Summary of the Interagency Oxygen Carrier State of the Science Meeting

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26 June 17

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Purpose

- Results of the Interagency meeting on Oxygen Carriers
- Review clinical development experience and status of new products in clinical development with potential for trauma

The Meeting

- Three day meeting co-sponsored by US DoD, BARDA, NHLBI, and US FDA OCET (Feb 6-8, 2017, Fort Detrick, Maryland, USA)
- Over 140 participants from Industry, Academia, BARDA, FDA, NHLBI, DoD, and International
- 3 days, over 60 presentations and panel discussions

Goals of the Meeting

- What new drugs are in clinical development for an indication relevant to trauma and hemorrhage?
- Review and reassess previous trauma and surgery phase III clinical development efforts and consider lessons-learned
- Re-assess the unmet military medical need
- Identify new directions for new (and old) products in clinical development

Unmet Need

Military Need

- 24% of combat deaths are due to hemorrhage, potentially preventable
- Recent Evacuation Times: 42 to 90 min

Early Transfusion is Key

- Retrospective analysis of 482 MEDEVAC patients (Afghanistan 2012-2015)
- Prehospital transfusion w/in 35 min reduced 24h mortality (4% vs 22%; p<.01)

Future Conflicts are Expected to Involve Delayed or Prolonged Evacuation:

- Distance (e.g. Pacific, Africa)
- Discontinuous "windows" of air superiority versus near-peer enemy
- More independent small unit operations

Required Capability: Prolonged Field Care

- **Stabilize casualties up to 72 h in out-of-hospital environment**

Eastridge et al., 2012; Shackelford and Del Junco, 2016; Joint Enroute Care Committee, 2014

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Current US Military Guidelines

Treat Hemorrhagic Shock (as soon as possible)

- Whole Blood
- 1:1:1 (Red Cells: Plasma: Platelets)
- 1:1 (Red Cells: Plasma)
- Plasma

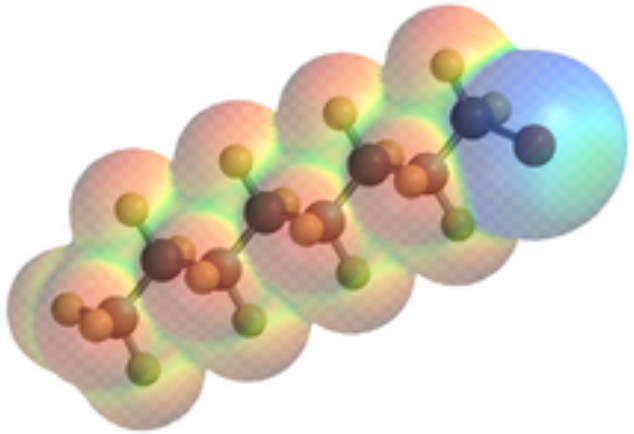
Not Always Logistically Possible

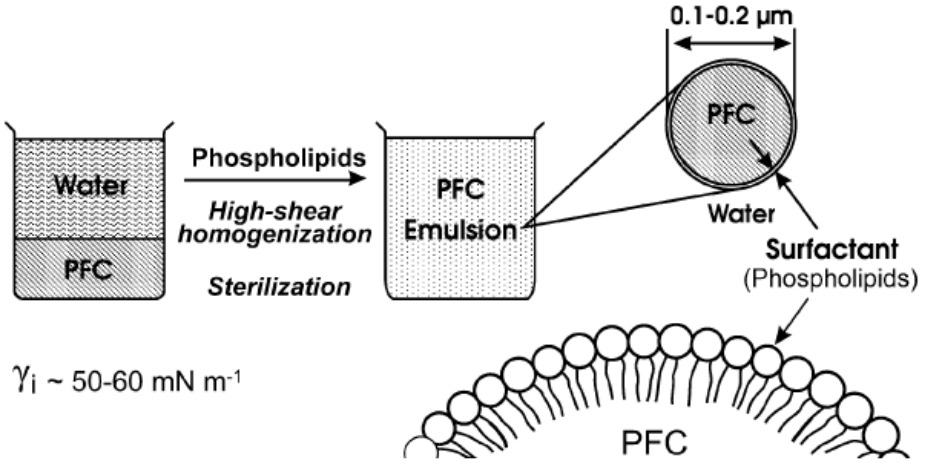
- Dried plasma in development in US
- What happens when blood or red cells are not available or significantly delayed?
- **A potential role for oxygen carriers when transfusion is needed but not possible or significantly delayed – a bridge to transfusion**

Two Categories of Oxygen Carriers - Basic Characteristics

Perfluorocarbons (1/2)

- Perfluorocarbons are hydrocarbon chains with flourines replacing hydrogens (all or most)
- F-C is the strongest single bond in molecular compounds – inert and stable
- Most hydrophobic substances invented (also lipophobic)
- Intravascular formulations must be emulsified
- Not metabolized – excreted by exhalation





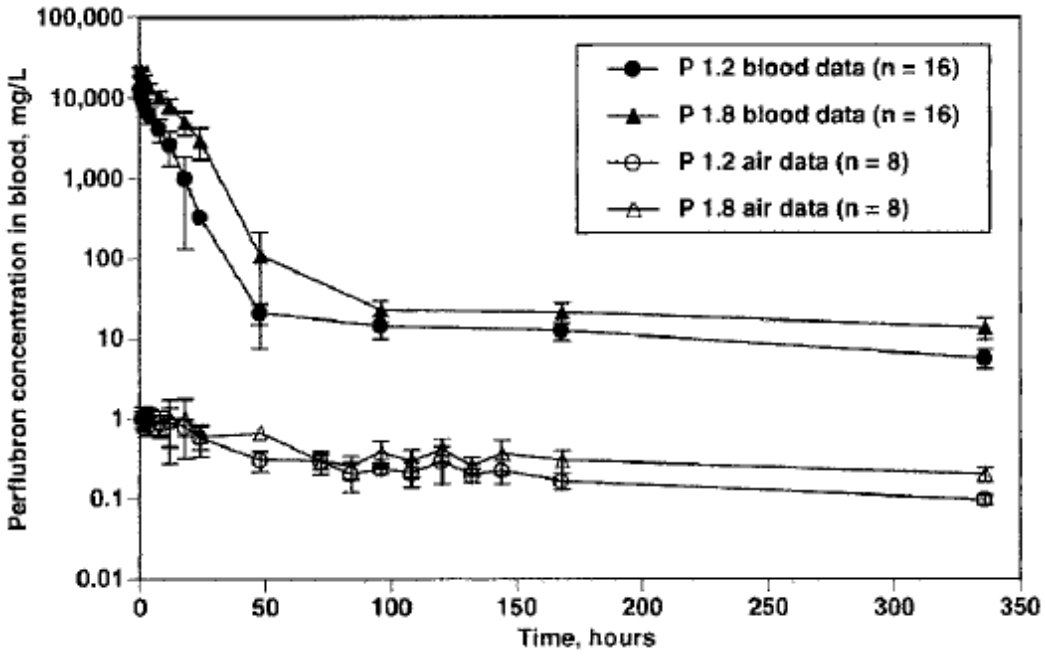
Riess, 2005

Perflurocarbons (2/2)

