Perfusione ex-situ del cuore nel DBD e prospettive nel DCD

Prof Ugolino Livi, MD, FECTS
University of Udine

STATI GENERALI
20 ANNI 1999·2019
RETE NAZIONALE TRAPIANTI

6·7·8 NOVEMBRE
ROMA

Organ Care System

OCS: Udine experience

47 CASES (89% UTILIZATION, 42 UTILIZED GRAFTS) SINCE 2007	
DONORS	
Mean age, years	47 ± 11
Age>55 years	9 (21%)
CAD	3 (7%)
Expected IT > 5h	23 (55%)
Cardiac arrest	8 (19%)
Reports use of narcotics	4 (10%)
RECIPIENTS	
Mean age, years	56 ± 12
ECMO	8 (19%)
VAD	7 (17%)
REDO	4 (10%)
Mean cold ischemic time, min	133 ± 27
Mean OCS time, min	282 ± 76
Mean total «out of body» time, min	**400 ± 101**

Potential OCS benefits

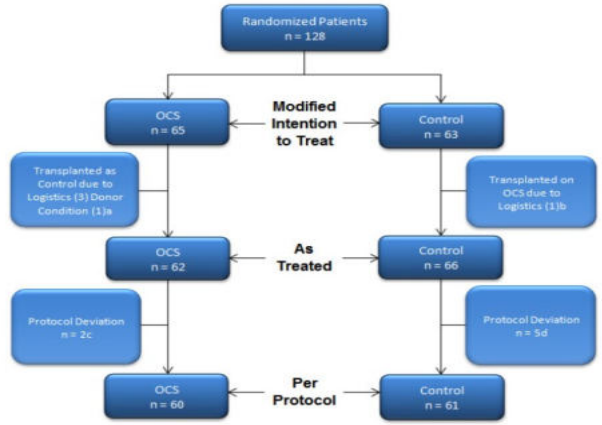
• By reducing ischemic time	**PRESERVATION**	(PROCEED II)
• By increasing acceptance of organs	**ASSESSMENT**	(EXPAND)
• By improving graft quality	**RECONDITIONING**	?
• By decreasing incidence of CAV	**I-MODULATION**	?
• By creating new heart	**REGENERATION**	?

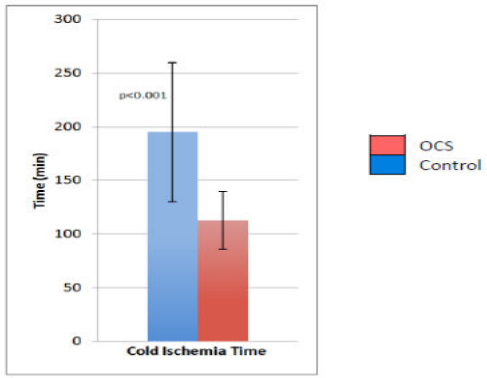
OCS (Heart) Clinical Trials

	Year	N	Donor
PROTECT	2006-7	22	Standard
PROTECT II	2007-8	20	Standard
PROCEED	2007-8	15	Standard
PROCEED II	2011-2013	128	Standard
EXPAND	2015-2019	93	Extended

PROCEED II Findings

- 30 day patient and graft survival are similar when the donor heart preserved on OCS vs on ice
- No different in secondary endpoints of cardiac –related SAE, Rejection, ICU stay
- Cold ischemia time significantly shorter, despite longer total preservation time





THE LANCET

Available online 14 April 2015
In Press, Corrected Proof — Note to users

Articles

Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial

Prof Abbas Ardehali, MD[a], Prof Farshad Esmailian, MD[b], Prof Mario Deng, MD[a], Prof Edward Soltesz, MD[c], Prof Eileen Hsich, MD[c], Prof Yoshifumi Naka[d], Prof Donna Mancini, MD[e], Prof Margarita Camacho, MD[e], Prof Mark Zucker, MD[e], Prof Pascal Leprince, MD[f], Prof Robert Padera, MD[g], Prof Jon Kobashigawa, MD[g], for the PROCEED II trial investigators[†]

Prolonged OCS Heart Perfusion

DONOR HEART TIMINGS	CASE 1	CASE 2
Clamp to clamp time	10 Hr 11 min	17 Hr 03 min
• *OCS perfusion time*	*8 Hr 23 min*	*15 Hr 55 min*
• *Total ischemic time*	*43+52min*	*68 min*

RECIPIENT OUTCOME	CASE 1	CASE 2
• VA-ECMO	17.5 Hr	44 Hr
• Ventilation	3 Days	5 Days
• Hospital length of stay	15 Days	24 Days
• Follow-up	> 1 yr	7 months

Case 1: Stamp NL et al. Heart Lung Circ. 2015 Jun;24(6):611-3
Case 2: Kaliyev R et al. Artif Organs. 2019 Mar;43(3):319-320

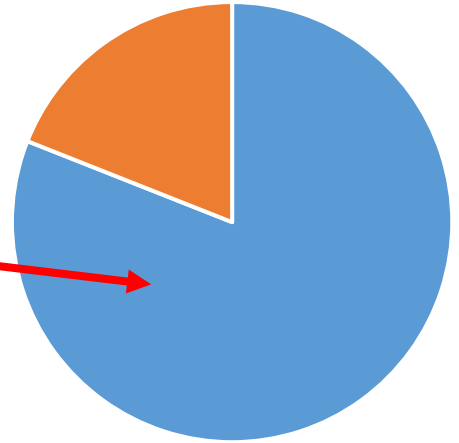
OCS Heart EXPAND Trial

Marginal Donors

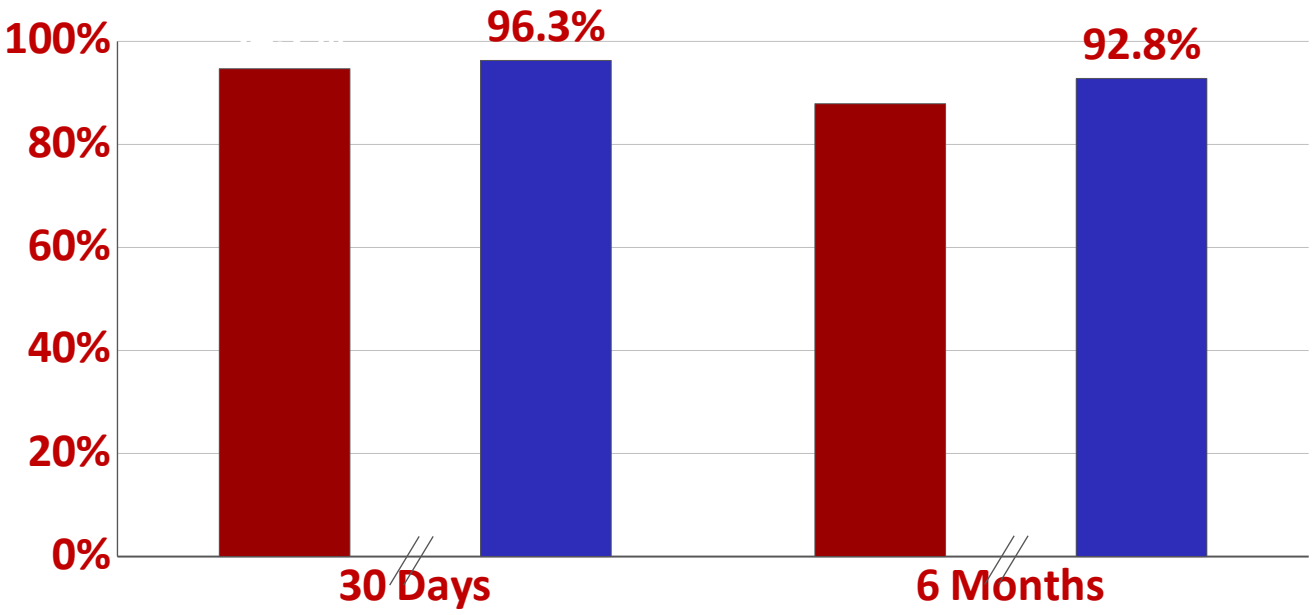
- **Single Arm Study:**
  - Because these hearts are not routinely being utilized and could not be compared to standard criteria hearts
- **Donor Criteria** – Targeted donor hearts that may benefit from perfusion
  - Extended ischemia time >4 hours
  - Older donors >45 y
  - Down time ≥ 20 mins
  - LVH hearts 12-16 mm thickness
  - Non specific CAD
- **Effectiveness Endpoints:**
  - Primary: Composite of patient survival at day 30 and freedom from severe PGD in the first 24 hours
  - Secondary: Donor hearts utilization rate
- **Safety Endpoint:**
  - Rate of moderate and severe PGD up-to day 30 post transplant

