Harvard's Brigham and Women's hospital cultured and expanded normal mouse islets in 3-D scaffolds in vitro for 40 days that were still able to reverse hyperglycemia when transplanted into diabetic mice.

Biomaterials and Cell Transplantation for Treatment of Ischemic Heart Failure

Just as with islets, disparate clinical results have been reported for stem cell transplantation for ischemic congestive heart failure with equally careful investigators reporting moderate improvement in cardiac function in one large study and no effect in the other.[12-13] Cells used for these trials included bone marrow stem cells, skeletal muscle stem cells, adipose tissue derived cells and cardiac progenitor cells.[14] Cardiac repair scaffolds are either engineered in vitro or in situ, but the situation they are to be used in is especially challenging: Infarcted myocardium is an unfavorable environment for cell engraftment not just because its circulation is compromised, as it is also subject to unusual shear and loading stresses.

In 1997, Freed and her colleagues[15] at MIT began using 3-D polyglycolic acid scaffolds to culture rat cardiac myocytes in a bioreactor system, resulting in interconnected, contracting constructs. Three years later Leor et al.[16] at the Ben-Gurion University of the Negev's Cardiac Research Center in Beer-Sheva, applied 3-D alginate scaffolds seeded with cardiomyocytes as an epicardial patch, resulting in intense neovascularization and attenuation of post-infarction left ventricular dilatation. Christman and her associates[17] at the Universities of California, Berkeley and San Francisco's Joint Bioengineering Group, reported using fibrin glue to transplant myoblasts into infarcted myocardium in 2004. The glue solidifies rapidly after mixing with thrombin and calcium and therefore stays where it is placed. Their results showed that intra-infarct injections of myoblasts in the glue improved cell survival and preserved cardiac function during healing. A year later Kofidis[18] at Stanford, showed that intra-myocardial injections of embryonic stem cells suspended in Matrigel™, which solidifies at body temperature, also improved post-infarction cardiac function. Their gel approach retained the geometry of the left ventricle's lateral wall preventing the distortion that has sometimes been observed with rigid scaffolds. Last year, Yu et al.[19] at UC's Joint Bioengineering group, used RGD modified alginate to generate an in situ solidified scaffold by injecting it into an infarct along with both endothelial cells and calcium. The RGD-modified alginate enhanced the vascular response in vivo and improved cardiac function compared to unmodified alginate-endothelial cell control injections



a. Porous RGD-alignate SEM; b. seeding for culture expansion; c. placement on rat LV infarct; d. disc after 7 days with margin vascularity.

We modified polyvinylidene-difluoride purified alginate with cyclic RGD and then freeze/thawed the solution to generate highly porous, cyclic RGDfK scaffold discs. We seeded them with 1 million human-bone-marrow derived stem cells that were cultured for 24 hours, before the scaffolds were placed on coronary-artery-ligation induced LV infarcts in athymic rats. At 1 week, RGD scaffolds showed significantly greater within-scaffold cell survival, greater vascularity and better contractile function in vitro compared to unmodified scaffolds. Hearts with RGD scaffold covered infarcts also exhibited better cardiac function than saline controls and those patched with unseeded scaffolds. Shachar-Goldenberg and the Beer-Sheva group[20] just reported using a macroporous, RGD modified, alginate scaffold, similar to ours but seeded with neonatal rat cardiac cells. Within 6 days, the cardiomyocytes in the RGD scaffolds had organized themselves into typical myofiber bundles and the fibroblasts in the culture surrounded the bundles in a manner similar to normal myocardium.

In vitro engineered heart tissue constructs (EHTs) have been made by mixing type I collagen with neonatal rat heart cells that are then preconditioned on a stretching device.[21] The resulting contractile loops are combined and transplanted into infarcted myocardium. These EHTs formed thick cardiac muscle layers after implantation and exhibited near immediate electrical coupling without inducing an arrhythmia. They mitigated further dilatation and improved cardiac function when compared to non-contractile heart constructs.

Ott et al.[22] at the Mass General perfused hearts with detergents and enzymes to remove the resident cells while retaining the myocardium's extracellular architecture. Repopulating these hearts with cardiac cells in vitro resulted in contractile tissue, albeit with less efficacious pump function than a normal heart, that could be used as contractile cardiac patches and even as a complete heart in a lightly loaded, in vitro circuit.

Temperature sensitive materials such as poly (N-isopropylacrylamide) (PNIPAAM), which is a solid at body temperature, but liquefies below 20°C, have been used as a platform for growing an interconnected sheet of mesenchymal cells.[23] Lowering the temperature permits the material to flow away without distorting the cells. Transplanting these cell sheets onto the epicardial surface of an infarct induces local angiogenesis and improves cardiac function. Hamdi[24] and his Paris Surgical Research Laboratory colleagues performed a head to head comparison of multiple, intra-infarct injections of human skeletal myoblasts vs. overlaying of a skeletal myoblast-seeded collagen scaffold or a bilayered fibrin-myoblast cell sheet on similar sized lateral wall infarcts in rats. Echocardiographic ventricular function and histologic quantification of angiogenesis were done after 1 month. Rat hearts treated with both cell constructs exhibited better functional recovery and more angiogenesis activity than those receiving direct cell injections. The skeletal myoblast seeded collagen scaffold was the most effective of the three treatments in enhancing angiogenesis and reducing fibrosis. Small scale clinical trials of bone marrow stem-cell seeded collagen scaffold patches overlain on left ventricle lateral wall infarcts in combination with direct cell injections have shown the procedure to be safe and to enhance the effect of the injections.[25] The cell-seeded collagen matrix buttresses the infarct scar with viable tissue and helps to normalize local cardiac wall stress, limiting ventricular remodeling and improving diastolic function.

Future Direction

Multiple cell types and tissue engineering approaches have been used successfully to introduce restorative cells in degenerated

tissues, but there are still many questions and issues that need to be addressed before the technology can be widely applied in patients. Never the less, exciting things are happening, mostly on the cellular side. The US government's reluctance to fund embryonic stem cell research has spurred a great deal of adult stem cell research. One of the most promising aspects is the insertion of transcription factors into the DNA of adult cells that reprogram them to be inducible pluripotent stem (iPS) cells that are in every way similar to embryonic stem cells.[26] Murine iPS cardiomyocytes have similar responses, calcium channel ion expression and contractility to embryonic stem cell derived cardiomyocytes.[27] Pfannkuche et al.[28] at Cologne University's Institute for Neurophysiology, found that this similarity included shared immaturity of sarcoplasmic reticulum and weaker beta-adrenergic responses than normal ventricular tissue of comparable age.

