Alcor A-3523

Case Report

Prepared by:

Linda Chamberlain, Co-Founder and Director of Special Projects, Alcor Life Extension Foundation

July – 2023

Table of Contents

1. Summary

Information was derived from multiple sources and was all converted to Mountain Standard Time (MST). For de-identification, dates are not shown. T-0 represents the date of cardiac arrest (if more than a few moments before pronouncement) or pronouncement of legal death, T-X represents occurrences on dates before T-0, and T+X represents occurrences on dates following T-0.

A-3523 was a 74-year-old member with neuro cryopreservation arrangements. The death certificate gave the cause of death as respiratory failure following chronic obstructive pulmonary disease, end state heart disease, and end-stage Alzheimer's disease. The member was pronounced legally deceased in Florida at 13:48 hrs on T-0 days in 2022.

After stabilization and field blood substitution, the patient was air transported to Alcor for cryoprotectant perfusion and cryogenic cooldown. The patient arrived at Alcor on T+1 days at 21:39 hrs. The cryogenic cooldown was initiated on T+2 days at 02:24 hrs and terminated on T+5 days at 18:29 hrs. CT scans were made of the patient's brain at liquid nitrogen temperature on T+27 days at 11:50 hrs. The patient was transferred to long-term maintenance at liquid nitrogen temperature on T+65 days at 13:16 hrs.

2. Patient Assessment and Deployment

T-1 days

The member had been in a long-term care facility for several years and on Alcor's Watch List for some time. There had been no reported decline in the member's status prior to a call at 20:28 hrs from the member's family stating that the member's breathing was uncharacteristically rapid, heavy, and with gurgling sounds. Additionally, the attending nurse had indicated the member was actively dying and potentially within 24 hours of clinical death.

Alcor's Deployment Committee deployed Suspended Animation (SA) one of Alcor's strategic partners for providing standby, stabilization, and Transport (SST) and field blood substitution. SA had been deployed for a different member, also in Florida, but that member was stable enough for SA to redeploy instead to this member. At 21:43 hrs SA had arrived at this member's bedside.

3. Standby and Stabilization

T-0 days

The member's vital signs at 01:27 hrs were as follows: heart rate (HR) 99/min, respiratory rate (RR) 36/min and shallow, capillary oxygen saturation (SpO_2) 93% on 3 liters (L) of oxygen (O_2), blood pressure (BP) 111/70. And at 04:47 hrs the vital signs were as follows: HR 101/min, RR 28/min, SpO_2 93% on 5 L of O_2, BP 125/71. The MRD spoke to the family at 06:21 hrs and learned the family had asked the care facility to provide comfort care only for the member.

At 10:52 hrs the vital signs were as follows: HR 100, RR 28, SpO_2 82% on 3.5 L, BP 105/59 with shallow breaths. At 15:10 hrs the vital signs were as follows: HR 87, RR 30, SpO_2 82% on 3.5 L, BP 100/63.

The family called Alcor at 12:56 hrs to report that they had said their goodbyes to the member and were going home. The facility nurse turned off the member's oxygen at 13:10 hrs as directed by the attending physician. The member went into cardiac arrest at approximately 13:47 hrs and was pronounced legally deceased at 13:48 hrs.

The SA team consisted of three individuals. The patient was placed in the portable ice bath (PIB) at 13:50 hrs. At 13:51 hrs mechanical chest compression with the Autopulse device was initiated to support blood circulation and improve external cooling. The EZIO intraosseous device was placed in the right tuberosity of the patient' right leg for vascular access to administer the stabilization medications, the patient was intubated, and an additional 120 lbs. of water ice was applied to the patient's head and body.

At 13:52 hrs a ventilator was attached to the ET tube and a Capnograph device was attached to the ventilator to record $EtCO_2$ data for after case analysis and comparison with other cases. Concurrently, the administration of stabilization medications was initiated (see the below Table of Medications Administered for the names of the medications, doses, and times of administration).