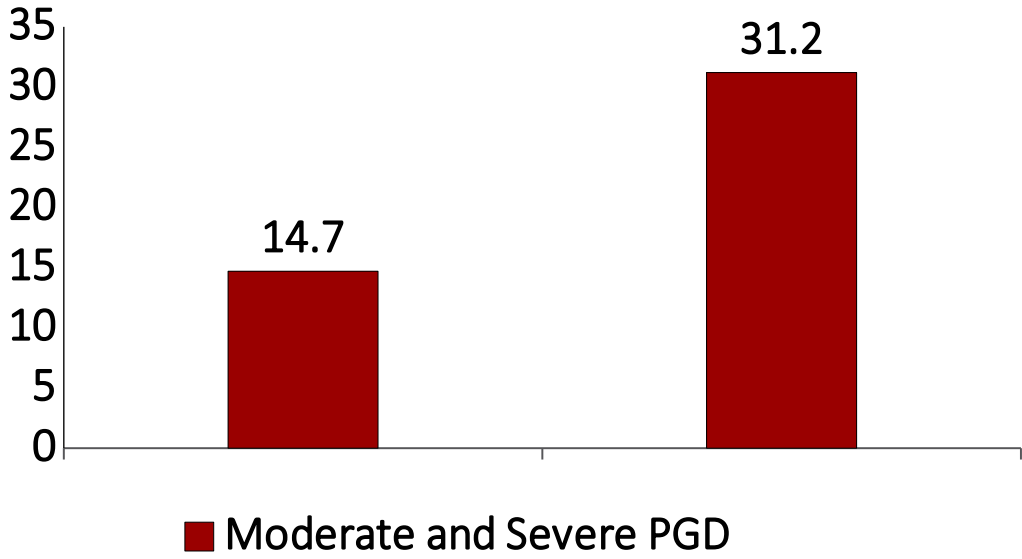
OCS HEART EXPAND TRIAL

Marginal Donors



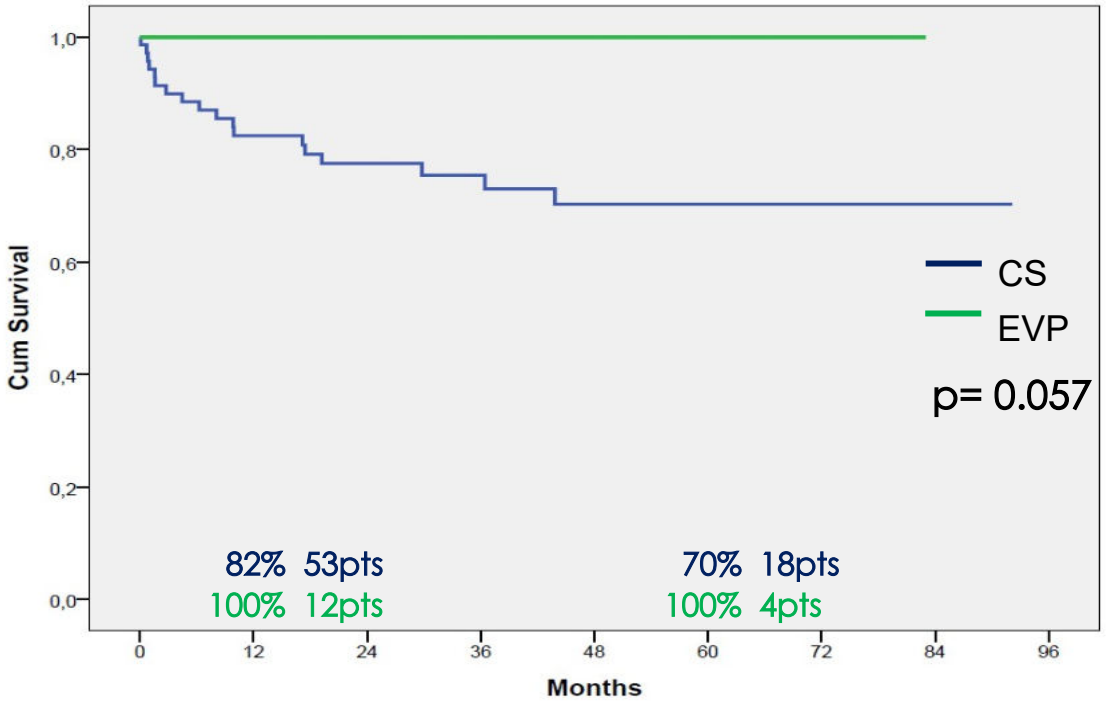
81% Utilization Rate





Udine experience: Marginal donors

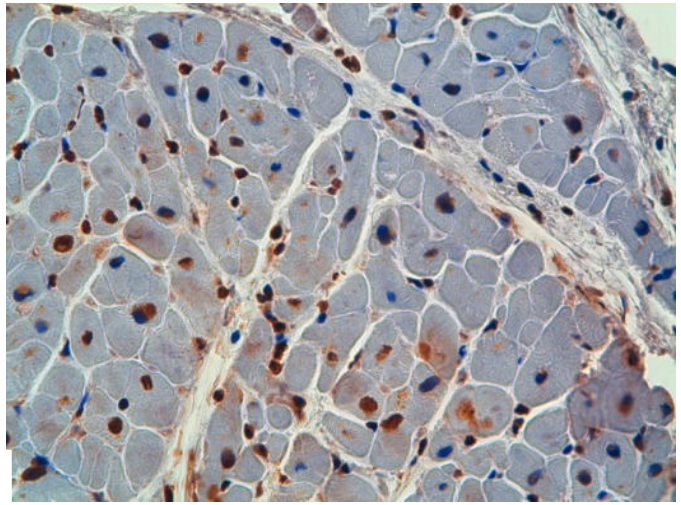
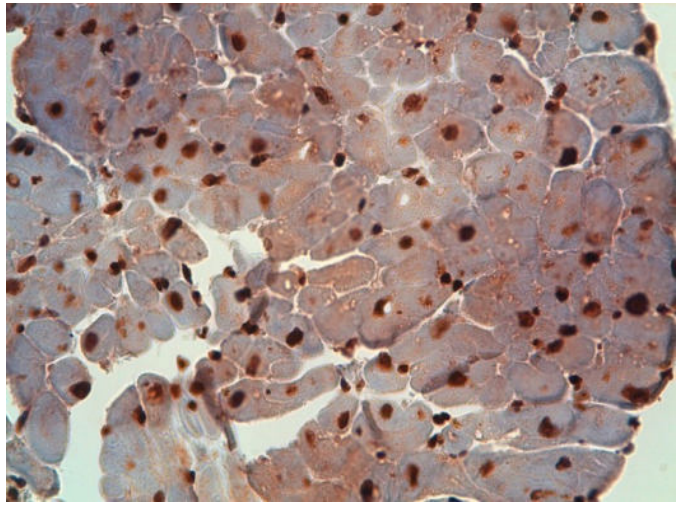
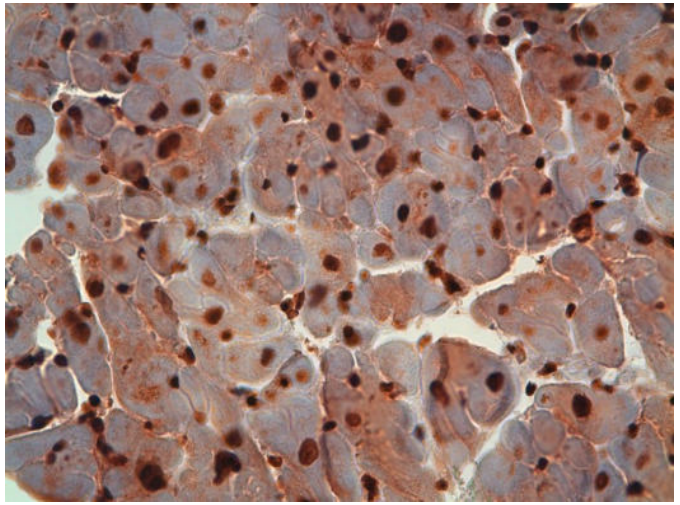
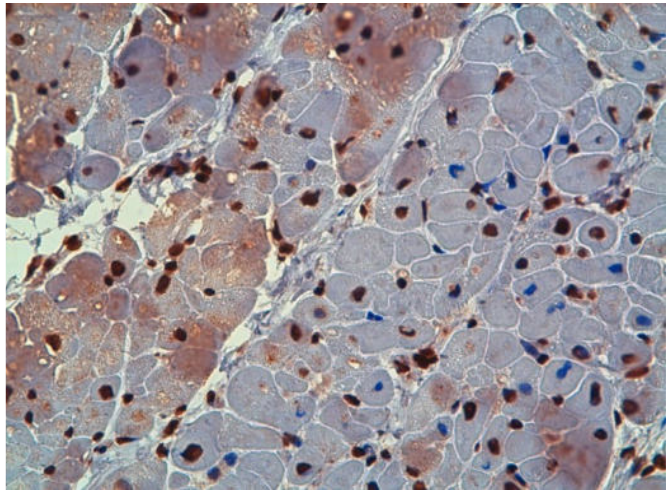
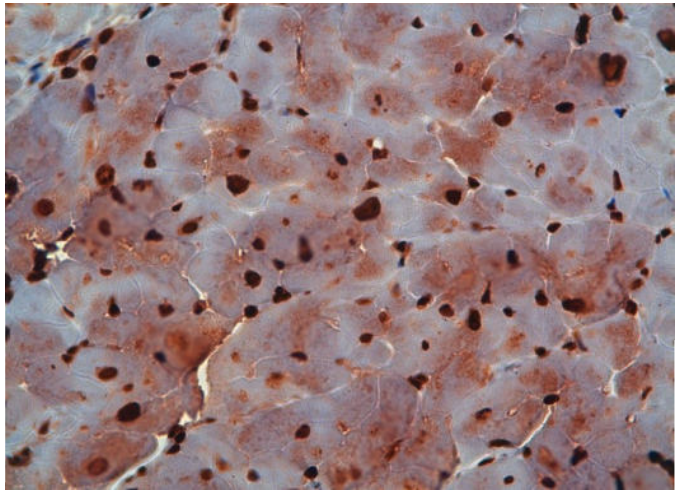
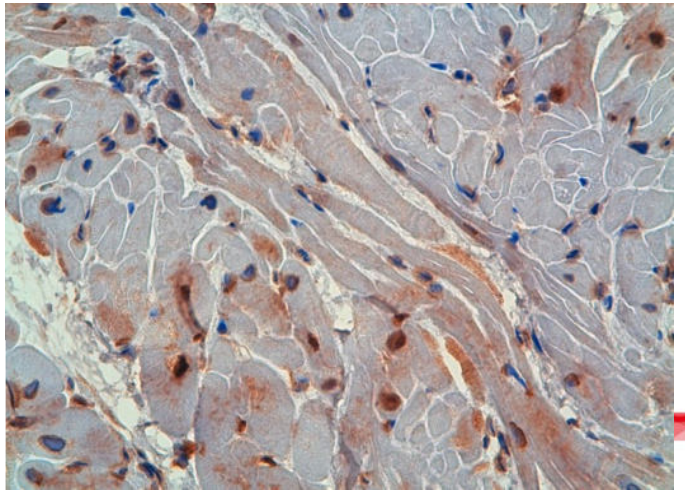
Peri-operative data	CS (n=72)	EVP (n=17)	p
CPB time (min), median (min-max)	210 (136-394)	176 (125-413)	0.03*
Ischemic time (min), median (min-max)	229 (91-365)	138 (103-231)	<0.01*
Total Surgical Time (min), median (min-max)	450 (247-840)	400 (220-615)	0.05*



Early outcome	CS (n=72)	EVP (n=17)	p
Overall complications, % (n)	88% (63)	65% (11)	0.04*