- Nonpolar nature enhances solubility of gases ($CO_2 > O_2 > N_2$)
- Oxygen carrying capacity is a function of the molecular mass provided and the oxygen partial pressure

Clearance

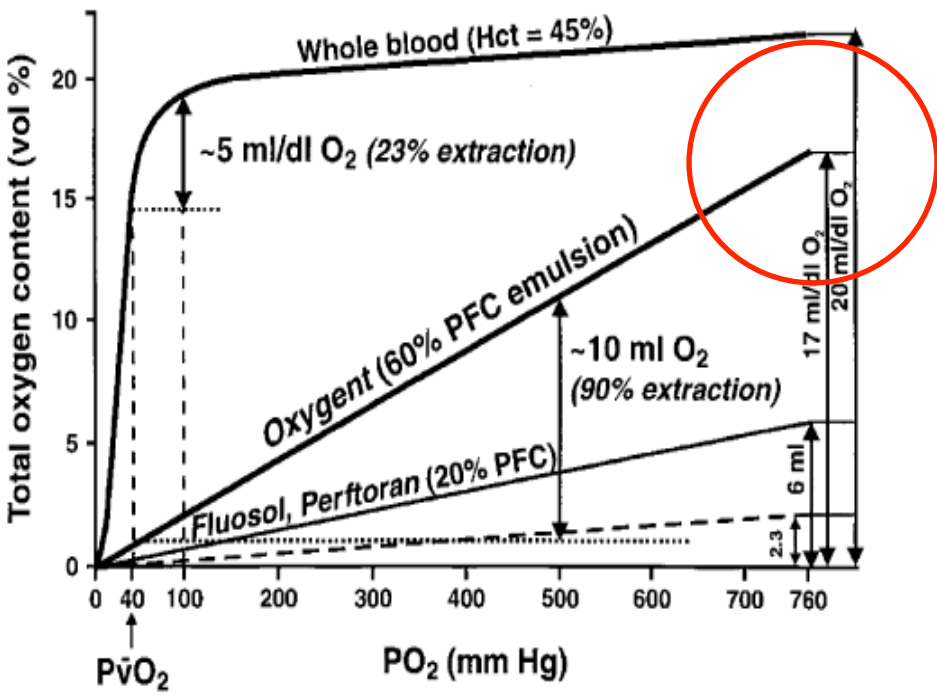
- Two-phase clearance (hours)
- Initial half-life (Intravascular)
  - Saturable RES clearance (hrs)
- Terminal half-life (days-wks)
  - Taken up in tissues
  - Transport by plasma lipids to lung for exhalation



Leese et al., 2000; Reiss, 2005, 2006

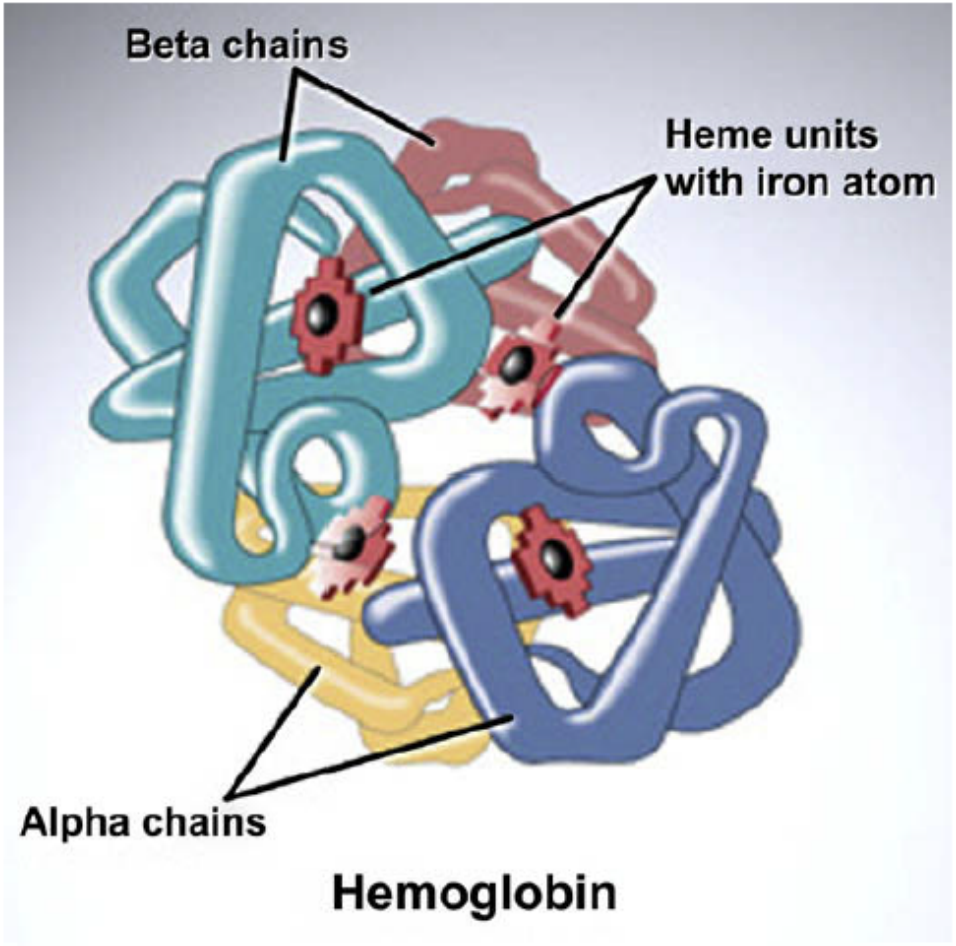
Formulation Challenges and Side Effects

- Challenges for Oxygen Delivery
  - O2 solubility very high but carrying capacity related to O2 tension and PFC content
  - Need to load high levels of PFC to increase O2 delivery
  - Need to breath FiO2 = 1.0 for most applications
- Primary Side Effects
  - Flu-like symptoms, Fever, Vomiting
  - Immune suppression
  - Complement activation
  - Transient thrombocytopenia (a few days)
- Large particle size and higher PFC loading - more side effects



