UNIVERSITY OF BIRMINGHAM

Research at Birmingham

Searching for the Optimal Fluid to Restore Microcirculatory Flow Dynamics After Haemorrhagic Shock

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Table 1. Animal characteristics of included studies

Species	Animals	Studies	Weight range	Awake, %	References
Rat	1149	31	120–460 g	0	(20-22, 33, 38-40, 44, 46, 48, 49, 52-55, 62, 66, 70-75, 79-81, 86-90)
Hamster	518	26	40–75 g	100	(23-32, 45, 47, 50, 58, 60, 61, 63, 68, 69, 76-78, 82-85)
Rabbit	94	3	0.8–3.5 kg	0	(51, 57, 59)
Pig	74	4	7–45 kg	0	(41-43, 56)
Mouse	68	2	25–30 g	0	(64, 65)
Dog	56	5	22–35 kg	0	(34-37, 67)

Table 2. Summary of haemorrhage protocol targets and timings

Haemorrhage protocol	N	Target % of blood volume lost	Target MAP, mmHg	Target % of MAP	Target mL/kg bled	Period of shock, minutes	References
Volume controlled haemorrhage							
% of total blood volume lost	27	50 (50–50)	N/A	N/A	N/A	60 (60–60)	(24-32, 51, 56, 58, 61-63, 71-74, 76-78, 82-85, 89)
Bled a specific volume of blood per kg	6	N/A	N/A	N/A	30 (30–30)	40 (30–57.5)	(41, 42, 46, 48, 86, 87)
Pressure controlled haemorrhage							
Bled to a target MAP	26	N/A	40 (37.5–40)	N/A	N/A	60 (45–60)	(20-22, 33, 38-40, 44, 45, 47, 49, 52-55, 59, 64-67, 75, 79-81, 88, 90)
Bled to both % of total blood volume and target MAP	11	50 (40–50)	40 (37.5–48.8)	N/A	N/A	45 (36–60)	(23, 34-37, 43, 50, 57, 60, 68, 69)
Bled to a % of MAP	1	N/A	N/A	50	N/A	30*	(70)

All values are expressed as median, with interquartile range in brackets unless otherwise specified
*This is a single value rather than a median

Table 3. Anatomical location and techniques for microcirculatory parameter acquisition

Anatomical location	Studies	References					
		Intravital microscopy	Laser speckle contrast imaging	Sidestream darkfield microscopy	Laser Doppler flowmetry probe	Electron microscopy	Orthogonal polarization spectral imaging
Dorsal skin fold	26	(23-32, 45, 47, 50, 58, 60, 61, 63, 68, 69, 76-78, 82-85)					
Bowel / mesentery	15	(33, 39, 46, 48, 49, 62, 70, 89)	(86, 87)	(59, 67)	(44)	(52)	(75)
Liver	9	(20, 21, 38, 53, 55, 66, 79)	(86, 87)		(38)		
Cremaster	5	(64, 65, 71, 73, 74)					
Skeletal muscle	6	(22, 39, 40, 57)	(86, 87)				
Conjunctiva	4	(34-37)					
Sublingual	5			(41, 42, 56)	(43, 56)		
Kidney	5	(39)	(86, 87)		(44, 54)		
Pancreas	2	(80, 81)					
Brain	2				(44, 54)		
Internal spermatic fascia	1	(88)					
Buccal mucosa	1			(67)			
Ear chamber	1	(51)					
Heart	1				(44)		