MAV hours, median (min-max)	48 (10-408)	24 (12-192)	0.02*
MAV >72 hours, % (n)	24% (17)	6% (1)	0.09
High dose inotropic support, % (n)	22% (16)	12% (2)	0.27
ECMO, % (n)	3% (2)	0	0.65
IABP, % (n)	22% (16)	6% (1)	0.11
AKI, % (n)	28% (20)	18% (3)	0.30
PGF, % (n)	15% (11)	12% (2)	0.53
Rejection ≥ grade 2, % (n)	13% (9)	12% (2)	0.65
Atrial Fibrillation, % (n)	17% (12)	6% (1)	0.24
ICU stay (days), median (min-max)	6 (2-83)	5 (2-20)	0.04*
Hospital stay (days), median (min-max)	33 (14-279)	32 (19-102)	0.71
Early death, % (n)	7% (5)	0	0.34

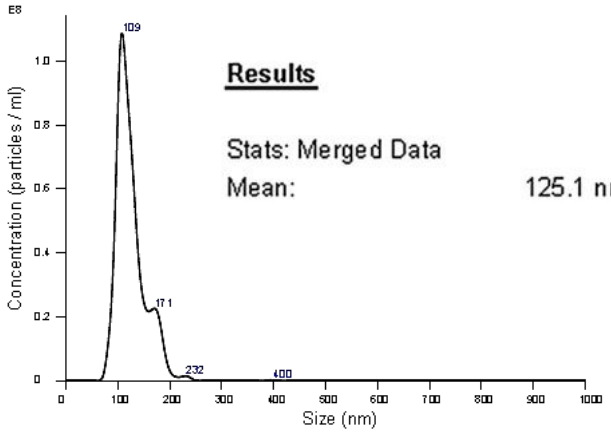
Histological analysis

Mithocondrial dysfunction - PARKIN

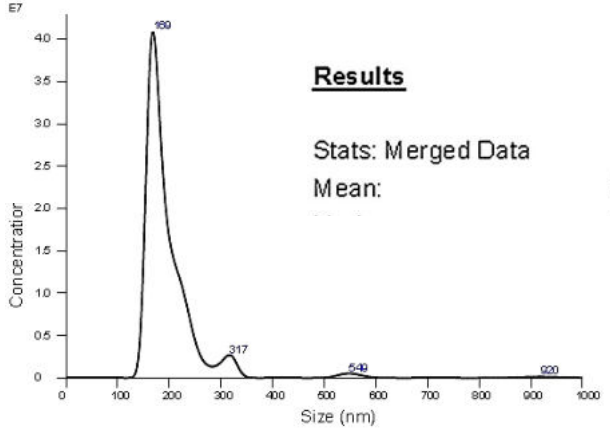
	Explant	Pre Implant	Post Declamp
Cold Ischemia			
OCS			