A nasopharyngeal temperature (NPT) thermocouple was placed in the patient's left nare at 13:54 hrs and approximately 5 gallons of water was added to the PIB so that the surface conduction cooling device (SCCD) would circulate the water and improve cooling. At 13:55 hrs the SCCD was started, and the face mask was placed to allow ice water to flow over the patient's face. At 13:57 MST the patient was covered and transported to the mobile operating vehicle in the parking lot where prior authorization had been granted by the facility to perform the stabilization. The patient was loaded into the operating vehicle at 14:00 hrs where the medications were concluded and the presurgical cooling continued. An additional 100 lbs. of water ice was added to the PIB.

4. Field Surgery and Washout

Chest compressions were discontinued at 14:21 hrs with the patient's nasal temperature at 23°C and the initial sternal cutdown began.

At 14:37 hrs the perfusion circuit was fully set up and primed, including a 5 L/min pump providing room air to the circuit oxygenator. At 14:44 hrs the Mobile OR generator stopped functioning, leaving all surgical and perfusion equipment to rely on battery backups. SA received permission to run an extension cord to an exterior outlet at the facility. At 14:54 hrs the power was restored.

At 15:05 hrs the sternal saw was used to access the chest. Due to extensive scar tissue, it was difficult to cut through the sternum with the saw, and additional manual dissection was required to access the heart. At 15:46 the patient was cannulated and connected to the open circuit bypass with a 22 French (Fr) curved aortic cannula and a dual stage 29-37 venous cannula through the

right atrium which was fed down the inferior vena cava. At 15:50 hrs 250,000 IU streptokinase was added to the washout solution to help break up blood clots.

Once on bypass the perfusionist limited the perfusate pressure from the heart canulae to 98mmHg. At 15:54 hrs a thoracic temperature probe was placed in the abdomen for additional temperature data. At 16:01 hrs recirculation of perfusate began. There were noticeably lower temperatures with the arterial perfusate and the venous return perfusate compared to the nasopharyngeal temperature (NPT) thermocouples (see the Discussion section).

At 16:23 hrs the patient was taken off bypass with an arterial perfusate temperature of 0.3°C, venous perfusate temperature of 1.1°C, thoracic temperature of 5.3°C, left NPT of 9°C, and right NPT of 13°C. The cannulae were now removed, and the purse strings tied off. The sternum was reapproximated with three #6 stainless steel wires. and the skin stapled. The patient was then transferred to a local funeral home, with the intention to transport to Alcor the next day. The patient already had approximately 220 lbs. of ice in the PIB, and an additional 30 lbs. of ice was added to the patient's head during transport to the funeral home.

5. Patient Transport

The patient arrived at the funeral home at 18:10 hrs and was transferred from the PIB into a Ziegler case with approximately130 lbs. of water ice at 18:10 hrs. The NPT was 9°C and the thoracic temperature was 4°C. The patient was then transferred into the funeral home's temperature-controlled room that was kept at a constant 2.8°C.

T+1 days

At 06:12 hrs the patient was booked on a commercial flight to Phoenix, AZ. At 07:06 hrs the Ziegler case was placed into the air shipping tray with insulation. The remaining ice in the Ziegler case was inspected, and due to the overnight storage in the 2.8°C cooler no additional ice was needed. R-19 insulation was placed on the top, bottom, and sides of the Ziegler case to ensure good insulation for the long transit time.

The patient was checked into the cargo department at the airport at 08:00 hrs and departed the airport at approximately 09:55 hrs. The plane arrived in Phoenix at 20:28 and the patient was transported to Alcor by the Arizona funeral director. The long transit time was a result of flight delays (see the Discussion section).