Table 4. Summary of study findings regarding haemoglobin-carrying fluids

Study	Animal	Method	Test fluid(s)	Control fluid(s) or sham	Endpoint(s)	Main finding
A. Haemoglobin based oxygen carriers						
Bi 2004	Rat	IVM	PEG-Hb (3 different volumes)	Whole blood (2 different volumes) and dextran and HC	Velocity, flow, diameter	HBOC superior to non Hb carrying fluid, and equivalent to whole blood
Botzlar 1996	Hamster	IVM	U-PBHb 11g/dL and 13g/dL	Dextran and LR	Diameter, FCD	HBOC superior to non Hb carrying fluid
Cabrales 2009	Hamster	IVM	PBH 13g/dL and PBH 4g/dL	Albumin	Velocity, flow, diameter, FCD	HBOC superior to non Hb-carrying fluid
Cheung 2001	Canine	IVM	Bovine Hb glutamer-200	Whole blood	Velocity, diameter	HBOC equivalent to whole blood
Cheung 2004	Canine	IVM	Oxyglob	Whole blood and 6% hetastarch	Velocity, diameter	HBOC equivalent to non Hb-carrying fluid and whole blood
Cheung 2006	Canine	IVM	Oxyglobin	Whole blood and NS and 6% hetastarch	Velocity, diameter	HBOC equivalent to non Hb-carrying fluid and whole blood
Cheung 2007	Canine	IVM	Oxyglobin	Whole blood and NS and 6% hetastarch	Velocity, diameter	HBOC equivalent to non Hb-carrying fluid and whole blood
Gulati 1998	Rat	LDF	DCLHb (3 different concentrations)	LR	Velocity, perfusion	HBOC superior to non Hb carrying fluid
Hermann 2007	Hamster	IVM	Recombinant Hb wild type and rcombinant Hb nitric-oxide scavenging	6% dextran	Velocity, diameter, FCD	Nitric-oxide scavenging HBOC superior to HBOC or non Hb carrying fluid
Hungerer 2006	Hamster	IVM	DCLHb	Whole blood and dextran	Velocity, FCD	HBOC equivalent to whole blood and non Hb carrying fluid
Kerger 1997	Hamster	IVM	Cell-free o-raffinose cross-linked oligomerized Hb	Whole blood and LR and dextran	Velocity, flow, diameter, FCD	HBOC superior to non Hb-carrying fluid and equivalent to whole blood
Kubulus 2009	Rat	IVM	Hb glutamer-200	Whole blood	Velocity, flow, diameter, PVD	HBOC equivalent to whole blood
Kumar 1997	Rat	LDF	DCLHb	LR	Velocity, perfusion	HBOC superior to non Hb carrying fluid
Nolte 1997	Hamster	IVM	DCLHb	Whole blood and dextran	Velocity, diameter, FCD	HBOC superior to whole blood and non Hb carrying fluid
Ortiz 2014	Hamster	IVM	Bovine Hb glutamer-250 at 4, 8, and 12 g/dL	Whole blood	Velocity, flow, FCD	HBOC superior to whole blood; lower Hb preparations superior to higher
Palmer 2011	Hamster	IVM	Polymerised human Hb and polymerised bovine Hb	HSA	Velocity, flow, FCD	HBOC superior to non Hb carrying fluid; bovine preparation of Hb superior to human
Sakai 2002	Hamster	IVM	Vesicle-encapsulated Hb 3g/dL and 7g/dL	Whole blood and HSA	Velocity, flow, diameter, FCD	HBOC superior to non Hb carrying fluid but inferior to whole blood
Vazquez 2011	Hamster	IVM	Oxyglobin	HES	Diameter, FCD	HBOC superior to non Hb carrying fluid
von Dobschuetz 1999	Rat	IVM	DCLHb	Whole blood and HES	FCD	HBOC superior to non Hb carrying fluids and equivalent to whole blood
Wettstein 2003	Hamster	IVM	PEG-Hb	Whole blood and HES	Velocity, flow, diameter, FCD	HBOC superior to whole blood and non Hb carrying fluid
Wettstein 2004(a)	Hamster	IVM	PEG-Alb	PEG-Hb (from earlier experiment)	Velocity, flow rate, diameter, FCD	HBOC equivalent to non Hb carrying fluid
B. Red cells and whole blood						
Casali 2002	Rat	IVM	Whole blood	LR	Velocity	Whole blood superior to crystalloid
Kao 2010	Rat	IVM	Whole blood and whole blood/EPO	NS and NS/EPO	Flow, perfusion	Whole blood superior to crystalloid
Ni 2013	Rabbit	SDF	Whole blood and LR and whole blood/LR	Sham	TVD, PVD, PPV, MFI	Blood and crystalloid combined superior to either fluid on its own
Paxian 2003	Rat	IVM	PRBC and perflubron emulsion/HES and PRBC/perflubron emulsion	LR and HES and whole blood	Diameter, velocity, flow	O_2 emulsion superior to whole blood, red cells, or non-oxygen carrying fluid
Sakai 1999	Hamster	IVM	Whole blood	HSA	Velocity, flow, diameter, FCD	Whole blood is superior to colloid
Villela 2009	Hamster	IVM	High O_2-affinity PRBC (50mmHg)	Low O_2-affinity PRBC (10mmHg)	Velocity, flow, diameter, FCD	Lower O_2 affinity of red cells superior to higher