Mouse and human fibroblasts can be reprogrammed to a pluripotent state with a combination of four transcription factors. This raised the question of whether transcription factors could directly induce other defined somatic cell fates, and not just an undifferentiated state. Tateishi et al.[29] at the University of North Carolina, Chapel Hill's Howard Hughes Medical Institute, induced pluripotent (iPS) insulin producing cells from human skin fibroblasts that reacted to differing glucose levels. Viebuchen,[30] working in Marius Wernig's laboratory at Stanford's Institute for Stem Cell Biology and Regenerative Medicine, hypothesized that combinatorial expression of neural-lineage-specific transcription factors could directly convert fibroblasts into neurons. They needed only three factors to efficiently convert mouse embryonic and postnatal fibroblasts into functional neurons in vitro. These induced neuronal (iN) cells expressed multiple neuron-specific proteins, generated action potentials and formed functional synapses.

Too much pluripotential could create immortal malignancies, so there is an important issue of control, but think of a patient's own iN cells generating scaffold-oriented, autologous repair tissues for high spinal cord injuries. The immense, worldwide interest in biomaterials, tissue engineering, and cell therapy that dominates this review is bound to produce a major clinical breakthrough within this decade that will affect a sea change in the management of degenerative diseases, like type I diabetes, ischemic heart failure, and maybe amyotrophic lateral sclerosis.

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A Prologue for the Next Quantum Leap in Heart Transplantation

Erik Sylvin and David D'Alessandro

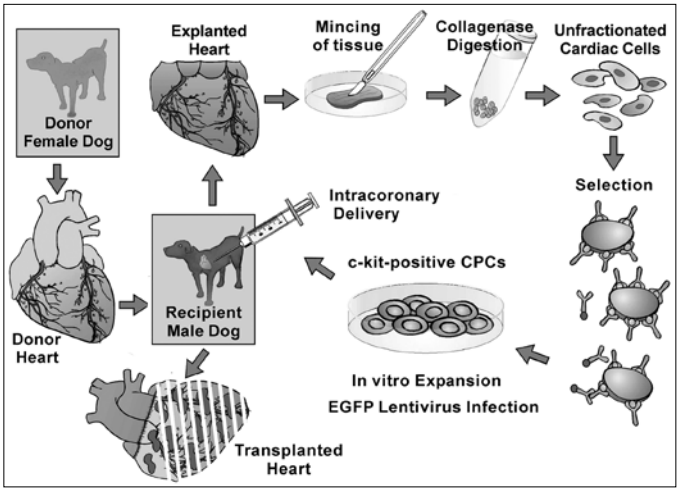
Erik Sylvin David D'Alessandro

The heart is not a terminally differentiated organ. It has a population of stem cell like cardiac progenitor cells (CPCs) that are capable of regenerating all of the elements that constitute the heart.[1] CPCs should be able to maintain organ homeostasis but their reparative capabilities are apparently overwhelmed in the setting of elderly patients with severe heart failure, which explains its increasing prevalence. We have developed a canine heart transplantation model to study the repair potential inherent in CPCs. Successful translation of this work could be the next quantum leap for cardiac transplantation.

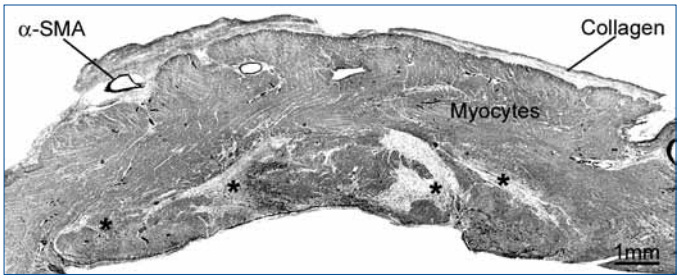
High-resolution confocal microscopy, coupled with stem cell immunohistochemical purification protocols that exploit cell proliferation markers (Ki67 and BrdU), cardiac myocyte proteins, and mitotic spindle microtubules have unequivocally identified multiplying cardiac muscle cells in both diseased and non-diseased hearts.[2-5] Last year, Bermann and his colleagues,[6] at the Karolinska Institute, used the integration of carbon-14 into human DNA, from Cold War nuclear bomb testing to establish the age of cardiomyocytes in humans. They found that cardiomyocytes renew, with a gradual decrease from 1% annually, at the age of 25 to 0.45% at the age of 75. These observations, together with the recognition of age and cardiac failure related myocyte telemorase activity, telomeric shortening, and apoptosis define a declining natural turnover of resident myocardium.[7]

In 2002, Federico Quaini, Piero Anversa, et al.[8] at New York Medical College in Valhala, NY examined eight deceased donor human hearts from female donors that had been transplanted into male recipients, looking for recipient Y-chromosome marked cells. Between 7 and 10% of the myocytes and vascular cells had the Y-chromosomes, indicating recipient-derived myocardial regeneration. This group went on to provide indisputable evidence that the heart possesses CPCs capable of repairing senile, stressed, and damaged myocardium, both in animals and in humans.[3,9,10] CPCs bear the c-kit cell surface marker, are self renewing, clonogenic and multipotent. They reside in niches within the myocardium and under the right conditions can assume myocyte, as well as smooth muscle cell and endothelial cell lineages to form coronary vessels in vitro and in vivo. Clinical utility of CPCs has been limited by the need to isolate them from atrial and ventricular tissue biopsies. Subsequent in vitro culture expansion can take several months to accumulate sufficient cells for effective therapy, restricting their application to chronic conditions.

Our orthotopic transplant model makes the entire native heart available for isolating c-kit positive cells to start expansion culturing from a large base number. The model's immune mediated damage also provides a stimulating milieu for homing, differentiation, and engraftment in what amounts to a functioning biologic scaffold. The experimental group consisted of female heart allografts in four immunosuppressed male recipients. The native heart was cut into pieces and dissociated in a collagenase solution. Rabbit anti-c-kit coated magnetic immuno-beads sorted out the CPCs for serial culture and subsequent injection into both coronary arteries, catheterized from a carotid artery. Two animals received two CPC injections and two received four CPC injections, consisting of $7x10^6$ cells each, given at weekly intervals.