Nanoparticle tracking analysis

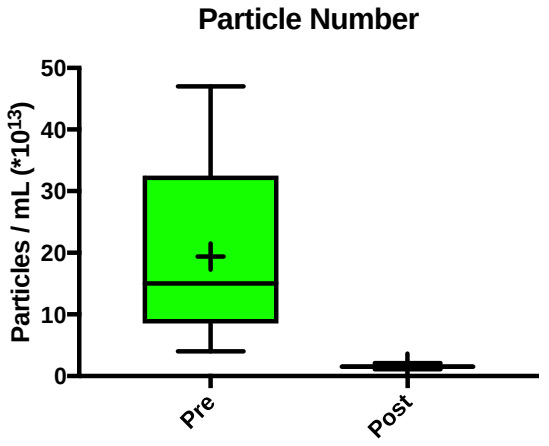
- OCS



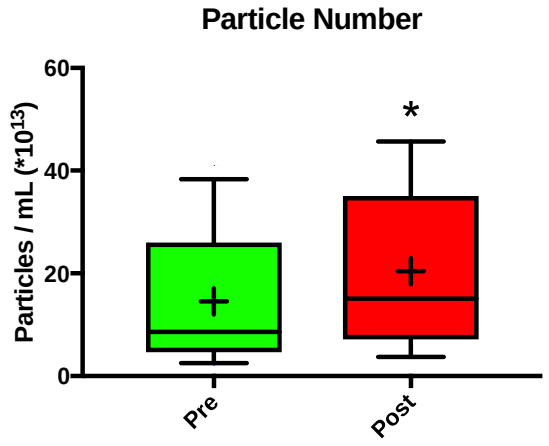
- Cold Ischemia



OCS



Cold Ischemia

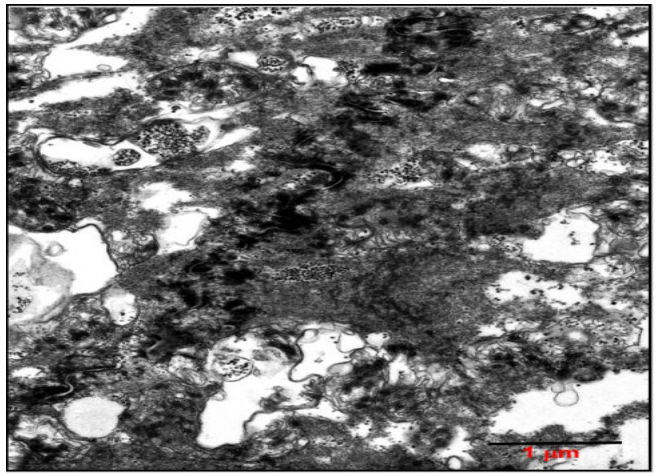


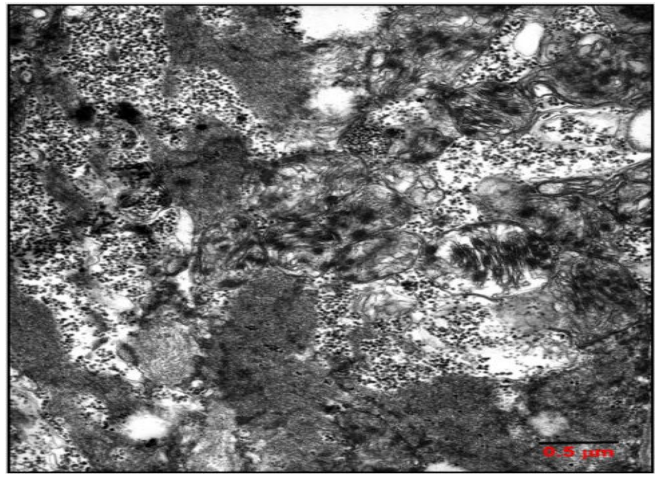
STATI GENERALI
RETE NAZIONALE TRAPIANTI
20 ANNI 1999-2019

Ultrastructural analysis

Transmission Electron Microscopy

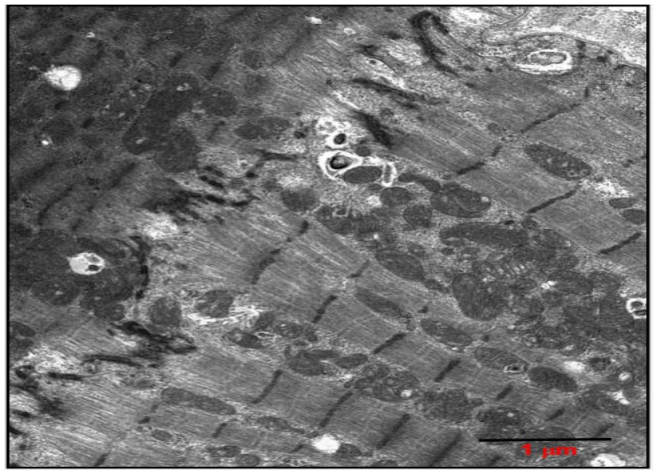
Cold storage

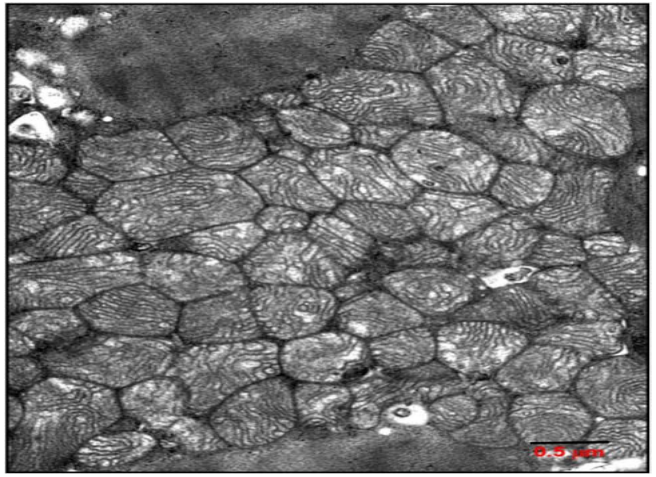




Hearts preserved in Cold Storage showed cardiomyocyte degeneration, with maximal cell suffering after heart reperfusion

OCS





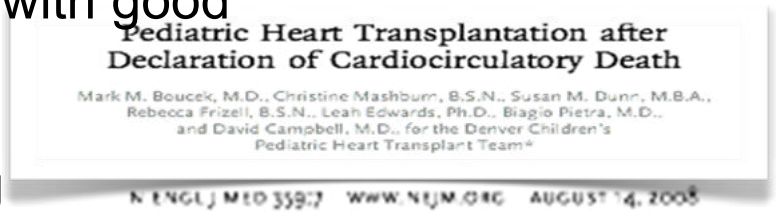
Conversely, after ex-vivo reperfusion, cardiomyocyte showed well-preserved organelles also after heart reperfusion

Experience with DCD hearts

Lower 1966, Virginia - Resuscitated human cadaver heart and transplanted it into a baboon where it provided satisfactory circulatory support for several hours.

1967 •First heart Tx was technically a DCD donor heart

2008 •3 DCD hearts used in paediatric heart transplants with good outcomes, all alive at 6 months; Denver, USA


Pediatric Heart Transplantation after Declaration of Cardiocirculatory Death

Mark M. Boucek, M.D., Christine Mashburn, B.S.N., Susan M. Dunn, M.B.A., Rebecca Frizell, B.S.N., Leah Edwards, Ph.D., Biagio Pietra, M.D., and David Campbell, M.D., for the Denver Children's Pediatric Heart Transplant Team*