Techniques: IVM: intravital microscopy; LDF: laser doppler flowmetry; SDF: sidestream darkfield microscopy
Endpoints: PVD: perfused vessel density; TVD: total vessel density; PPV: proportion of perfused vessels; MFI: microvascular flow index; HI: heterogeneity index; FCD: functional capillary density;
Fluids: HBOC: haemoglobin based oxygen carrier; PRBC: packed red blood cells; LR: Ringer's lactate; NS: normal saline; HTS: hypertonic saline; HSA: human serum albumin; HC: haemorrhage control; HES: hydroxyl-ethyl starch; PBH: polymerized bovine haemoglobin; PEG-Alb: polyethylene glycol-conjugated albumin; PEG-Hb: polyethylene glycol-conjugated haemoglobin; UPBHb: ultrapurified polymerised bovine haemoglobin solution; DCLHb: diaspirin cross-linked haemoglobin; Hb: haemoglobin; EPO: erythropoietin

Table 5. Summary of study findings regarding osmotic/oncotic pressure

Study	Animal	Method	Test fluid(s)	Control fluid(s) or sham	Endpoint(s)	Main finding
Bauer 1993	Rat	IVM	7.2% HTS/Dextran and 7.2%HTS/10% HES	LR and Sham	Velocity, flow, diameter	Hypertonic and isotonic fluids are equivalent
Cabrales 2005(b)	Hamster	IVM	5% PEG-Alb	10% HES and HC	Velocity, flow, diameter, FCD	Length of time of oncotic pressure is important
Cabrales 2008(b)	Hamster	IVM	4% PEG-Alb	5% HSA and 10% HSA	Velocity, flow, shear stress, diameter, FCD	Increased plasma expansion superior with conjugated molecule
Cryer 2005	Rat	IVM	7.2% HTS/6% dextran and NS/dextran	NS	Velocity, flow, diameter	Effects of hypertonic/hyperosmotic fluid last longer than isotonic
Gierer 2004	Rat	IVM	10% HES and 7.2% HTS/6% HES	NS	FCD	Hypertonic/hyperosmotic fluid is superior
Kao 2011	Rat	IVM	7.5% HTS and 7.5% HTS/EPO	NS and NS/EPO and LR and LR/EPO	PVD	Hypertonic crystalloid inferior to isotonic
Komori 2005	Rabbit	IVM	HES	LR	Velocity, flow, diameter	Hypertonic/hyperosmotic fluid is superior
Maier 2009	Porcine	LDF and SDF	Gelatine and 7.2% HTS/6% HES	6% HES	Flow, capillary density, MFI	Hypertonic and isotonic fluids equivalent
Mazzoni 1990	Rabbit	IVM	HTS/dextran	LR	Diameter	Hypertonic/hyperosmotic fluid is superior
Paes-da-Silva 2003	Rat	IVM and LDF	7.5% HTS and 5% BSA and NS/HES and HTS/HES	Whole blood and NS	Diameter, flow	Hypertonic/hyperosmotic fluid is superior
Pascual 2001	Mouse	IVM	Pentastarch and LR	Sham	Diameter, velocity, shear rate	Hypertonic and isotonic fluids equivalent
Pascual 2002	Mouse	IVM	7.5% HTS and LR	Sham	Velocity, shear rate, shear stress	Hypertonic and isotonic fluids equivalent
Scalia 1990	Rat	IVM	HTS/dextran and dextran	Whole blood	Diameter	Hypertonic fluid superior to whole blood
Vajda 2004	Rat	OPS	HTS/HES	NS	Velocity, flow, FCD	Hypertonic/hyperosmotic fluid is superior
Vollmar 1994	Rat	IVM	10% HES and 7.2% HTS/10% HES	LR and Sham	Velocity, perfusion	Hypertonic/hyperosmotic fluid is superior
Vollmar 1996	Rat	IVM and LDF	10% HES and 7.2% HTS/10% HES	LR	Velocity, diameter, flow, FCD	Hypertonic/hyperosmotic fluid is superior
Wu 2015(b)	Rat	LSCI	NS and HTS and gelatine and HES	Sham and HC	Flow	Hypertonic/hyperosmotic fluid is superior
Zakaria 2006	Rat	IVM	Whole blood/NS and whole blood/HTS and HTS/NS	Sham	Diameter, flow	Hypertonic/hyperosmotic fluid is superior when given with whole blood
Zhao 2009	Rat	IVM	NS and HTS and HTS/dextran	Sham and HC	Shear rate	Hypertonic/hyperosmotic fluid improves RBC deformability