Hemoglobin-Based Oxygen Carriers (HBOC)

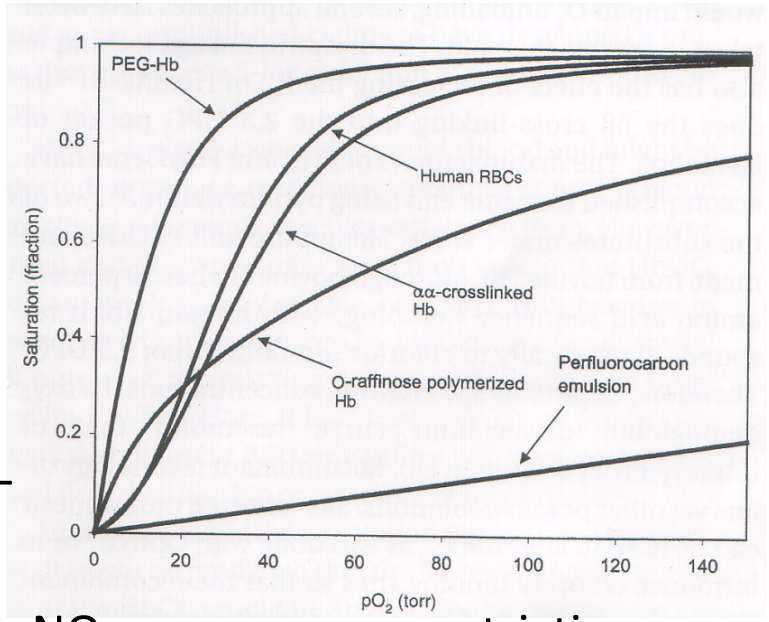
- HBOC based on bovine or human Hb
- Product Variations
  - Cross-linked
  - Polymerized
  - PEGylated



Napolitano, 2009

HBOC Primary Characteristics

- **Primary Toxicities**
  - Cardiac events
  - Vasoconstriction
  - Jaundice
- **Primary Manufacturing Approaches**
- **Free Hb tetramer** dissociates into dimer –
  - vasoconstriction and kidney toxicity
- **Cross-linked Hb** – extravasates, scavenges NO, causes vasoconstriction
- **Polymerized Hb**
  - Increased molecular size
  - Improved intravascular time
  - Reduced vasoconstriction
  - Eliminated kidney toxicity
- **PEGylation** – increases molecular size with similar reduced toxicity



New Generation of O2 Carriers

RBC Substitute

Quantitative Oxygen Carrying Capacity - To deliver O2 from the lungs to tissues
in place of red cells after significant hemorrhage.

- High doses (eg. 13 g/dL Hgb; 60% PFC) to maximize O2 carrying capacity
- Dose limited by toxicity

Oxygen Delivering Therapeutic

Augmented Oxygen Diffusion – Enhanced diffusion of O2 from existing RBC to ischemic tissues. O2 carriers offload O2 from existing RBC, increasing O2 diffusion through the plasma phase to reach obstructed or low flow vascular beds where RBC may be excluded by size.

- Low doses (e.g. 4 g/dL Hgb; 2% PFC) using modified molecules with enhanced O2 carrying or other characteristics
- Reduced side effects expected

Clinical Development Experience
Oxygen Carriers

Fluosol-DA (20% PFC)

(Green Cross Corp., Osaka, Japan, and Alpha Therapeutic, Los Angeles, CA, USA)

- 1989-90 - Approval in US, Japan, Europe; adjunct to provide distal oxygenation in percutaneous transluminal coronary artery balloon angioplasty (Castro and Briseno, 2010; Young et al., 1990)
- Not clinically effective in treating severe anemia in 13 patients with religious objections (Gould et al., 1986; Tremper et al., 1982)
- Side Effects (Systemic Use): Increase PAP due to plasma volume expansion, 35% decrease WBC, sensitivity in 2/7 patients (Gould et al., 1986)
- Emulsifier - 2.7% Pluronic F-68 caused complement activation (Castro and Briseno, 2010)
- Removed from the market in 1994
  - poor user acceptability (frozen, mixing, improved catheters)
  - >14,000 patients treated

Oxygent (60% PFC)

(Alliance PharmaceuticalCorporation, San Diego, CA, USA)

- Natural emulsifier, higher PFC loading, smaller particle size (0.2 uM)
- Phase 1 - Prospective, randomized, dose escalation study in 48 normal volunteers
  (Leese et al., 2000, Noveck et al., 2000)
- Reduced side effects:
  - 28%WBC decline 24 h
  - 17% decline plt d 3-7
  - Mild fever in only 5/36 dosed subjects
  - No complement activation

Anesthesiology 2002; 97:1338–49

Use of Perflubron Emulsion to Decrease Allogeneic Blood Transfusion in High-blood-loss Non-Cardiac Surgery

Results of a European Phase 3 Study

- RCT, single-blind, multicenter (34) trial (1998-2000)
- 492 patients non-cardiac surgery with expected blood loss >/= 20 ml/kg (cancer, major abdominal, orthopedic)
- Treatment Groups
  - ANH with 1.8g/kg Oxygent to allow ANH to Hgb 5.5 g/dL (FiO2 = 1.0)
  - Control ANH to 8.0 g/dL (FiO2 = 0.4)
- Primary Outcome: Number and frequency of allogeneic RBC units transfused
- Results
  - PFC (n=241) group received 26% fewer allogeneic transfusions (1.5 versus 2.1 U at 24h; p<.01)
  - PFC patients – 21% more avoided allogenic transfusion (p<.05)
  - Plts decreased ~25% days 3-7 post-op
  - PFC more overall SAE than controls (38% vs 21%; p<.05)
  - Mortality 4% vs 3% (NS)

Oxygent in Cardiac Surgery

(Hill et al., 2002, 2005)