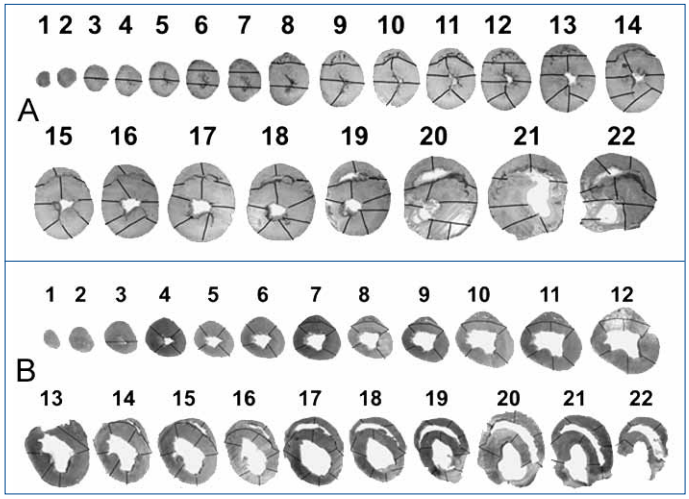


The Quaini-Anversa construct of a female graft in a male recipient would allow Y-chromosome tracking of the CPCs' fate, but we added a second marker by transfecting the male CPCs with a lentivirus expressing enhanced green fluorescent protein (EGFP). We evaluated the ability of EGFP-CPCs to commit to myocytes, smooth muscle cells and endothelial cells by the detection of transcription factors specific for the three lineages and confirmed engraftment and survival with polymerase chain reaction. Random sampling of kidney, spleen, lung, and liver tissues did not reveal DNA sequences for EGFP suggesting that CPCs are specific to repairing cardiac tissue.



Ventricle wall section after 4 CPC injections and 73 days. Asterisks indicate ECFP expressing structures in subendocardial damaged areas. α-SMA=alpha smooth muscle actin.

The animals were sacrificed 30-73 days after transplantation. The heart was sampled from base to apex in 19-22 approximately 4-mm slices, analyzing several segments from each slice. Our data suggest that nearly 50% of rejected non-immunocompatible donor myocardium was restored by tissue derived from the EGPF-CPCs.[11] Newly formed cardiomyocytes acquired adult characteristics and were integrated both structurally and functionally within the transplanted heart. The EGPF expressing myocytes were small and resembled fetal or neonatal myocytes in the two dogs that received two CPC injections and were euthanized 30 and 45 days after transplantation. Some EGPF-positive myocytes achieved the adult phenotype and were in-



Sections from two transplanted hearts: cross line indicate individual analyzed segments, 114 for heart A and 127 for hearrt B.

distinguishable from resident cells in the two dogs that received four CPC injections and survived for 70 and 73 days.

Regions of complete cardiac repair were found together with areas of immature myocytes, suggesting that tissue replacement was ongoing and these cells represented myocytes formed earlier and later in the regeneration process. Recipient immunocompatible regenerated areas were also observed across the entire spectrum of the transplanted heart's vasculature.

Conclusion

These findings have redirected our research to the near universal progressive graft dysfunction that follows human heart transplantation. The recipient's discarded heart may actually be rich in CPCs* that could not meet the repair needs of the severely damaged heart but might be a godsend for repairing the new heart's ongoing immune mediated damage. Repeated injections paced according to functional parameters might create a standoff. Over time, progressive chimerism might go well beyond 50%, creating a heart that will be increasingly resistant to immune-mediated injury.

We are now investigating the fate of transplanted CPCs in long term canine heart transplants, specifically looking for their effectiveness in mitigating allograft vasculopathy. We are also working within the NIH-sponsored Cardiothoracic Surgical Network to develop a Phase-1 safety and feasibility trial based on our experimental model. Successful translation of this research could profoundly affect cardiac transplantation and enrich the lives of heart transplant recipients.

*Ed note: Determining CPC depletion or abundance in discarded human hearts should be "Job One" prior to a Phase-1 trial.

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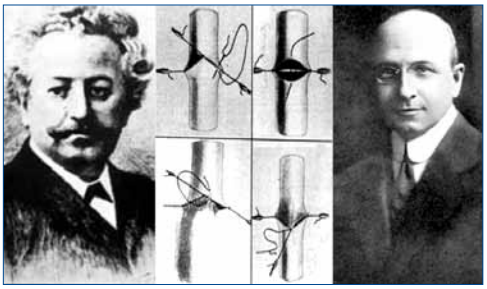
Xenotransplantation: Getting Closer

Adam Griesemer and Megan Sykes

Adam Griesemer

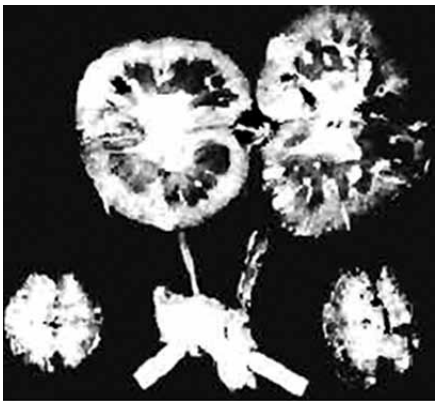
Megan Sykes

Xenotransplantation, as the joke goes, is the future of transplantation, and always will be. The history of xenotransplantation is as old as the history of transplantation itself. Ethical and cultural issues criminalized dissection of the human body well into the Age of Enlightenment, but animal tissue xenografts date at least back to the 16th century.[1] The first attempts at solid organ xenografts had to wait until the first years of the 20th century when Jaboulay and his student Carrel developed techniques for anastomosing small vessels. Jaboulay transplanted a pig kidney to the antecubital fossa of a woman in 1906, which is widely regarded as the first clinical attempt at solid organ transplantation. Although the kidney initially produced urine, it had to be removed after 3 days because the graft had thrombosed. Heterotopic organ transplantation persists today as a valuable laboratory technique, but clinical heterotopic organ transplants were essentially abandoned before the beginning of World War I.