6. Cryoprotectant Perfusion Surgery

The patient arrived in the Alcor operating room (OR) at 21:39 hrs. The initial nasopharyngeal temperature (NPT) was not recorded (see the Discussion section). At 21:44 hrs a hoist was used to move the patient from the shipper to a gurney for surgery to be performed while the patient was still in the body bag and packed with ice filled baggies. Styrofoam blocks were placed under

patient's shoulders at 21:48 hrs to raise the head for surgery. The patient was draped and the initial incision for cephalic isolation was made at 21:57 hrs.

The left carotid artery was exposed at 21:59 hrs and isolated at 22:01 hrs. The right carotid artery was exposed at 22:04 hrs and isolated at 22:05 hrs. Scalp cuts were made for both burr holes at 22:10 hrs. Using a Codman perforator, the left burr hole was started at 22:12 hrs using sterile water to cool the perforator and the skull. Several attempts were made to complete the burr hole because the perforator bit kept coming loose from the perforator. Both burr holes were finished at 22:16 hrs.

The surgeon returned to the cephalic isolation at 22:17 hrs and at 22:22 hrs had transected the spinal cord with an osteotome and mallet. The cephalic isolation was complete at 22:23 hrs. The cephalon weighed 5.23 kg at 22:24 hrs, before cryoprotectant perfusion. The cephalon was not weighed after perfusion, so it could not be determined whether the patient lost or gained weight, one indicator of perfusion quality (see the Discussion section). The cephalon was placed into the neuro ring and secured at 22:25 hrs. A purse string was placed in the left carotid artery at 22:29 hrs.

7. Cryoprotectant Perfusion

This was a neuro cryopreservation in the Alcor operating room (OR) using the bladder with gravity feed system rather than the OR perfusion pump system (see Discussion section). A clamp was opened to the perfusion tubing to allow the removal of air bubbles.

An 18 Fr red Robinson cannula was placed in the left carotid artery and tied off. Open circuit perfusion was initiated at 22:32 hrs on the left carotid artery (see the below Table of Concentrations (Brix) of nM22 Solution, for the times the bladders were started, the precalculated concentrations of each bladder in both Brix and molarity, and the refractive index of effluent samples taken). The arterial pressure was 39 mmHg. The left jugular vein was cannulated at 22:35 hrs with an 18 Fr red Robinson cannula to allow effluent to flow. The arterial perfusate temperature at 22:37 hrs was 2.9°C.

A purse string was placed in the right carotid artery at 22:38 hrs. The right carotid artery was cannulated with an 18 Fr red Robinson cannula. Effluent sampling for refractometry was started on the left jugular vein. The arterial pressure at 22:45 hrs was 76 mmHg. The right jugular vein was cannulated to allow effluent to flow at 22:46 hrs with an 18 Fr red Robinson cannula. Flow was detected from the right vertebral artery at 22:47 hrs. Step ramp perfusion was started with Bladder #4, with a molarity (M) of 1.29, to control edema.

The right vertebral artery was cannulated (size not recorded) at 22:53 hrs. The left vertebral artery could not be found, cannulated, or clamped off. A thermocouple was placed into the right burr hole and sutured to skin at 22:56 hrs.

Sidebar:
Per the cryoprotection protocol, the ramp is to be paused at 30 Brix (50% of the desired terminal concentration) to allow the patient to come to osmotic equilibrium. When the bladder system is used, bladders 6 & 7 represent the pause. The cephalic enclosure and the chiller are switched from +3°C to -3°C operation. At the end of the 30-minute pause, the ramp is resumed at the maximum addition rate (maximum without losing total volume in the circuit) to go to 105% of the desired end concentration (52.5 Brix) and held between 102% and 105% concentration until the terminal concentration is obtained.

The lid was placed on the neuro enclosure and the chiller was turned on at 23:05 hrs. The temperature of the enclosure was 15.6°C. Four minutes later the temperature of the enclosure was 2.9°C. At 23:08 hrs it was noted that the patient's skin was darkening (see the Discussion section).

The temperature in the neuro enclosure was 3.7°C. At 23:23 hrs the target temperature in both the enclosure and the heat exchanger were switched from +3°C to -3°C. Both corneas had collapsed (see the Discussion section) at 23:24 hrs.