- **Single center, RCT, single blind, dose escalation study - Phase II (1996-1997)**
- **Cardiac Surgical Patients with CPB**
  - ANH to Hct 20% plus 1.8g/kg or 2.7g/kg PFC or colloid (n=12/group)
  - After CPB but before cooling, subjects received PFC or crystalloid in CPB circuit
- **Results:**
  - No difference transfusion requirements
  - Well tolerated
  - Post-op platelets decreased in PFC groups post-op period
  - Cerebral blood flow increased with PFC (~15% $p<.05$)
  - Increased cerebral emboli with PFC (4-5x in high dose only; $p<.05$)
- **Cardiac Surgery Pivotal Trial**
  - Halted due to increased incidence of stroke in Oxygent group (Alliance press release Jan 2001)
  - Company suspended clinical development
  - Technology agreement with Double Crane Pharmaceutical for development in China (no reported activity)

Oxycyte™ (60% PFC)

(Tenax Therapeutics, ***Costa Mesa, CA, USA)***

- Clinical Trial Hypothesis - Improving brain oxygenation would improve outcomes in TBI *(not reducing transfusion)*
- 2005 - An Open Label, Proof of Concept Study, to Evaluate the Safety and Biological Effects of Oxycyte™ Perfluorocarbon in Patients With A Severe Head Injury Requiring Intracranial Pressure Monitoring-OX-CL-II-002 (GCS: 3-9)
  - N=4 - Oxycyte 3 mL/kg, at Fi02 = 0.50 for 24 hours
  - N=4 - Oxycyte 3 mL/kg, at Fi02 = 1.00 for 24 hours
  - FDA placed the US development project on clinical hold due to transient thrombocytopenia. Additional platelet studies required.
- 2009 – RCT, double blind, dose escalation to determine Safety and Tolerability of Oxycyte in Patients With Traumatic Brain Injury (TBI) (STOP-TBI) - Phase II European Study (Switzerland, Spain, France, Israel)
- Patients receive 1.0, 2.0, or 3.0 ml/kg Oxycyte (in three respective cohorts) or saline control
  - 1.0 ml/kg dose cohort complete (n=8) and approved to proceed to cohort 2.
- 2014 – FDA US lifted clinical hold
- 2014 – Tenax terminated STOP-TBI study and discontinued development program

HBOCs

Human Polymerized Hemoglobin for the Treatment of Hemorrhagic Shock when Blood Is Unavailable: The USA Multicenter Trial

Open label RCT at 21 centers (2004-2006)

Primary Outcome: 30 d mortality

Patients: Injured patients with SBP < 90 mm Hg randomized in the field

PolyHeme (10 g Hb/dL) – up to 6 U (500ml) PolyHeme at scene and for 12 hours post injury; RBC after 12h.

Control - crystalloid in the field; RBC in hospital

Noninferiority Hypothesis:
PolyHeme </= 7% mortality vs control

Results:
- Prehospital transport time – 26 minutes
- Protocol violations – 124 patients with major protocol violations

Parameter	HBOC	Cont	P
Patients	350	364	
ISS	19.9	19.4	NS
Mortality (%)	13.4	9.6	NS
Transfusion Avoidance (24h)	57%	48%	<.01
Time to first RBC (per protocol)	14.1h	1.5h	<.01
AE	93%	88%	<.05
SAE	40	35	NS
MI*	3%	1%	<.05
Hypertension (reported as SAE)	18%	12%	<.05
Mean SBP 6h	129	122	<.05
MOF	7.4%	5.5%	NS

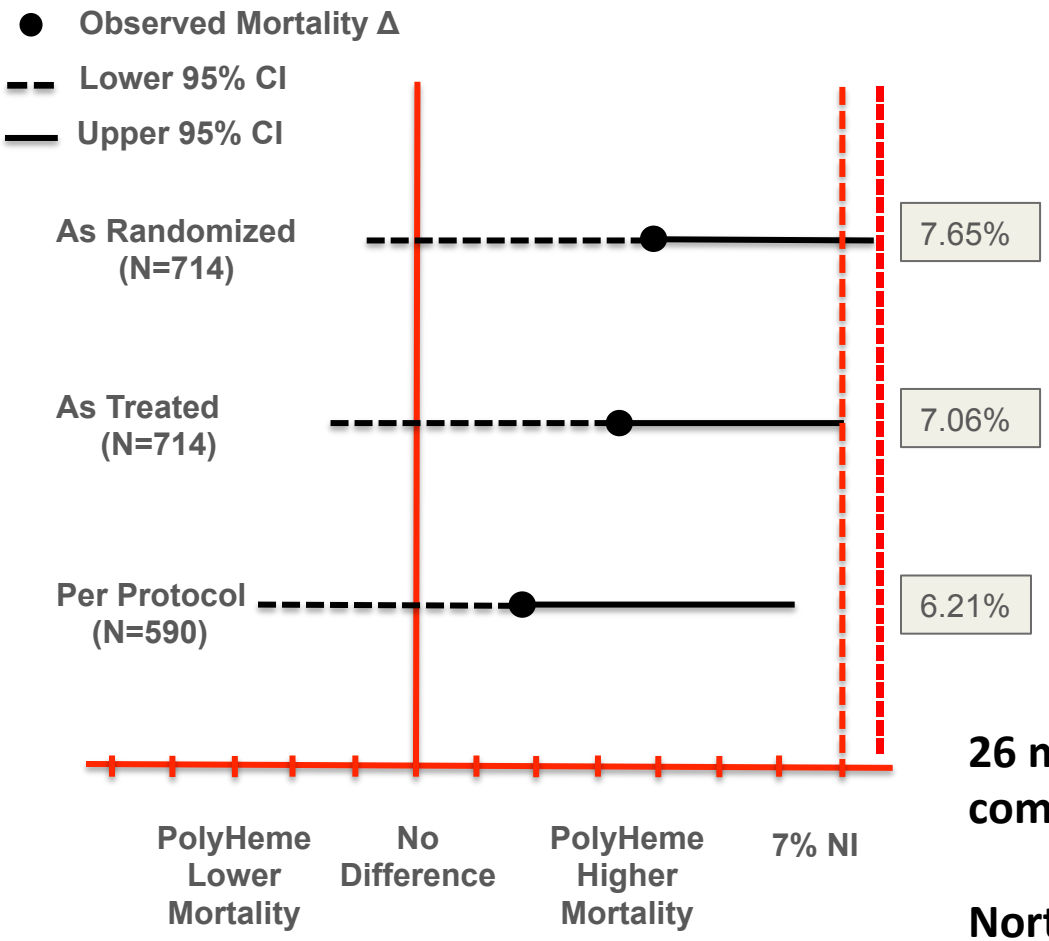
*Independent panel reviewed record and found no association with trt grp