Mathieu Jaboulay 1860-1913 | Carrel's triangulation anastomoses | Alexis Carrel 1873-1944

Greater understanding of histocompatability and interspecies relatedness led to the realization that primates might be better source animals with respect to the immunologic response they might provoke in a human recipient. Great strides were also made in the development of immunosuppressive drugs that would hold that immunologic response in check. These two developments led to a renewed attempt at



9-m0 Reemstma Chimp kidney showing no cortical necrosis or hemorrhage.

clinical xenotransplantation in the 1960's. In 1963, while at Tulane University, Keith Reemtsma transplanted a kidney from a rhesus monkey into a 43 year-old man using total body irradiation, prednisone, azathioprine, and actinomycin C as immunosuppression. Unfortunately, the patient died of pneumonia 63 days later[2]. Undaunted, he then transplanted a chimpanzee kidney into a 23 year-old teacher. This recipient survived for 9 months, when she also died from an infection, but with a functioning graft, which is the longest known survival of a solid organ xenograft in a human.[3]

20th Century Clinical Renal Xenografts

Year	Author	Location	Animal	No cases	Survival
1906	Jaboulay	Lyon	Pig	48-year C	3 days
1906	Jaboulay	Lyon	Goat	50-year C	3 days
1910	Unger	Berlin	Macaque	28-year C	32 hours
1913	Schonstadt	?	Monkey	Young C	60 hours
1923	Neuhof	New York	Lamb	1 person	9 days
1963	Hitchcock	Minneapolis	Baboon	65-year C	4 days
1963	Reemtsma	New Orleans	Rhesus monkey	43-year F	63 days
1964	Reemtsma	New Orleans	Chimpanzee	23-year C	9 mo
1964	Reemtsma	New Orleans	Chimpanzee	12 patients	63-270 days
1964	Starzl	Denver	Baboon	6 patients	18-98 days
1964	Hume	Richmond	Chimpanzee	1 man F	1 day
1964	Traeger	Lyon	Chimpanzee	3 patients	<49 days
1968	Cortesini	Rome	Chimpanzee	19 year old F	31 days

A few others, including James D. Hardy's unenviable experiences with a chimpanzee lung and a heart at the University of Mississippi, performed transplants from primates to humans with limited short-term success.[4] Since the 60's it has become accepted that primates are not a viable source of xenogeneic organs because of the risk of viral transmission between closely related species and doubts that their largely single-birth reproduction would satisfy the demand for donor organs[5] After it was established that the AIDS epidemic was the result of non-human primate to human viral transmission, the FDA declared what amounted to a moratorium on primate-to-human transplantation because of the potential for retroviral transmission[6].

Pigs as Source Animals

Most efforts in the field now focus on the use of porcine donors.[7] Domestic sows can produce two litters of 6 to 12 piglets annually that mature to have equivalent human size organs in less than a year, virtually assuring a sufficient organ supply should xenotransplantation become a reality. Since pigs are bred for slaughter to provide food, any ethical concern about breeding them for their organs is almost automatically moot. The first attempts at transplanting porcine organs in man consistently failed because of a significant evolutionary deletion that distinguishes humans from most other animals. Humans and Old World monkeys developed a frame shift mutation in the α-1,3-galacosyltransferase (GalT) gene rendering it inactive. New World monkeys and other mammals, including pigs, have a functional GalT gene, which expresses the sugar α-galactose-1,3-galactose (Gal) on most cell surfaces, including those of endothelial cells. Humans are thought to be sensitized to this antigen by Gal-expressing bacteria that are a natural part of our gut flora[8]. Anti-Gal antibodies make up 1% of total human serum antibodies, and up to 90% of our preformed antibodies against pig antigens are Gal directed.[9] Old World monkeys are similarly pre-sensitized as anti-Gal antibodies cause them to hyperacutely reject pig grafts.

Transgenic pigs were developed in 1990s, introducing human genes that coded for expressing various human complement regulatory proteins on the pig endothelial cells in an attempt to minimize anti-Gal antibody cell destruction. These proteins, such as Decay Accelerating Factor (DAF) interrupt the complement cascade and prevent complement-mediated antibody cell lysis. Baboons whose existing anti-Gal antibodies had been absorbed by perfusing their blood through a pig liver, or by plasmapheresis, did not hyperacutely reject kidneys from these transgenic for human complement (THC) pigs, but invariably rejected the kidneys, once their levels of anti-Gal antibodies reaccumulated.[10] Towards the end of the decade, pig genomes were identified that contained porcine endogenous retroviruses (PERV), and some of these retroviruses were able to infect human cells in vitro.[11] This caused the FDA to halt all pig-to-human transplantation for a year until researchers developed reliable methods for detecting PERV infected animals. As the 20th century closed, failing to overcome anti-Gal antibodies and transmissible retroviruses imbued xenotransplantation with what seemed like an indelible taint.

The Gal Knock-Out Era

Ten years later, xenotransplantation has morphed into a promising field that is at the verge of clinical trials. The beginning was realizing that in vivo PERV transmission to humans was not known to have occurred, that carefully isolated PERV-free or PERV non-transmitting pig colonies could be developed, and that cloning could potentially develop pigs that do not express Gal and are PERV resistant.[12,13] By 2004, investigators at PPL Therapeutics in Blacksburg, VA and Image Biotherapeutics in Cambridge, MA had performed targeted insertions into the α-1,3-galacosyltransferase gene, causing a mutation that renders the Gal gene non-functional.[14,15] These Gal knock-out (GalKO) pigs do not express Gal on cell surfaces and can produce anti-Gal antibodies themselves.[16]

In 2005, Shimizu and his colleagues[17] at Harvard and MGH's Transplantation Biology Research Center reported the first use of GalKO organs in pig-to-baboon heterotopic heart transplants. The hearts were not hyperacutely rejected and survived for 2 to 6 months to be rejected by T cells and low antibody levels against non-Gal antigens, with histology that showed widespread, small-vessel fibrin thrombi. The same investigators and, independently, the group at the University of Western Ontario in London, Canada, reported life-supporting GalKO pig-to-baboon kidney transplants in the same year, which only survived for a month or less before succumbing to cellular rejection and induced humoral antibodies.[18,19] These results were not substantially different from those achieved by absorbing anti-Gal antibodies prior to transplanting Gal positive organs. GalKO swine were not going to be a total solution, but their eliminating hyperacute rejection unmasked problems which directed new research opportunities. Pig endothelium does not display thromboregulatory molecules that primate blood components recognize, making pig endothelium less thrombo-resistant and accounting for the thrombotic microangiopathy observed in the GalKO-to-primate xenografts.[20] In vitro studies confirmed that