N ENGL J MED 359;7 WWW.NEJM.ORG AUGUST 14, 2008

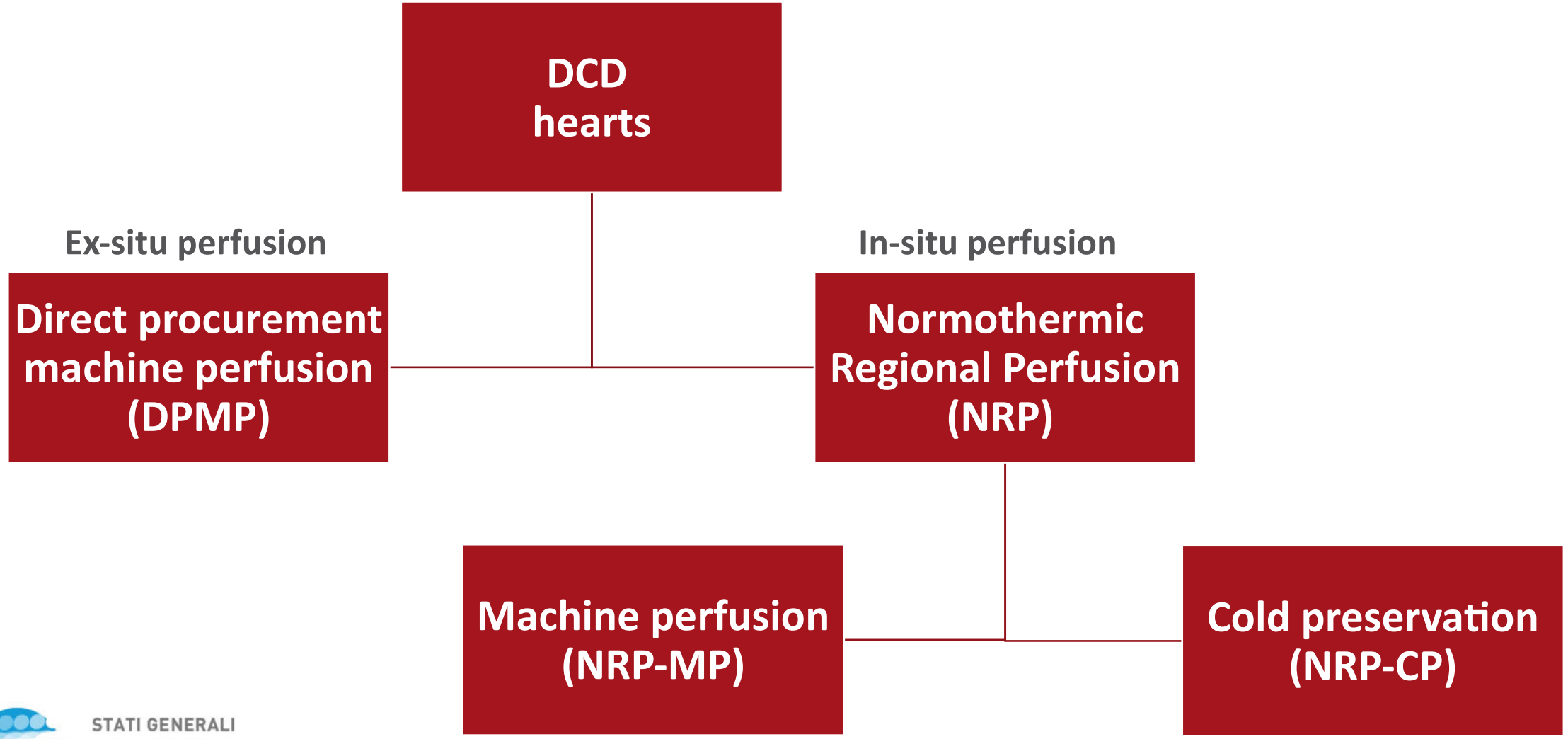
2009 •Human DCD heart successfully resuscitated using extracorporeal perfusion


CASE REPORTS

Cardiac Recovery in a Human Non–Heart-beating Donor After Extracorporeal Perfusion: Source for Human Heart Donation?

Ayyaz Ali, MRCS,[a] Paul White, PhD,[b] Kumud Dhital, FRCS,[a] Marian Ryan, RGN,[a] Steven Tsui, FRCS,[a] and Stephen Large, FRCP, FRCS[a]


Bruxelles: 3 cases
Leuven: 2 cases
North Sea
DENMARK
Kobenhavn
UNITED KINGDOM
Dublin
IRELAND
London
NETHERLANDS
GERMANY
Berlin
Brussels
BELGIUM
LUXEMBOURG
Prague
CZECH REPUBLIC
English Channel
Vienna
AUSTRIA
SWITZERLAND
Bern
LIECHTENSTEIN
SLOVENIA
Ljubljana
FRANCE
Bay of Biscay
SAN MARINO
MONACO
ANDORRA
ITALY
Rome
VATICAN
Madrid
PORTUGAL
SPAIN
Mediterranean Sea
Algiers
Tunis
MALTA
INDIAN
Port Hedland
Dampier
Carnarvon
Geraldton
Fremantle
Bunbury
News
Samsung GALAXY Note 4
SAMSUNG
+ Gear VR


DCD
hearts
Ex-situ perfusion
Direct procurement machine perfusion (DPMP)
In-situ perfusion
Normothermic Regional Perfusion (NRP)
Machine perfusion (NRP-MP)
Cold preservation (NRP-CP)

DPP – direct procurement and perfusion

- Declaration of death
- Reintubation and sternotomy
- Donor blood drainage and collection
- Transmedics OCS priming
- Cardioplegia into DCD heart
- Cardiac removal and instrumentation upon OCS

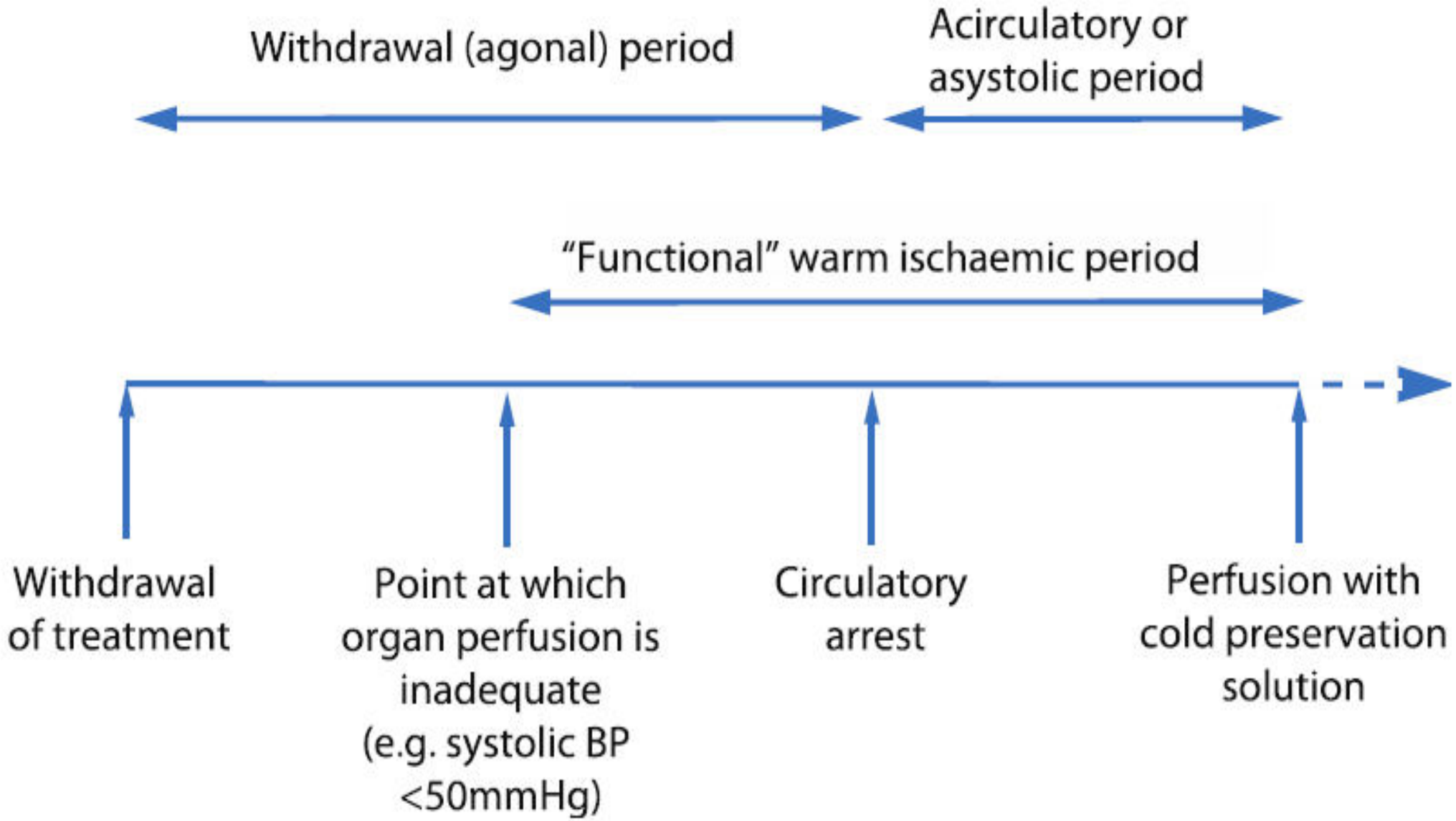
DRAWBACKS
Toxic milieu: blood hyperkalemic, hyperglycemic, hypoxic, hypercapnic, acidotic, high levels of cytokines and catecholamines. **Functional assessment**: perfusate lactate concentration <5mMol/L, (artery lactate > coronary sinus lactate). Questioned validity as metabolic marker (reliable indicator ?) **Small and dehydrated donors** → no enough blood to prime OCS **Time from withdrawal of life support to cardioplegia <30min**