Techniques: IVM: intravital microscopy; LDF: laser doppler flowmetry; LSCI: laser speckle contrast imaging; SDF: sidestream darkfield microscopy; OPS: orthogonal polarization spectral imaging
Endpoints: MFI: microvascular flow index; FCD: functional capillary density; PVD: perfused vessel density
Fluids: LR: Ringer's lactate; NS: normal saline; HTS: hypertonic saline; HSA: human serum albumin; HC: haemorrhage-only control; HES: hydroxyl-ethyl starch: BSA: bovine serum albumin; EPO: erythropoietin

Table 6. Summary of study findings regarding viscosity

Study	Animal	Method	Test fluid(s)	Control fluid(s) or sham	Endpoint(s)	Main finding
Cabrales 2004	Hamster	IVM	0.7% and 0.8% LVM alginate	5% HES and HC	Velocity, flow, shear stress, diameter, FCD	Higher viscosity superior to lower
Cabrales 2005(a)	Hamster	IVM	10% HES/0.3% alginate and 10% HES/0.6% alginate	10% HES	Velocity, flow, diameter, FCD	Higher viscosity superior to lower
Cabrales 2007(a)	Hamster	IVM	OxyRBC and MetRBC	Fresh plasma	Velocity, flow, shear stress, diameter, FCD	Higher viscosity superior to lower, independent of O2 carrying capacity
Cabrales 2007(b)	Hamster	IVM	High-MW HES	Low-MW HES and Sham	Velocity, flow, FCD	Higher viscosity superior to lower
Cabrales 2007(c)	Hamster	IVM	OxyRBC and MetRBC	10% HES	Velocity, flow, shear stress, diameter, FCD	Higher viscosity superior to lower, independent of O2 carrying capacity
Cabrales 2008(a)	Hamster	IVM	6% hetastarch /0.4% alginate	6% hetastarch and HC	Velocity, flow, shear stress, FCD	Higher viscosity superior to lower
Guerci 2014	Porcine	LDF	HES/7% HTS	LR	Perfusion	No difference between viscosities
Messmer 2012	Hamster	IVM	High MW Polymerised HSA (3 different concentrations)	HSA	Velocity, flow, shear stress, diameter, FCD	Higher viscosity superior to lower
Peruski 2014	Canine	SDF	HBOC-alginate (hyperviscous)	Standard HBOC	PVD, TVD, MFI, PPV	Higher viscosity equivalent to lower
Villela 2011	Hamster	IVM	LR-alginate	LR	Velocity, flow, diameter, FCD	Higher viscosity superior to lower
Wettstein 2004(b)	Hamster	IVM	PEG-BSA/PRBC at 4 and 8 g/dL	PEG-BSA	Velocity, flow, diameter, FCD	Higher viscosity superior to lower and more important than oxygen carrying capacity
Wettstein 2006	Hamster	IVM	5% HES and 10% HES and 20% HES	HC	Velocity, flow, FCD	Higher viscosity superior to lower