T-cell responses to xenogeneic tissue are stronger than those to allogeneic tissue suggesting need for more aggressive or specifically targeted chronic immunosuppression.[21]

Tolerance

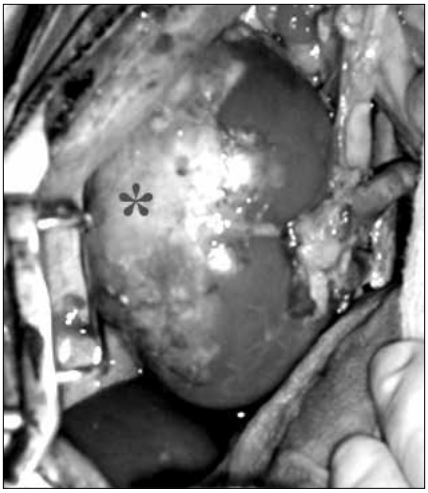
More immunosuppression would be a step backward towards more infections, arteriosclerosis, and skin cancers; whereas, tolerance induction is the ultimate in specific targeting. Scandling and his colleagues[22] at Stanford and Kawai et al.[23] at the Mass General Transplant Center, along with others, have induced tolerance to renal *allografts* with combined bone marrow and kidney transplantation. Transplanted postnatal thymus tissue has been used to treat juvenile patients with DiGeorge's syndrome* with in vitro evidence of tolerance to the donor thymus.[24-27] Tolerance without immunosuppression offers the added advantage of minimizing inflammatory molecule secretion that feeds into the coagulation cascade, mitigating thrombus related organ damage.

Mixed chimerism and thymus transplants are the current principal strategies for extending tolerance induction across xenogeneic barriers. Mixed chimerism is achieved by successful engraftment of donor bone marrow hematopoetic cells, so that the recipient will have two lineages of hematopoetic cells and be tolerant of tissues and organs from the bone marrow donor.[28] This is thought to occur through deletion of donor-reactive T-cell clones within the thymus from the display of donor antigens during negative selection of immature thymocytes.[29] Mixed chimerism prevented both hyperacute and T-cell mediated rejection of cardiac xenografts in several animal model pairings that also exhibited B-cell tolerance.[30,31] But, these results could not be replicated in pig-to-primate grafts. Low-level bone marrow chimerism was detectable, but primate anti-Gal antibodies prevailed.[32]

Switching to GalKO pig bone marrow donors in 2004 resulted in peripheral blood leukocyte chimerism for 5 days but no evidence of donor-specific unresponsiveness.[33] Recently, we found that GalKO bone marrow engraftment was associated with in vitro evidence of donor-specific unresponsiveness in 2 of 4 baboon recipients without our being able to identify peripheral blood chimerism. We concluded from this 50% success that additional measures to overcome innate immune and non-immune xenogeneic barriers should result in functional GalKO hematopoetic engraftment and donor specific tolerance to a transplanted solid organ.

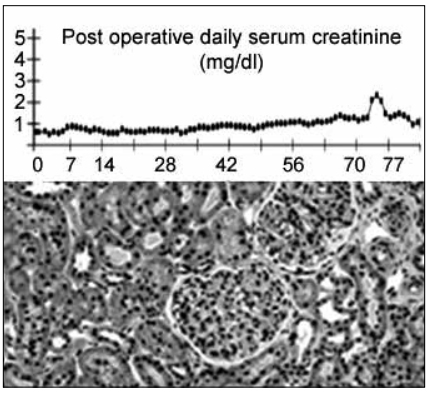
Yong-Guang Yang and Megan Sykes have shown that human macrophages recognize the cell surface antigen, CD47 as part of the innate immune system's self vs. non-self surveillance. Any cells, including bone marrow cells, that do not display human CD47 are recognized as invaders and phagocytosed.[34] Transgenic pig cells encoded for human CD47 evade detection and this fate.[35] Adding human CD47 to the GalKO pig is the obvious next step.

"Team Sykes"[36-40] at Harvard and the Mass General has extensively studied the alternative of thymus transplantation. They have shown that transplanting porcine thymus tissue into mice induces in vitro, donor-specific tolerance and subsequent permanent survival of donor-matched porcine skin grafts in mice that promptly rejected allogeneic mouse skin.[41,42] Swine allogeneic thymus needed to be transplanted as a vascularized graft and would induce tolerance to renal and cardiac grafts across class I and full major histocompatibilitiy complex (MHC) mismatched barriers.[43-48] Team Sykes is transitioning to CUMC along with their leader who is already well ensconced at the Medical Center, but commuting back and forth to Boston. Their productive research can be expected to grow and flourish despite Megan's acquisition of considerable organizational responsibilities.†



GalKO pig "thymokidney" implanted in baboon recipient, * Indicates subcapsular pig thymic tissue.

SCID mice studies have shown that pig thymus grafts can support normal human thymopoiesis, generating human T cells specifically tolerant of the pig donor's antigen, providing proof of principle for thymus-induced tolerance in humans. Baboon recipients of THC, Gal positive pig thymus and kidney exhibited in vitro evidence of donor-specific T-cell unresponsiveness.49 THC, GalKO pig thymus and kidney transplants survived for more than 80 days with normal renal function, while solitary kidney grafts were all rejected within 30 days. We have placed autologous thymus cells under the kidney capsule to make GalKO "thymo-kidneys" for transplantation into baboon recipients. The porcine thymus acquired the initial stages of baboon T-cell development, which was associated with pig-specific unresponsiveness in vitro.[50] Further genetic modifications of the THC, GalKO pig are underway to eliminate preformed non-Gal antigens and make the pig endothelium more thrombo-resistant to the primate coagulation cascade with an eye towards being ready for clinical trials.



Normal baboon creatinine, supported by GalKO thymokidney and co-transplanted kidney with normal biopsy.

Which Organ?