NRP – Normothermic Regional Perfusion

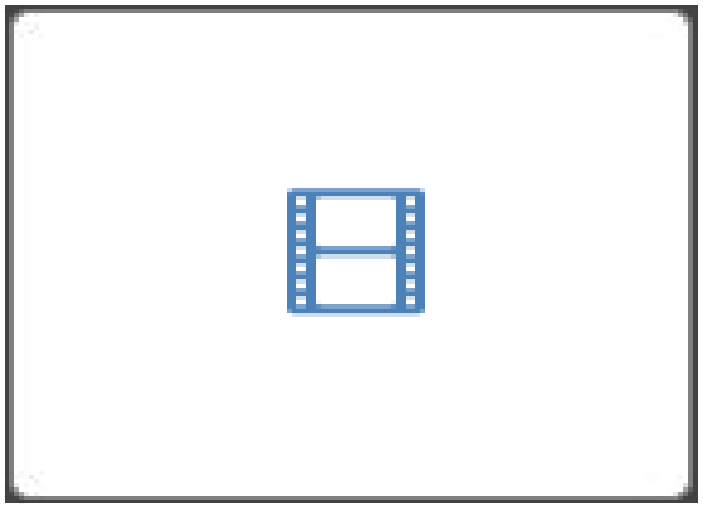
- Declaration of death
- Re-Intubation and sternotomy
- Heparin 30'000 UI into the RA and 20'000 UI into the PA
- Clamping of Aortic Arch Vessels
- Aortic – Right Atrial Cannulation → ECC
- DCD Heart Reconditioned
- NRP Discontinued (after 90 min)
- Cardiac Assessment with TEE and SG catheter
- Blood Collection to prime OCS

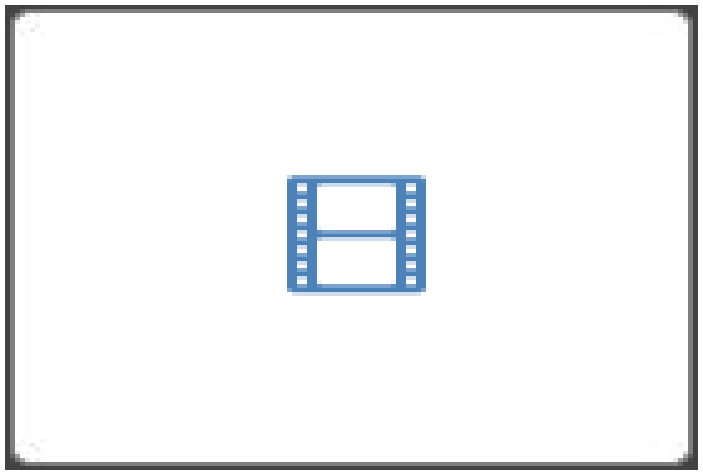
ADVANTAGES
Earlier reperfusion, shorter warm ischaemic time (on average 8 min) Time from withdrawal of life support to NRP up to 180 min Functional assessment as usual (TEE/SG catheter) More confidence in decision making Better donor organ utilization

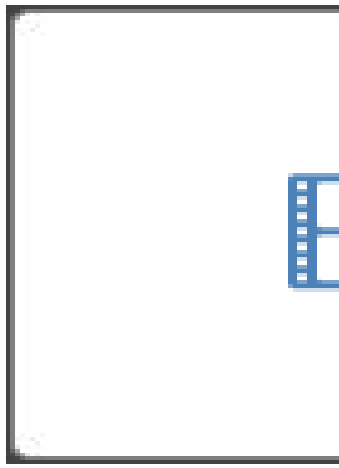
DRAWBACKS
More resources required (blood products, equipement, personal, etc) Detrimental effects on other organs? Legal and Ethical issues (heart perfusion within the donor)


Withdrawal (agonal) period
Acirculatory or asystolic period
"Functional" warm ischaemic period
Withdrawal of treatment
Point at which organ perfusion is inadequate (e.g. systolic BP <50mmHg)
Circulatory arrest
Perfusion with cold preservation solution

First DCD Human Heart Transplant







Initial 10 Mins on OCS

After 4 hours on OCS

Off Bypass in Recipient

Courtesy of Dr. Kumud Dhital, St. Vincent's Hospital, Sydney, Australia

Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series

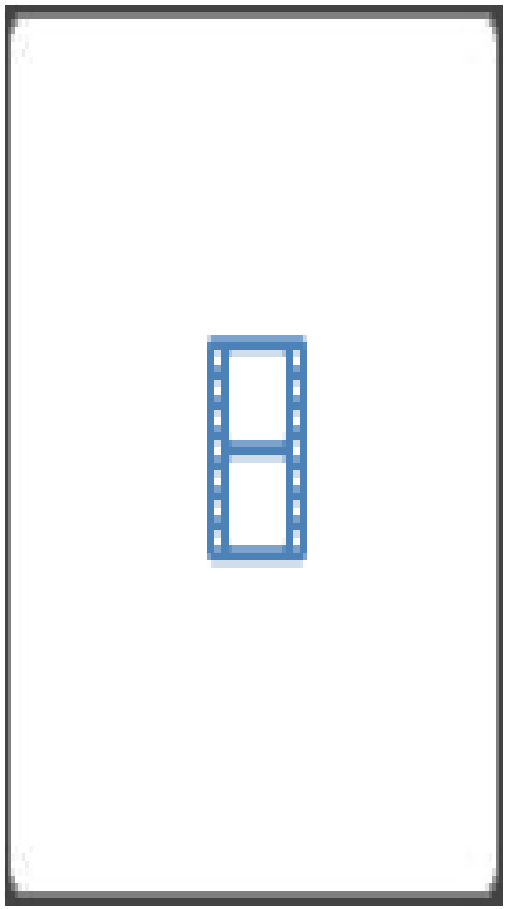
Kumud K Dhital, Arjun Iyer, Mark Connellan, Hong C Chew, Ling Gao, Aoife Doyle, Mark Hicks, Gayathri Kumarasinghe, Claude Soto, Andrew Dinale, Bruce Cartwright, Priya Nair, Emily Granger, Paul Jansz, Andrew Jabbour, Eugene Kotlyar, Anne Keogh, Christopher Hayward, Robert Graham, Phillip Spratt, Peter Macdonald

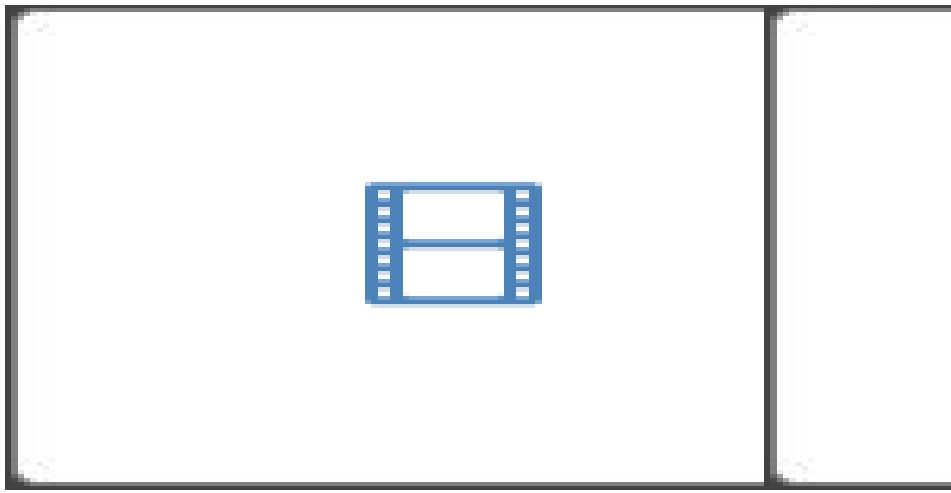
	Recipient 1	Recipient 2	Recipient 3	Donor 1	Donor 2	Donor 3
Age (years)	57	43	57	26	26	27
Sex	Male	Female	Male	Male	Male	Male
Diagnosis	Familial DCM	Viral DCM	ARVD*	Hypoxia	Trauma	Trauma
Blood group	A	A	O	A	A	O
Height (cm)	163	176	170	183	173	182
Bodyweight (kg)	71	70	79	92	70	79
Ejection fraction (%)	20	18	19	75	50	NA
LVEDD (mm)	84	61	67	..	..	..
TPG (mm Hg)	7	5	8	..	..	..
Creatinine concentration (µmol/L)	99	135	149	..	..	..
eGFR (mL/min BSAc)	44	65	42	..	..	..
Total bilirubin concentration (µmol/L)	30	60	42	..	..	..