Techniques: IVM: intravital microscopy; LDF: laser doppler flowmetry; SDF: sidestream darkfield microscopy
Endpoints: PVD: perfused vessel density; TVD: total vessel density; PPV: proportion of perfused vessels; MFI: microvascular flow index; HI: heterogeneity index; FCD: functional capillary density
Fluids: HBOC: haemoglobin based oxygen carrier; PRBC: packed red blood cells; LR: Ringer's lactate; HSA: human serum albumin; HTS: hypertonic saline; MW: molecular weight; HC: haemorrhage-only control; HES: hydroxyl-ethyl starch; OxyRBC: oxygen-carrying red blood cells; MetRBC: methemoglobin red blood cells; PEG-BSA: pegylated bovine albumin; LVM: low viscosity high-mannuronic acid

Table 7. Summary of study findings regarding attenuation of inflammation

Study	Animal	Method	Test fluid(s)	Control fluid(s) or sham	Endpoint(s)	Main finding
Bauer 1993	Rat	IVM	7.2% HTS-Dextran and 7.2% HTS/10% HES	LR and Sham	Velocity, flow, diameter	Modified fluid can reduce leucocyte adhesion and restore microcirculatory flow
Bauer 1995	Rat	IVM	HES-desferoxamine conjugate	HES and Sham	Velocity, flow	Modified fluid to scavenge free radicals can reduce leucocyte adhesion and restore microcirculatory flow
Corso 1999	Rat	IVM and LDF	6% dextran and 7.2% HTS/10% dextran	LR	Velocity, flow, shear stress	Hypertonic fluid no difference in flow but attenuates leukocyte adhesion
Horstick 2002	Rat	IVM	20% albumin	NS	Velocity, shear rate	Albumin has anti-inflammatory properties
Maier 2004	Rat	IVM	Gelatine and 5%HSA and SPS	Sham	Diameter, velocity, flow	Serum protein solution can reduce inflammation
Pascual 2001	Mouse	IVM	Pentastarch and LR	Sham	Diameter, velocity, shear rate	Hypertonic fluid attenuates leukocyte adhesion
Pascual 2002	Mouse	IVM	7.5% HTS and LR	Sham	Velocity, shear rate, shear stress	Hypertonic fluid attenuates leukocyte adhesion
Vollmar 1994	Rat	IVM	10% HES and 7.2% HTS/10% HES	LR and Sham	velocity, perfusion	Hypertonic fluid attenuates leukocyte adhesion
Yada-Langui 2004	Rat	IVM	HTS	LR	Velocity, shear rate	Hypertonic fluid attenuates leukocyte adhesion

Techniques: IVM: intravital microscopy; LDF: laser doppler flowmetry;
Fluids: LR: Ringer's lactate; NS: normal saline; HTS: hypertonic saline; HES: hydroxyl-ethyl starch; SPS: serum protein solution; HSA: human serum albumin