Moore et al., 2009

US Multicenter Trauma Trial

Non-Inferiority Outcomes

Slide courtesy of S. Gould



NI margin selected based on medical literature, study feasibility and give some allowance for a potential prehospital benefit

Mortality

	HBOC	Ctrl	P
As Randomized	13.4	9.6	NS
Per Protocol	11.1	9.3	NS

26 min prehosp time precluded comparison with significant delay in RBC

Northfield discontinued the program

HBOC-201 as an Alternative to Blood Transfusion: Efficacy and Safety Evaluation in a Multicenter Phase III Trial in Elective Orthopedic Surgery

Jonathan S. Jahr, MD, Colin Mackenzie, MD, L. Bruce Pearce, PhD, Arkadiy Pitman, MS, and A. Gerson Greenburg, MD, PhD

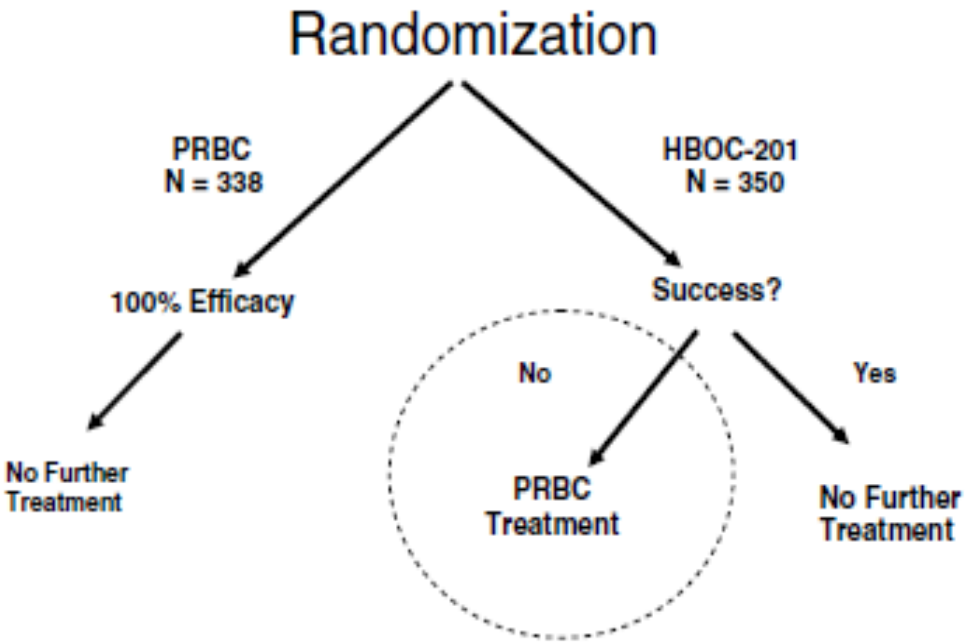
RCT, single blind, multicenter (46) study conducted on 3 continents

Primary Endpoints:
- Efficacy: RBC transfusion avoidance >/= 35% at 6wk
- Safety: Safety (AE)

Patients: Orthopedic surgery patients expected to require >/= 2 U RBC by day 3 post-op (1997-1999)

Treatments:
- Randomized within 3 d surgery, at first transfusion decision

- RBC versus HBOC-201 up to 10 units



Jahr et al., 2008

Results

Group	N	6 wk TX Avoid (%)	Mort (%)	SAE(%)	AE (%)	Hypertension AE (%)	MI (%)
HBOC-201	350	59	3	32	95	17	4
RBC	338		2	25	91	7	2
P			NS	<.05	<.01	<.01	NS

Subgroup analysis - SAE imbalance isolated to high transfusion need patients (those receiving 10 U HBOC plus RBC, versus patients receiving >3 U RBC w/o HBOC)

AE imbalance associated with age > 80, volume overload, and under-treatment in patients that received both HBOC-201 and RBC (high TXN requirement)

Hypertension: Mean SBP following HBOC loading dose (2U) <10 mm Hg over baseline

Authors conclusion: High need patients should only receive HBOC if RBC not available

SANGART Oxygen Therapeutic Phase 2b Trauma Study

- Double blind RCT, multicenter (38 sites, 14 countries)
- Study Hypothesis: MP4OX will reverse the lactic acidosis by enhancing perfusion and oxygenation of ischemic tissues , reducing MOF and improving outcomes
- Primary Outcomes: Safety - SAEs to 28 d; Efficacy - 28 day mortality
- Trauma patients with severe hemorrhage + blood Lactate ≥ 5.0 mmol/L
- Treatments: 1 U (250 mL) MP4OX (4.3 g/dL PEGylated Hgb) vs 250 ml saline

Results:

- No efficacy
- No hypertension
- No imbalance in cardiac AEs
- Dose too low?

Efficacy Parameter	MP4OX (n=155)	Ctrl (n=158)	P
Mortality Rate (ITT)	12%	14%	0.86
Discharged, Alive d 28	57%	50%	0.18
SAE	28.8%	31.1%	NS