	Donor 1	Donor 2	Donor 3
Withdrawal parameters			
Location of withdrawal	Operating theatre	Intensive care unit	Anaesthetic bay
Withdrawal to systolic blood pressure <50 mm Hg (min)	7	5	11
Withdrawal to SaO_2 <50% (min)	8	2	1
Withdrawal to cessation of circulation (min)	16	10	11
Observation period (min)	2	2	5
Warm ischaemic time (min)*	28	25	22
OCS parameters			
Pacing	Yes	Yes	No
Adrenaline infusion (µg/h)	5	5	5–7
Adenosine infusion (mg/h)	0–21	0–21	0–21
Total OCS perfusion time (min)	257	260	245
Total ischaemic time (min)†	90	96	107
A-V lactate at start of perfusion (mmol/L)	8·30–8·10	6·79–6·48	7·60–7·40
A-V lactate at end of perfusion (mmol/L)	3·60–3·60	2·80–2·30	2·69–2·54

Lancet 2015

DCD Udine project





Arrested 30’

15’ of reperfusion

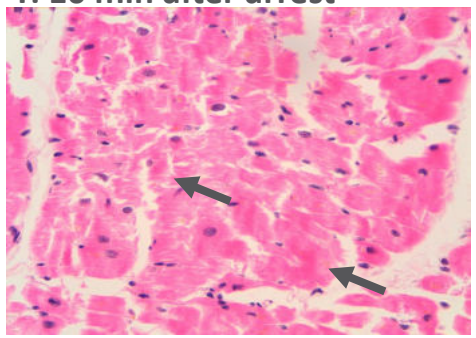
30’ of reperfusion

From arrest to reperfusion

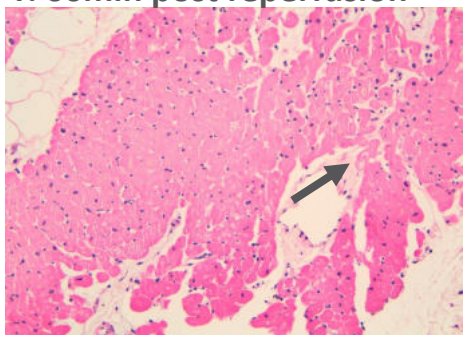
Histological findings

- Contraction bands

T: 10 min after arrest

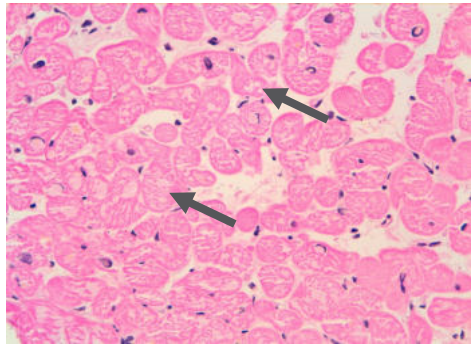


T: 60min post reperfusion

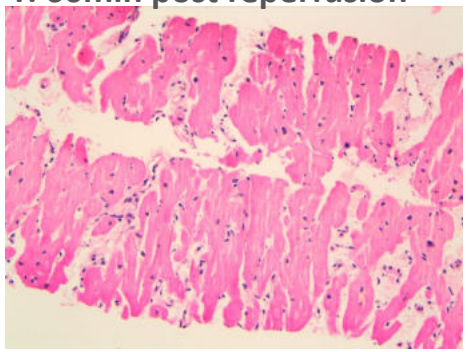


- Intracellular vacuolization

T: 10 min after arrest

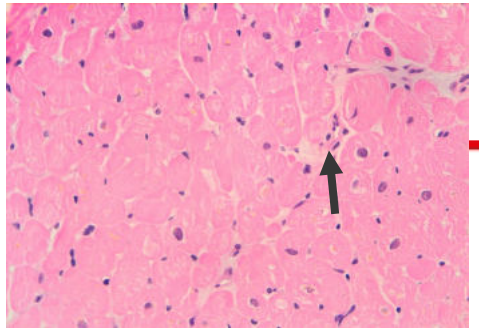


T: 60min post reperfusion

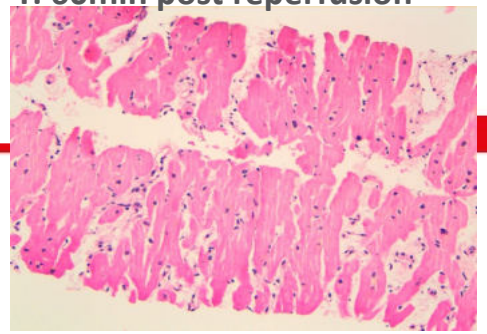


- Endothelial cells swelling

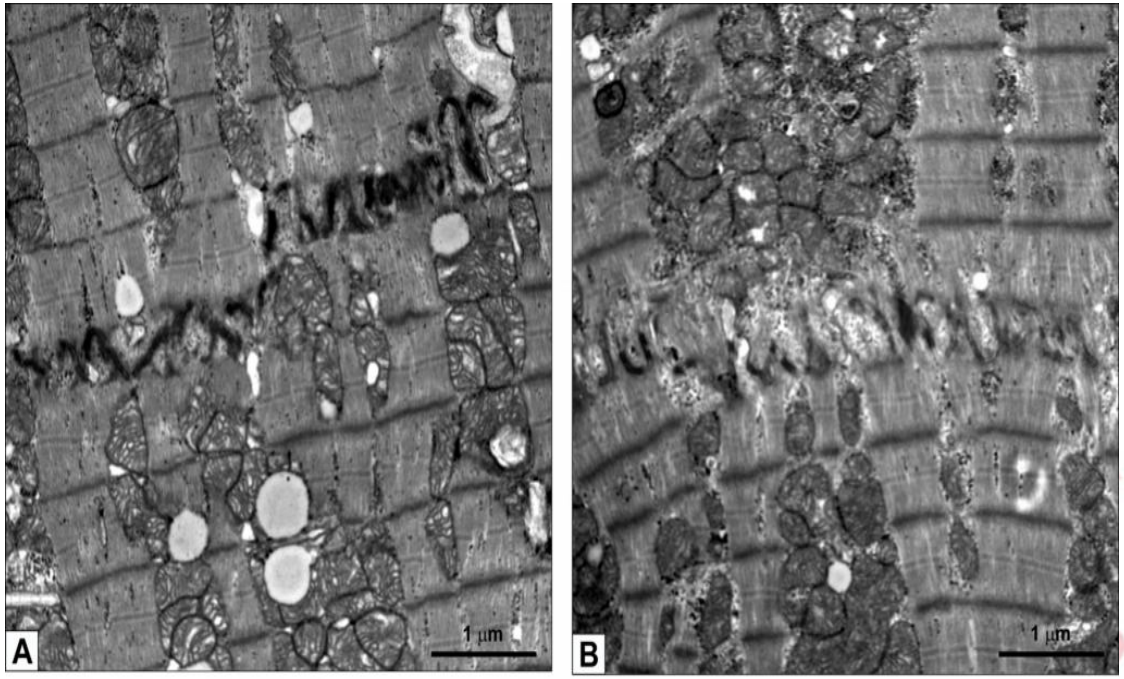
T: 10 min after arrest



T: 60min post reperfusion



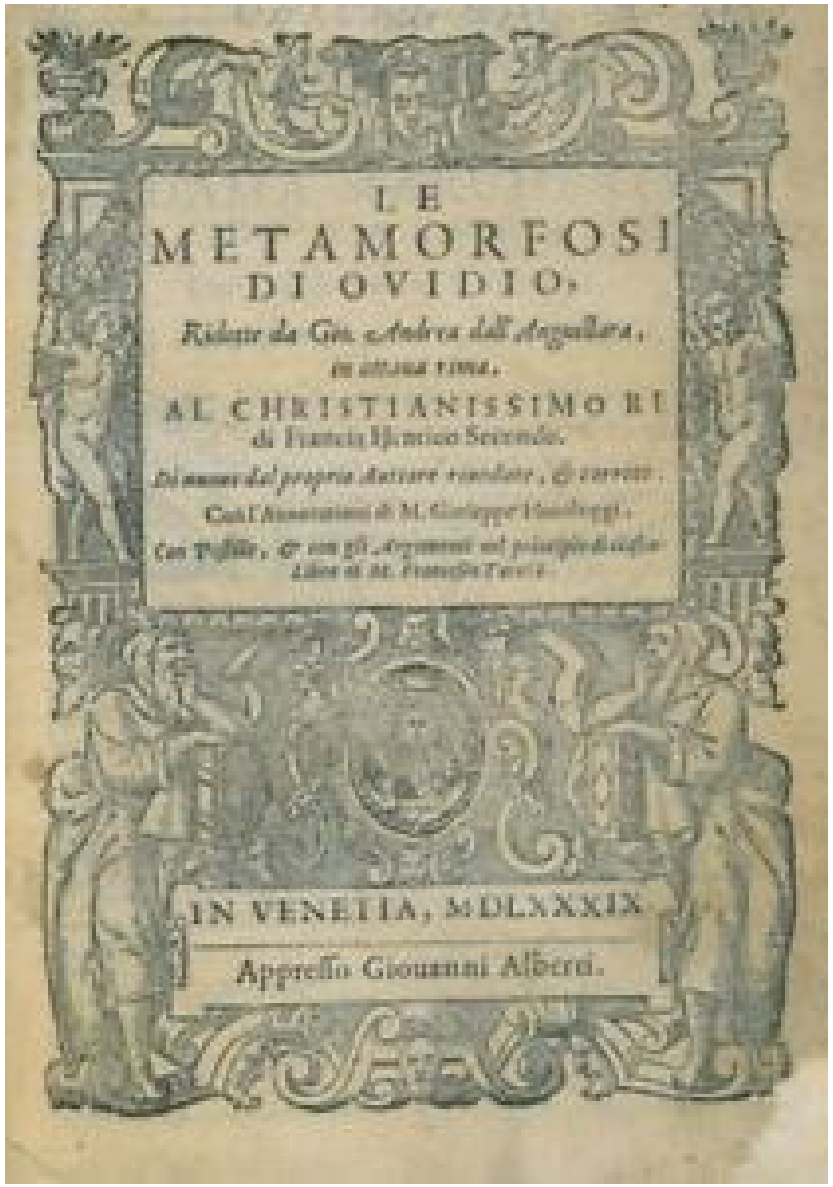
Ultrastructural changes



Bonetti A & Ortolani F. Histology & Electron Microscopy Unit, Department of Medicine, University of Udine, Italy

Omnia mutantur, nihil interit

Everything changes, nothing perishes



STATI GENERALI

RETE NAZIONALE
TRAPIANTI

6·7·8 NOVEMBRE

ROMA

American Journal of Transplantation 2016; 16: 33–43
Wiley Periodicals Inc.

doi: 10.1111/ajt.13446

Altered Immunogenicity of Donor Lungs via Removal of Passenger Leukocytes Using *Ex Vivo* Lung Perfusion

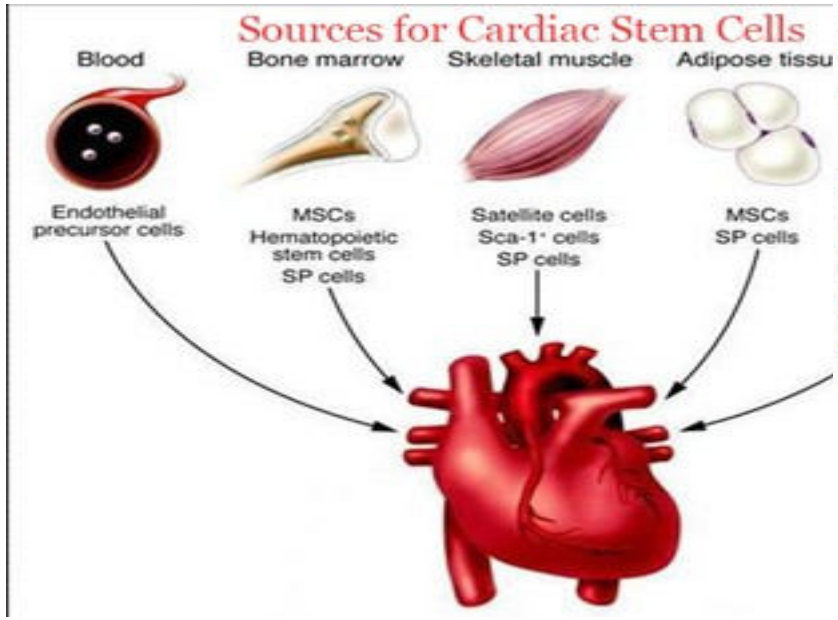
J. P. Stone[1,2], W. R. Critchley[1,2], T. Major[1,2], G. Rajan[2], I. Risnes[3], H. Scott[3], Q. Liao[4], B. Wohlfart[4], T. Sjöberg[4], N. Yonan[2], S. Steen[4] and J. E. Fildes[1,2,*]

Ex-vivo perfusion reduces

- **graft immunogenicity by decreasing the transfer of passenger leukocytes from the donor graft to the recipient**