The obvious candidates for the first clinical trial of pig-to-human organ transplantation are liver, heart, kidney, and pancreatic islets. Some have advocated beginning with the pig liver as a bridge to allogeneic transplantation to allow more time to become eligible for a human liver. It is interesting to ponder how this extension would be regarded by the MELD allocation scheme, but xenotransplant bridging would do nothing to alleviate the incredible shortage of allogeneic organs. The liver is also a problematic choice for the first xenogeneic organ graft, as it is the main source of complement and clotting factors, and has many other essential enzymatic functions. Pig-to-primate liver transplants have maintained complement function and clotting factors for up to 8 days so longer term studies in a non-human primate would be a prerequisite.[51]

*Deletion of locus q11.2 i.e., near the middle of chromosome 22, causes a variety of birth defects typically including incomplete palate closure (velo-pharyngeal insufficiency) and predilection to recurrent infections related to an absent or hypoplastic thymus.

†Director of P&S's Center for Translational Immunology, Director of Research for CUMC's Transplant Initiative, and Director of Bone Marrow Transplantation Research in the Medicine Department's Division of Hematology/Oncology.

The International Society for Heart and Lung Transplantation issued a position paper in 2000 recommending that nonhuman primate studies demonstrate a 60% survival for 3 months with at least 10 animals having reached this goal before beginning human trials of pig-to-human accessory heart transplants and greater than six months survival before beginning orthotopic replacements. The REMATCH trial of the HeartMate II LVAD's 50% 1-year survival and this year's INTERMACS registry report covering 1000 LVAD implants obviate any consideration of an accessory human or animal heart.[52,53] Clinical cardiac xenotransplantation cannot begin until nonhuman primate xenogeneic heart graft survival has matched its contemporary LVAD results.

Living on dialysis is an unpleasant life, but beginning human organ xenografting with the kidney is a minimalist gamble: if it fails, it's back to dialysis. Eighty-five percent of the 100,000 plus US allograft waiting list are seeking kidneys and would be willing to make that gamble if presented with reasonably durable non-human primate function. It is hard to imagine a population more amenable to recruitment into a clinical trial.

Cardona and his colleagues[54] at Emory University and Bernard Hering's group[55] at the University of Minnesota's Schulze Diabetes Institute have published prolonged survival of porcine islet xenotransplants in primates utilizing a powerful immunosuppressive regimen that relies on blocking co-stimulatory signals to T cells and preventing their activation. Hering[56] reviewed the literature last year, finding 15 reports of pig islet therapy involving 151 non-human primates, including 109 that were made diabetic before the transplant. Eventual T-cell rejection was the common ending despite aggressive immunosuppression, which would be more harmful than years of administrating exogenous insulin. Thymus induced tolerance could shift this balance in the future, but Hering says that he is preparing the necessary infrastructure to proceed with clinical islet xenotransplants within a few years, and he may be the first out of the gate.

A Race with Stem Cells

Organ shortfall drives stem cell as well as xenograft research. Transcription factor insertions have been remarkably successful in inducing differentiated cells into pluripotent iPS cells as noted by Hardy and Sondermeijer in this newsletter. The pitfall for stem cell therapy is its potential for tumor transmission or formation of an immortal tumor after transplantation, but its advantage, particularly with iPs cells, is a noteworthy autologous source from a just a snip of the recipient's dermis. With respect to forming stem cells into organs, they can be colonized on three-dimensional scaffolds and even become contractile. Ott and his colleagues at MGH have repopulated decellularized hearts with neonatal cardiac cells that responded to electrical stimulation with 25% of the beat strength of a fetal heart, but it is a long ways to something that looks and works like a kidney or liver.[57] Stem-cell therapy for degenerative dysfunction is a rapidly advancing technology and might lead to organ substitutes that could obsolete xenotransplantation. More likely there will be room for both and advantageous situations for each.

Conclusion

Xenotransplantation is approaching clinical trials, and it is especially fitting that CUMC's Center for Translational Immunology and Transplantation Initiative are well positioned to lead the way. As Thomas Starzl once said, "If xenotransplantation eventually works, no doubt the starting point will be traced to Keith Reemtsma."[58]

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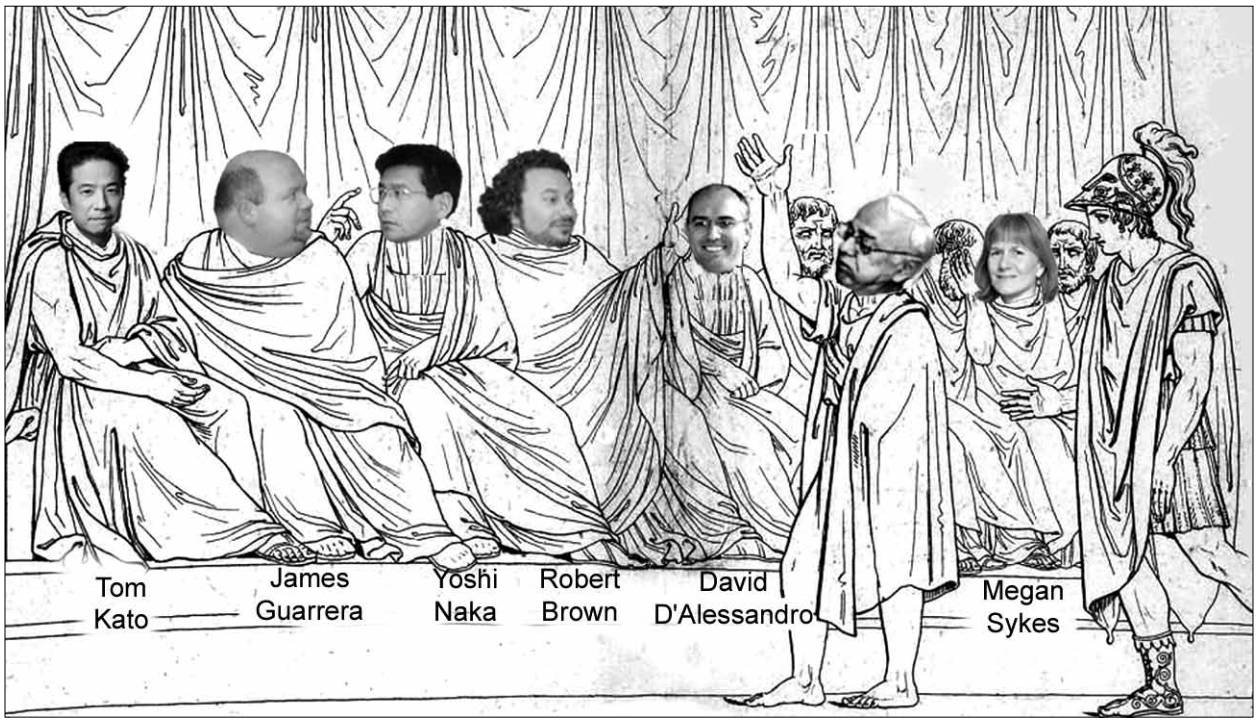
JJSS Banquet Pictures

Jean Emond, Megan Sykes, John Schullinger, Carol Conklin, Steve Libutti, and Foster Conklin.