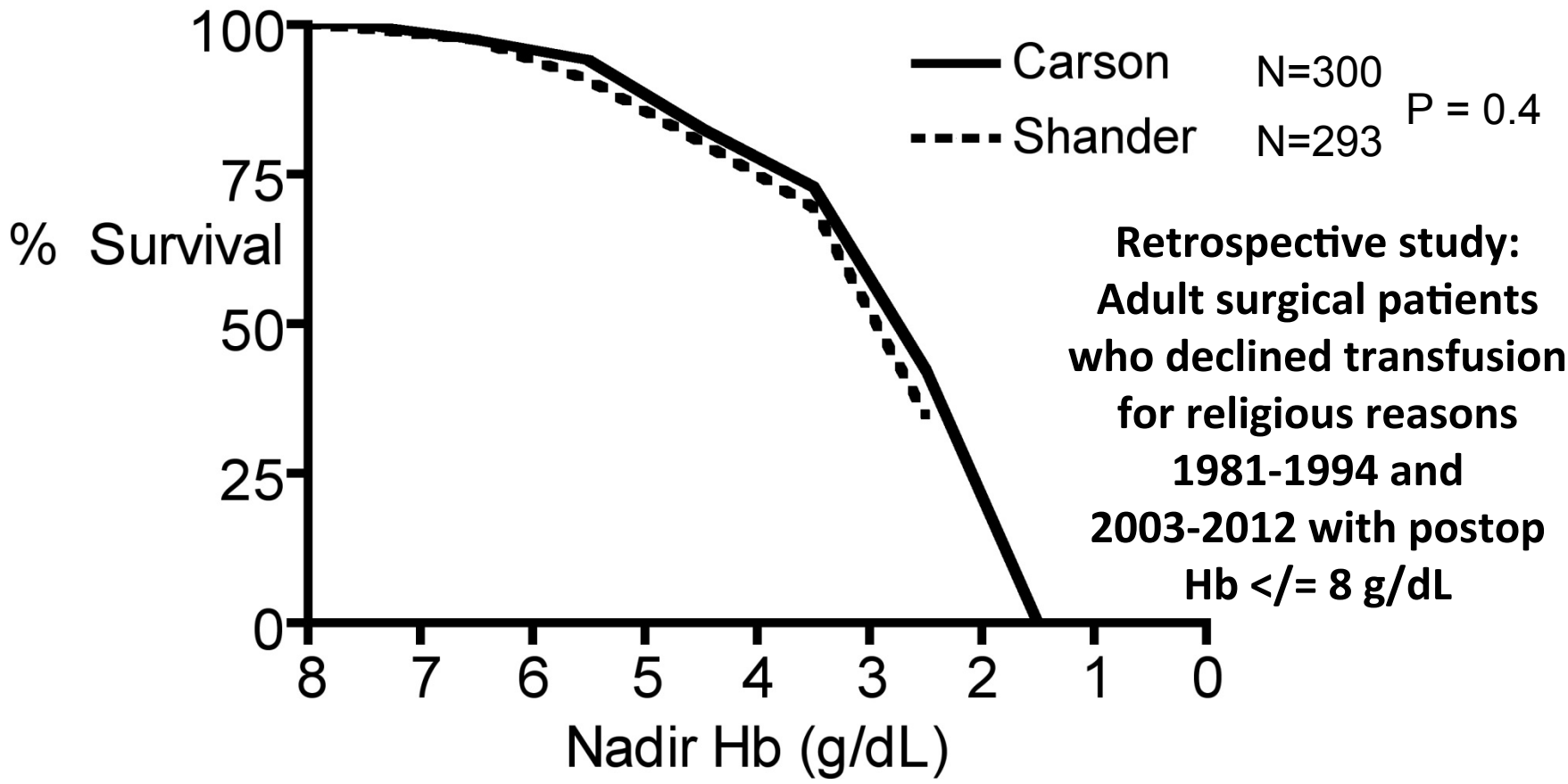
Source: Brohi et al. 2013 AAST presentation; Keipert, 2017

When Blood is Not an Option

- No trial has directly examined an oxygen carrier for use when transfusion is not possible or is significantly delayed (transfusion avoidance or short prehospital times)
- The risk-benefit analysis is very different when RBC not available
- Standard power RCT difficult or impossible in US, Europe
  - Northfield Trial – 26 min to hospital (almost no "delay")
  - Unethical to withhold or delay transfusion as control
- Can we get an idea of how HBOC may perform by examining existing data from untransfused surgical patients?

What is the Expected Mortality for Untransfused Surgical Patients? - Update

(Slide courtesy RB Weiskopf)

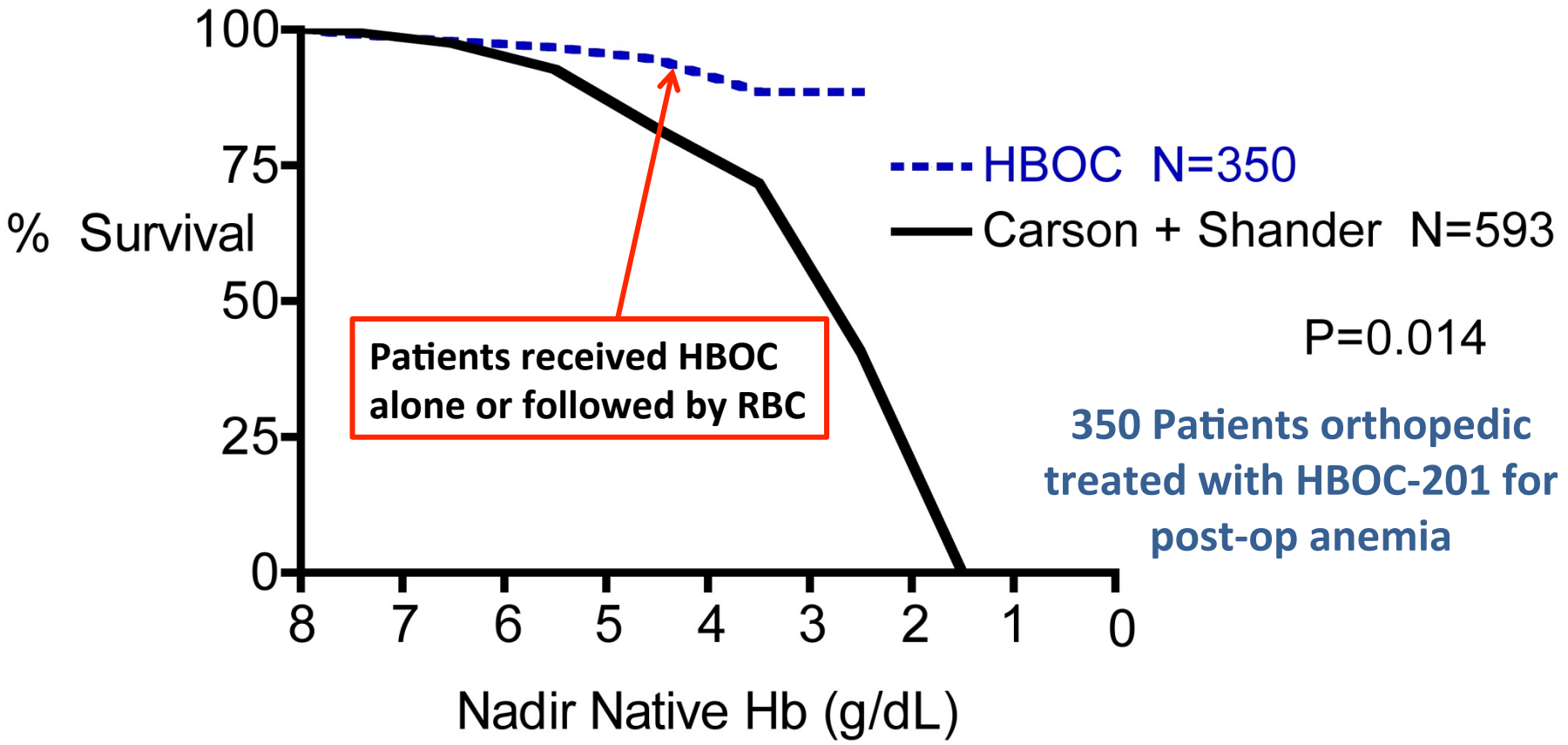


Retrospective study: Adult surgical patients who declined transfusion for religious reasons 1981-1994 and 2003-2012 with postop Hb </= 8 g/dL