- **ischemic time → reduction of I/R injury and activation of innate immunity system**

Reduced risk of rejection and CAV?

Stem cells immunomodulation and rigeneration



Immunomodulatory properties of mesenchymal stromal cells

Alma J. Nauta[1] and Willem E. Fibbe[1]

[1]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands

Mesenchymal stem cells (MSCs) are multipotential nonhematopoietic progenitor cells capable of differentiating into multiple lineag
have emer
modality f
pair. Furth
raised by
immunopr
display immunomodulatory capacities. Although the mechanisms underlying the immunosuppressive effects of MSCs have
vivo, the possible underlying mechanisms, the potential clinical use of MSCs as modulators of immune responses in

Review

Journal of INTERNAL MEDICINE

doi: 10.1111/j.1365-2796.2007.01844.x

Immunomodulation by mesenchymal stem cells and clinical experience

K. Le Blanc[1,2] & O. Ringdén[1,3]

From the [1]Division of Clinical Immunology, [2]Hematology Center and [3]Center for Allogeneic Stem Cell Transplantation, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

MSCs inhibit T-cell proliferation and prevent the development of cytotoxic T-cells

Abstract Le Blanc K, Ringdén O (Karolinska Univer- Sweden). Immu- cells and clinical 2007; **262**: 509–

Mesenchymal stem cells (MSCs) from adult marrow can differentiate *in vitro* and *in vivo* into various cell types, such as bone, fat and cartilage. MSCs preferentially home to damaged tissue and may have therapeutic potential. *In vitro* data suggest that MSCs have low inherent immunogenicity as they induce little, if any, proliferation of allogeneic lymphocytes. Instead, MSCs appear to be immunosuppressive *in vitro*. They inhibit T-cell proliferation to alloantigens and mitogens and prevent the development of cytotoxic T-cells. *In vivo*, MSCs prolong skin allograft survival and have several immunomodulatory effects, which are presented and discussed in the present study. Possible clinical applications include therapy-resistant severe acute graft-versus-host disease, tissue repair, treatment of rejection of organ allografts and autoimmune disorders.

Keywords: acute graft-versus-host disease, haematopoietic stem cell transplantation, immunomodulation, mesenchymal stem cells, tissue toxicity.