Henry and Matthew Spotnitz, Patricia Smith, and Kay Forde

Mark Hardy's **http**: **h**ot [future] **t**ransplantation **t**opics **p**anel*



Mark Hardy: What's the potential role of hepatocytes for either transplantation to correct metabolic defects or as bridge to whole liver organ transplantation?

Robert Brown: Hepatocyte transplantation is plagued by need for immunosuppression and the necessity of using human donor organs as a cell source, which could instead be used for whole organ transplantation. If a source of hepatocytes could be developed from self-propagating stem cells, the situation could change rapidly. Porcine and human hepatoblastoma cell-line hepatocytes attached to columns have been tried for extracorporeal perfusion in patients with acute liver failure, as a bridge to either recovery or to liver transplantation. These options, however, do not appear ready for human application due to risks of transmitting virus in the first instance, or hepatoblastoma in the second.

Mark Hardy: As a follow-up to xenogeneic cells for liver replacement, could you compare this technology to that of xenotransplanting islets, hearts or kidneys?

Robert Brown: Acute liver failure is a condition where a xenotransplant may be applicable as a bridge to allogeneic transplantation or to allow the autogenous liver to regenerate. We could repopulate the liver with porcine cells and, when the autogenous cells recover, let the porcine cells be rejected. Whole liver xenotransplantation for liver failure is down the line due to the problem of acute rejection. Pancreatic islets will probably constitute the first attempts at cell, or even organ xenotransplantation in the US. If good survival of xenogeneic islets can be demonstrated, then it may be feasible to use xenogeneic cells or livers as a bridge to allogeneic transplantation, if we believe that the organs are sufficiently compatible.

Mark Hardy: It was Keith Reemtsma's dream to use allogeneic and xenogeneic islets. We have transplanted human islets here at Columbia as have our colleagues at Weill-Cornell. Although investigators at the Universities of Minnesota, Miami, and Alberta have shown good results with islet transplants, our success was very short lasting. Recently, the Minnesota group demonstrated 7-8 month survival of xenogeneic islets in a primate model, suggesting that this technology is making progress, as the large diabetic population's need cannot be met by the foreseeable allogeneic donor programs.

Mark Hardy: In most centers, organ perfusion preservation means near freezing University of Wisconsin "UW" solution, developed by Fred Belzer in 1987.[1] James Guarrera is at the cutting edge of liver preservation. Should it be hypothermic, normothermic, and different for different organs?

James Guarrera: Hypothermia and UW have been standard for many years. Kidneys are now perfused in many regions in the US, much less so in Europe, and are at the forefront of investigations of machine perfusion of organs that was developed in the 1960s. Initially, it was not enthusiastically embraced, but with the improvements in preservation solutions, such as UW, perfusion has become standard of care for prolonged cold ischemia.[2] Successful kidney machine perfusion has renewed interest in liver machine preservation. We have "pumped" 26 livers that were placed in CUMC recipients. This is now the only published experience in the world, but we are working on expanding to other centers as part of a multi-institutional study. Some investigators are using normothermic machine perfusion for hearts. I think this is risky because if there is a problem with the normothermic flow, there will be immediate warm-ischemia organ damage, so constant vigilance by an expanded perfusion team is an absolute necessity. Trials of normothermic organ preservation are likely to be done first for hearts and lungs because their ischemia tolerance is lower in that of the abdominal organs, which could justify the additional expense associated with a larger team. The field of organ preservation is also ripe for the implementation of gene therapy and stem cell modifi-

*Tomoaki Kato, Surgical Director, Liver Disease and Transplantation; James V. Guarrera, Surgical Director, Adult Liver Transplantation; Yoshifumi Naka, Director, Cardiac Transplantation and Mechanical Circulatory Support; Robert S. Brown Jr., Chief, Department of Medicine's Division of Hepatobiliarly and Abdominal Transplant Surgery; David D'Alessandro, Director, Clinical Trials, Montefiore Medical Center; Mark A. Hardy, Director Emeritus and Founder of CUMC's Transplant Program; Megan Sykes, Director, Center for Translational Immunology and Research Director for CUMC's Transplant Initiative.

cation, and modulation and normothermic organ perfusion is a good platform for these studies.

Yoshi Naka: James' concern is real: we have lost organs because the Transmetics™ system we are testing needs constant attention. Despite these difficulties, we envision many possibilities with this normothermic perfusion device. The shortage of donor organs has made acceptance of 4 hours of ischemia routine. Perfusion may not impact survival much, but we are intensely studying ischemia-reperfusion injury in hearts. We have developed a novel cardiac preservation solution relying on ATP, but this "Columbia" solution has not been tried clinically. Cell transplant for repair of infracted tissue is being investigated by cardiologists and cardiac surgeons. The results remain controversial and ill defined. Some surgeons are testing human stem cell transplants for treatment of non-ischemic cardiomyopathy. We have not obtained successful results with cardiomyocyte stem cell transplants, and it is clear that much more research on this issue is required.

Jim Chandler *(in the audience)*: Dr. Kato, your ex-vivo tumor excisions and visceral reimplantation procedures are fascinating. At the University of Colorado there is a huge interest in gut-ischemia-reperfusion mesenteric lymph's pro-inflammatory cytokines. Experimentally, complete diversion of the gut lymph avoids the severe lung injury associated with hemorrhagic-shock-ischemia-reperfusion in rodents. No matter how cold your viscera are on the back table, you cannot avoid warm reperfusion when you reimplant. Since you cannot reconnect the mesenteric lymphatics you are fortuitously mimicking the Colorado gut-lymph diversion model.