Weiskopf et al.: Transfusion 2017; 57: 207; data from:
Carson et al.: Transfusion 2002: 42: 812
Shander et al.: Transfusion 2014: 54: 2688

Mortality of Acute Severe Untransfused Anemia Surgical Patients

(Slide courtesy RB Weiskopf)



Weiskopf et al.: Transfusion 2017; 57: 207; data from:
Carson et al.: Transfusion 2002: 42: 812
Shander et al.: Transfusion 2014: 54: 2688
Jahr et al.: J Trauma 2008; 64: 1484

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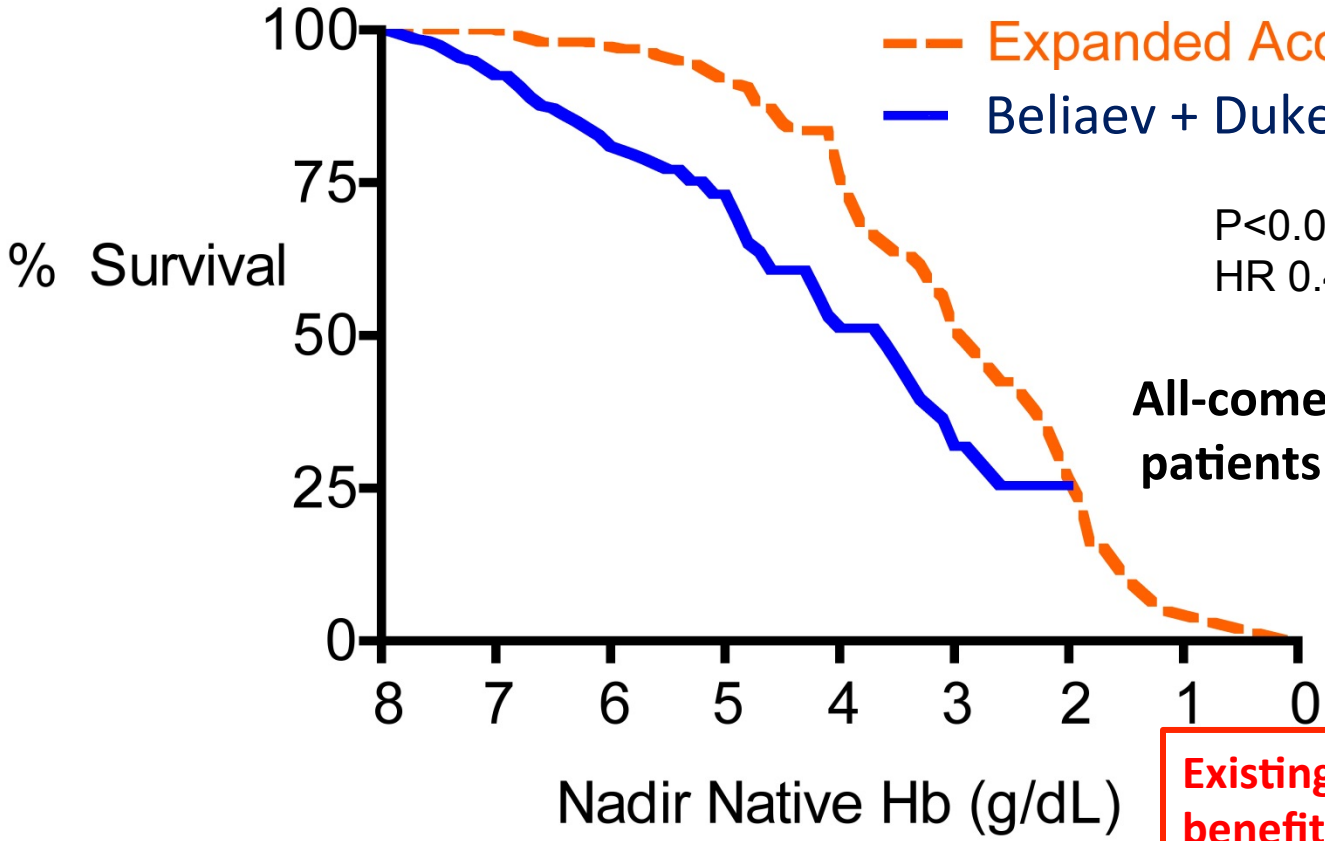
Mortality of Acute Severe Untransfused Anemia
All Hospitalized Patients

(Slide courtesy RB Weiskopf)



All-comer untransfused anemic patients (surgical and medical)

Existing data suggest a survival benefit for HBOC-201 when transfusion is not possible or significantly delayed

Beliaev et al.: Vox Sang 2012; 103: 18
Duke Univ: unpublished data
Weiskopf et al.: Transfusion 2017; 57: 207; data from

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Current Products (World-wide)

Hemopure (bovine polyHb, 13 g/dL)

(HBOC-201)

Approved in South Africa 2001 for use when RBC not available or possible

602 patients treated – 1-19 U range (limited availability)

Guidelines published (Mer et al., 2016)

US - Expanded Access Protocol Using HBOC-201 for patients with life threatening anemia, when blood is not an option. 140 patients treated.