Table 8. Summary of study findings regarding restorative properties of the fluid

Study	Animal	Method	Test fluid(s)	Control fluid(s) or sham	Endpoint(s)	Main finding
Kozar 2011	Rat	Electron microscopy	Fresh plasma	LR and Sham and HC	Glycocalyx thickness	Plasma can restore the endothelial glycocalyx
Torres 2013	Rat	IVM	FFP	LR and HES and HC and sham	Velocity, diameter	Plasma can restore the endothelial glycocalyx
Torres 2014	Rat	IVM	1:1 PRBC/LR and 1:1 washed PRBC/LR and whole blood	LR and Sham	Glycocalyx thickness, flow	Constituents of plasma can restore the endothelial glycocalyx
Torres 2015(a)	Rat	IVM	FFP	NS and 3% HTS and Sham	Glycocalyx thickness	Plasma can restore the endothelial glycocalyx
Torres 2015(b)	Rat	IVM	FFP	NS	Glycocalyx thickness, flow	Plasma can restore the endothelial glycocalyx and flow

Techniques: IVM: intravital microscopy
Fluids: PRBC: packed red blood cells; LR: Ringer's lactate; NS: normal saline; HTS: hypertonic saline; HC: haemorrhage-only control; HES: hydroxyl-ethyl starch; FFP: fresh frozen plasma

Supplementary Table 1. Summary of information recorded during extraction of data from eligible studies

Category	Extracted data
Study characteristics	Author, year, type of publication, country of origin, design
Animal characteristics	Species, sex, number, weight, housing, awake or anaesthetised
Haemorrhage protocol	Percentage of volume of blood lost, mean arterial pressures, length of time of shock
Microcirculation monitoring	Anatomical location, technique used, endpoints recorded
Intervention and control	Allocation of intervention, hypothesis, fluids delivered, control arm, haemorrhage only or sham
SYRCLE assessment	Sequence generation, baseline characteristics, allocation concealment, housing, blinding, random outcome assessment, incomplete data, selective outcome reporting, other sources of bias
Translatability	Choice of sample size, flow of animals, appropriate control, dose-response relationship, matching to human manifestation, characteristic pathway, matching age, replication in different models, independent replication, different species

Supplementary Table 2. Resuscitative fluid comparisons in included studies

Studies	Sham (no haemorrhage)	Haemorrhage only	Blood product(s)	Oxygen carrier(s)	Crystalloid(s)	Colloid(s)
Torres 2013	✔	✔	✔		✔	✔
Kozar 2011	✔	✔	✔		✔	
Zhao 2009, Wu 2015(b)	✔	✔			✔	✔
Wu 2015(a)	✔	✔			✔	
Paxian 2003	✔		✔	✔	✔	✔
Von Dobschuetz 1999	✔		✔	✔		✔
Kubulus 2009	✔		✔	✔		
Paes-da-Silva 2003	✔		✔		✔	✔
Ni 2013, Torres 2014	✔		✔		✔	
Torres 2015(a), Zakaria 2006	✔		✔		✔	
Bauer 1993, Pascual 2001, Vajda 2004, Vollmar 1994	✔				✔	✔
Bauer 1995, Cabrales 2007(b), Maier 2004	✔					✔
Gulati 1998	✔			✔	✔	
Pascual 2002, Yada-Langui 2004	✔				✔	
Bi 2004		✔	✔	✔		✔
Cabrales 2004, Cabrales 2005(b), Wettstein 2006		✔				✔
Cheung 2001, Ortiz 2014, Wettstein 2004(b)			✔	✔		
Cheung 2006, Cheung 2007, Kerger 1997			✔	✔	✔	✔
Cheung 2004, Hungerer 2006, Nolte 1997, Sakai 2002, Wettstein 2003			✔	✔		✔
Casali 2002, Kao 2010, Torres 2015(b)			✔		✔	
Cabrales 2007(c), Sakai 1999, Scalia 1990			✔			✔
Cabrales 2007(a), Villela 2009			✔			
Peruski 2014				✔		
Kumar 1997				✔	✔	
Botzlar 1996				✔	✔	✔
Cabrales 2009, Hermann 2007, Palmer 2011, Vazquez 2011, Wettstein 2004(a)				✔		✔
Kao 2011, Villela 2011					✔	
Corso 1999, Cryer 2005, Gierer 2004, Gonzalez 2012, Gonzales 2016, Guerci 2014, Horstick 2002, Komori 2005, Mazzoni 1990, Vollmar 1996					✔	✔
Cabrales 2005(a), Cabrales 2008(a), Cabrales 2008(b), Maier 2009, Messmer 2012						✔