Tom Kato: Work on ex-vivo organ manipulation and operations were based originally on experience in multiorgan transplantation.[3] Based on these experiences we had established the approach to ex-vivo operations by flushing and reperfusing the organs and allotting time for cold ischemia of organs to be transplanted. This is not the same as the ischemia-reperfusion injury seen in trauma patients because the organs are immediately flushed with cold preservation solution as in donor surgery and maintained at regular organ transplant preservation temperature. In that way, the safety and functional recovery was already previously well-studied. Interestingly, the intestinal lymphatics gradually re-establish cisterna chyli connections.

Mark Hardy: Dr. Kato, yours is an autologus situation: ischemia-reperfusion is a major issue in allogeneic situations since it can trigger rejection.

Megan Sykes: Dr. D'Alessandro, your work with female canine heart allografts that then receive intracoronary injections of label-transfected, male-recipient cardiac progenitor cells (CPCs) has produced some very interesting data. My question is what happens over time when you have recipient CPCs residing in the donor graft? You imply that there is progressive replacement of donor myocytes by autologus cells.

David D'Alessandro: We have done four animals: two received two injections and two received four injections of autologous male CPCs. Animals receiving four injections survived longer and their myocytes were larger, suggesting replacement. This is not a long-term survival model, but the autologus cells were there at the time of sacrifice, compared to other cell types, for which we could not find evidence of survival. However, what happens long-term remains an open question.

Tom Kato: I have a question for the whole panel. End of life decisions are now being made so that people withdraw care early so that we now see more DCD organs relative to organs from brain dead donors. This will present a challenge for the field in the long run. Is there anything that we can do about this situation?

Robert Brown: We have to get better at preserving organs after DCD or change the legal definition of when death can be declared. In the short term I think the answer lies with better preservation methods, but in the long term we hope that there will be more acceptances of alternate definitions of death.

James Guarrera: We are attempting to address this with preservation improvements and plan to apply our pump technology to DCD livers in the future. Right now, biliary complications limit the use of DCD livers. Those who make policy realize that DCD organs won't help increase the numbers of donor organs much more. However, I think that we can develop technology to revive organs in vitro and that may surmount problems with DCD organs.

Mark Hardy: The use of ECMO, as it becomes more practical and miniaturized, may come into play. Donors may be maintained by ECMO in the same way as the femoral catheter perfusion of DCD donors helps to maintain abdominal organs until recovery. The public acceptance of when we can obtain deceased donor organs and under what circumstances may change in the future, as it has in the past.

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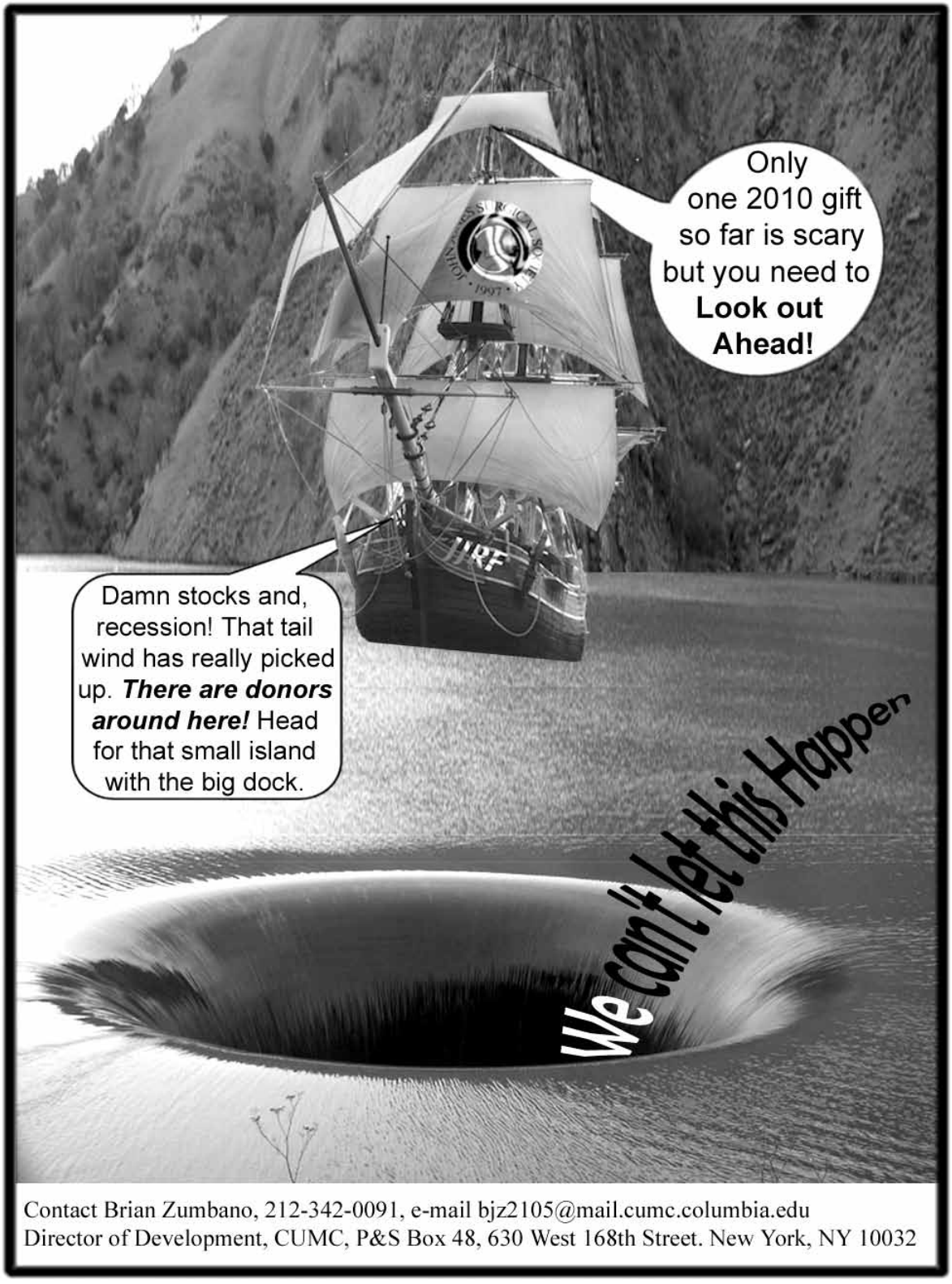

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