HbO2Therapeutics exploring clinical development pathway for BNAO with FDA, using EA IND data and a reasonable prospective study (Z. Zafarelis personal communication)

Veterinary use Oxyglobin approved EU (1998) and US (1999) for canine anemia. >150,000 animals treated (multiple species)

Perfortoran

- 20% PFC - Approved for temporary intravascular oxygen carrier for hemorrhagic shock and perfusion of human organs
  - Russia 1996; Kazahkstan 1998; Ukraine 2005; Kirzygh Republic 2006
  - Mexico 2005 (Pending a GMP facility)
- Indications include blood loss, microcirculatory disorders, TBI, burns, CPB, TBI, others
- Most reports available only in Russian
- >30,000 treated as of 2016
- Licensed as Vidaphor - FluorO2 Therapeutics
- Planning GMP manufacturing in US
- Expects Mexican approval, followed by Central America
- Full range of development expected if seek US approval
- Capital investment needed

Maevsky et al., 2005;
Latson, 2017 presentation

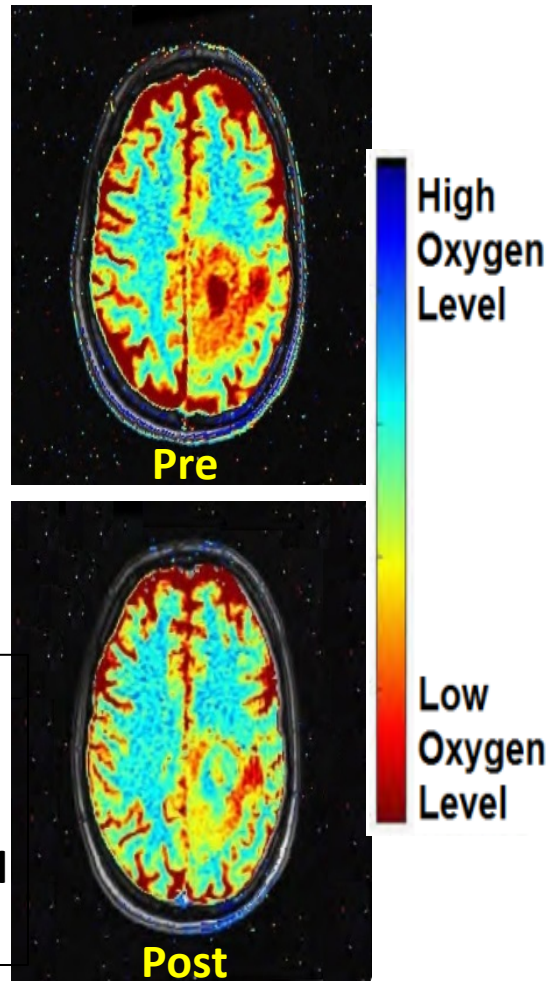
New Products in Clinical Development
Oxygen Therapeutics

PFC Oxygen Therapeutic

2% PFC - Dodecafluoropentane emulsion (DDFPe)

- Terminal half-life 90 minutes
- Used as an oxygen therapeutic
  - Improves perfusion and oxygenation of ischemic tissues
  - Carries > 100x more O_2 than other fluorocarbons (low dose – 2% PFC)
- Clinical trials:
  - Ph Ib/II Oncology
  - Ph I Stroke
- Preclinical
  - Hemorrhage, other

NuvOx Pharma



MRI - increased oxygenation in tumor with no change in normal tissue

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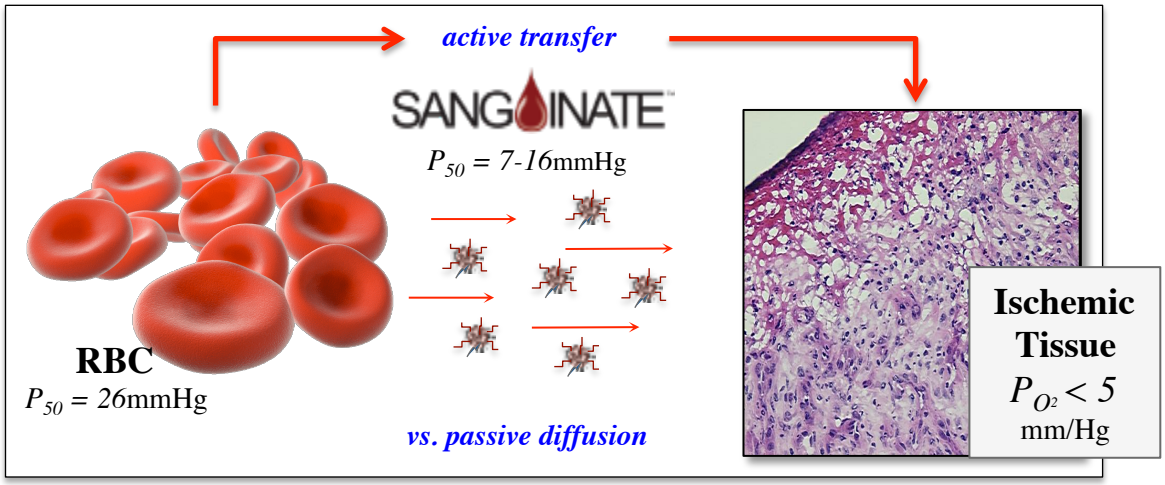
SANGUINATE™

Prolong Pharmaceuticals – PEGylated bovine carboxyhemoglobin

Hgb 4 g/dL



- P_{50} is between the P_{50} of RBCs and low P_{O2} of ischemic tissue
- Designed to actively transport oxygen from RBC to essential sites with hypoxia
- Phase II and III clinical trials for sickle cell, delayed graft function, thalassemia world-wide
- Blood not an option expanded access IND – 29 patients
- Open-label Phase 1 Safety Study of SANGUINATE™ Infusion in Patients With Acute Severe Anemia Who Are Unable to Receive RBC Transfusion – complete (103 patients)

Conclusions

- There is a military need for an oxygen carrier for use as a bridge to transfusion when blood is not available
- Oxygen Carriers have failed in development as a replacement for RBC
- Future development should focus on use for <u>when blood transfusion is significantly delayed or not possible</u>
- A large US RCT of HBOC in trauma when RBC are not available does not appear feasible

Conclusions

- Data from patients that cannot receive RBC but have received HBOC (Hemopure) suggest a survival advantage
- Hemopure approved in South Africa. Company planning US development for when blood not an option. If successful, maybe available in 5-10 years.
- No PFC for quantitative O2 delivery expected in US w/in 10 year

Conclusions

- Both a PFC-based (NuVox Pharma) and Hgb-based (Sanguinate, Prolong Pharmaceuticals) Oxygen Therapeutics are in development in the US, with potential market within 5-10 years
- Potential trauma role to mitigate MOF, ischemia-reperfusion suggested
- Role in significant hemorrhage is not yet determined

Thank You

- Questions and Comments