Supplementary Table 3. Assessment of bias based on the SYRCLE's risk of bias tool for animal studies

Author	Year	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding (caregivers)	Random outcome assessment	Blinding (outcome)	Incomplete outcome data	Selective outcome reporting	Other sources of bias	Total
Bauer	1993		*					*				**2**
Bauer	1995	*	*					*				**3**
Bi	2004	*	*						*			**3**
Botzlar	1996		*									**1**
Cabrales	2004	*	*									**2**
Cabrales	2005 (a)	*	*									**2**
Cabrales	2005 (b)	*	*									**2**
Cabrales	2007 (a)	*	*						*			**3**
Cabrales	2007 (b)	*	*						*			**3**
Cabrales	2007 (c)	*	*						*			**3**
Cabrales	2008 (a)	*	*						*			**3**
Cabrales	2008 (b)	*	*						*			**3**
Cabrales	2009	*	*						*			**3**
Casali	2002											**0**
Cheung	2001	*	*						*			**3**
Cheung	2004	*	*									**2**
Cheung	2006	*	*									**2**
Cheung	2007	*	*						*			**3**
Corso	1999		*									**1**
Cryer	2005		*									**1**
Gierer	2004		*									**1**
Gonzalez	2012	*										**1**
Gonzalez	2016	*	*					*				**3**
Guerci	2014	*	*						*			**3**
Gulati	1998		*									**1**
Hermann	2007	*	*									**2**
Horstick	2002	*	*									**2**
Hungerer	2006	*	*									**2**
Kao	2010	*	*						*			**3**
Kao	2011	*										**1**
Kerger	1997		*						*			**2**
Komori	2005		*									**1**
Kozar	2011		*									**1**
Kubulus	2009	*	*					*				**2**
Kumar	1997		*									**1**
Maier	2004	*						*				**2**
Maier	2009	*	*					*				**3**
Mazzoni	1990	*	*						*			**3**
Messmer	2012	*	*						*			**3**
Ni	2013	*										**1**
Nolte	1997	*	*									**2**
Ortiz	2014	*	*						*			**3**
Paes-da-Silva	2003	*	*									**2**
Palmer	2011	*	*						*			**3**
Pascual	2001	*	*					*				**3**
Pascual	2002	*	*		*			*				**4**
Paxian	2003		*									**1**
Peruski	2014	*	*					*				**3**
Sakai	1999		*		*				*			**3**
Sakai	2002		*		*							**2**
Scalia	1990		*									**1**
Torres	2013	*	*									**2**
Torres	2014	*	*						*			**3**
Torres	2015 (a)		*									**1**
Torres	2015 (b)		*									**1**
Vajda	2004	*	*									**2**
Vazquez	2011	*	*						*			**3**
Villela	2009	*	*						*			**3**
Villela	2011	*	*						*			**3**
Vollmar	1994	*	*					*				**3**
Vollmar	1996	*	*									**2**
von Dobschuetz	1999	*	*									**2**
Wettstein	2003	*	*						*			**3**
Wettstein	2004 (a)		*									**1**
Wettstein	2004 (b)	*	*									**2**
Wettstein	2006		*						*			**2**
Wu	2015 (a)	*	*									**2**
Wu	2015 (b)	*	*									**2**
Yada-Langui	2004	*										**1**
Zakaria	2006	*	*									**2**
Zhao	2009	*	*									**2**

Supplementary Table 4. Assessment of translatability according to most frequent recommendation for preclinical researc

Author	Year	Choice of sample size (power calculation)	Randomized allocation of animals	Blinded outcome assessment	Flow of animals through experiment	Selection of appropriate control groups	Dose-response relationship	Baseline characteristics	Matching mechanism, chronicity	Characteristic pathway	Age matching	Different transgenics/ strains	Different research groups	Replication in different species	Total
Bauer	1993			*				*		*					**3**
Bauer	1995		*	*		*		*		*					**5**
Bi	2004		*		*		*	*		*					**5**
Botzlar	1996					*		*		*					**3**
Cabrales	2004		*			*		*		*					**4**
Cabrales	2005 (a)		*					*		*					**3**
Cabrales	2005 (b)		*			*		*		*					**4**
Cabrales	2007 (a)		*		*			*		*					**4**
Cabrales	2007 (b)		*		*	*		*		*					**5**
Cabrales	2007 (c)		*		*			*		*					**4**
Cabrales	2008 (a)		*		*	*		*		*					**5**
Cabrales	2008 (b)		*		*			*		*					**4**
Cabrales	2009		*		*			*		*					**4**
Casali	2002									*					**1**
Cheung	2001		*		*	*		*		*					**5**
Cheung	2004		*			*		*		*					**4**
Cheung	2006		*		*			*		*					**4**
Cheung	2007		*		*			*		*					**4**
Corso	1999				*	*		*		*					**4**
Cryer	2005							*		*					**2**
Gierer	2004					*		*		*					**3**
Gonzalez	2012		*												**1**
Gonzalez	2016		*	*				*		*	*				**4**
Guerci	2014		*		*			*		*					**4**
Gulati	1998						*	*		*					**3**
Hermann	2007		*				*	*		*					**4**
Horstick	2002		*		*			*		*					**4**
Hungerer	2006		*					*		*					**3**
Kao	2010		*		*			*		*					**4**
Kao	2011		*												**1**
Kerger	1997				*			*		*					**3**
Komori	2005					*		*		*					**3**
Kozar	2011					*		*		*					**3**
Kubulus	2009		*			*		*		*					**4**
Kumar	1997					*		*		*					**3**
Maier	2004		*	*			*			*					**4**
Maier	2009		*	*				*		*					**4**
Mazzoni	1990	*	*		*	*		*		*					**6**
Messmer	2012	*	*		*			*		*					**5**
Ni	2013		*			*				*					**3**
Nolte	1997		*			*		*		*					**4**
Ortiz	2014		*		*		*	*		*					**5**
Paes-da-Silva	2003		*			*		*		*					**4**
Palmer	2011		*		*			*		*					**4**
Pascual	2001		*	*		*		*		*					**5**
Pascual	2002		*	*		*		*		*					**5**
Paxian	2003					*	*	*		*					**4**
Peruski	2014		*	*				*		*					**4**
Sakai	1999				*			*		*					**3**
Sakai	2002						*	*		*					**3**
Scalia	1990					*		*		*					**3**
Torres	2013		*			*		*		*					**4**
Torres	2014		*		*	*		*		*					**5**
Torres	2015 (a)					*									**1**
Torres	2015 (b)							*							**1**
Vajda	2004		*			*		*		*					**4**
Vazquez	2011		*		*			*		*					**4**
Villela	2009		*		*		*	*		*					**5**
Villela	2011		*		*			*		*					**4**
Vollmar	1994		*	*		*		*		*					**5**
Vollmar	1996		*							*					**2**
von Dobschuetz	1999		*			*		*		*					**4**
Wettstein	2003		*		*			*		*					**4**
Wettstein	2004 (a)							*		*					**2**
Wettstein	2004 (b)		*					*		*					**2**
Wettstein	2006				*	*		*		*					**4**
Wu	2015 (a)		*			*		*		*					**4**
Wu	2015 (b)	*	*			*		*		*					**5**
Yada-Langui	2004		*			*				*					**3**
Zakaria	2006		*			*		*		*					**4**
Zhao	2009		*			*		*		*					**4**