The arterial pressure was 71 mmHg at 23:53 hrs. The tripod was raised approximately 2" to raise the arterial pressure to 79 mmHg. The patient's skin continued to tan uniformly.

Air bubbles in the sample line caused fluctuations in the RI readings at 24:20 hrs. This was corrected by reducing the flow rate.

T+2 days

The arterial pressure was 84.5 mmHg at 01:11 hrs. The tripod was lowered approximately 4" and the pressure was reduced to 81.45 mmHg. At 01:14 hrs air bubbles again accumulated in the left refractometer sampling line which again caused the left venous RI to fall. The air bubbles were cleared, and concentration rose quickly (see the Discussion section).

The 30-minute countdown to termination of perfusion started at 01:24 hrs.

Sidebar:
Per protocol, a 30-minute countdown to the termination of cryoprotection is initiated, after which the final sub-zero terminal concentration ramp is resumed. Per the cryoprotection protocol, the normal endpoint criterion for whole body patients is over 100% for over 30 minutes from the venous return and for neuro patients, it is over 100% for over 30 minutes from both jugular veins. The addition pump speed is minimized, with frequent corrections, to compensate for latency.

An eyebolt was placed in the patient's vertebrae at 01:54 hrs for transferring the patient into the cooldown dewar. The end point for termination of perfusion was to have 49.9 Brix concentration on both jugulars, the terminal concentration needed to vitrify. The left venous RI reading kept falling off due to bubbles in the sampling lines. Therefore, perfusion continued until Bladder #17 was exhausted.

The cephalon was not weighed post perfusion (see the Discussion section).

Cryoprotectant perfusion was terminated at 02:11 hrs. The final RI readings were 51.22 Brix (103% of perfusate concentration needed to vitrify (CNV) from the right jugular vein and 51.95 Brix (105% of CNV) from the left jugular vein. At 02:13 hrs the final temperatures were: Arterial -2.8°C, Right venous -1.6°C, Left venous -0.9°C, NPT -1.1°C, Burr hole -1.6°C, Neuro box -2.2°C.

8. Cooling to Liquid Nitrogen Temperature

The patient was placed in the cooldown dewar at 02:19 hrs. Computer controlled cryogenic cooldown was initiated at 02:24 hrs on T+2 days, plunging to -110°C and descending thereafter at -1°C/hour to liquid nitrogen temperature. On T+5 days at 18:29, an uneventful cooldown was terminated. On T+27 days, CT scans were made of the patient's brain while in liquid nitrogen. On T+65 days, the patient was transferred to long-term maintenance at liquid nitrogen temperature.

9. Timeline and Time Summaries

Timeline

T-0	13:47	Cardiac arrest (time estimated)
T-0	13:48	Pronouncement of legal death
T-0	13:50	Start of ice bath cooling
T-0	13:51	Start of mechanical chest compressions
T-0	13:51	Placement of intraosseous device
T-0	13:51	Placement of airway
T-0	13:52	Administration of first medication (200 mg propofol)
T-0	13:53	Administration of 1st dose of sodium citrate (200 mL)
T-0	13:56	Administration of 2nd dose of sodium citrate (200 mL)
T-0	14:00	Transport patient to mobile operating vehicle
T-0	14:20	Administration of final medication (200 cc Decaglycerol)
T-0	14:21	Termination of cardiopulmonary support (NPT = 23°C)
T-0	14:21	Start of field surgery
T-0	14:44	MOV generator failed
T-0	14:54	Power restored
T-0	15:45	End of field surgery
T-0	15:46	Start of open circuit washout
T-0	16:01	Start of closed circuit washout
T-0	16:23	Completion of closed circuit washout
T-0	16:30	Begin transport of patient to funeral home (estimate)
T-0	18:05	Patient arrived at funeral home estimate)
T-0	18:10	Patient placed in Ziegler case with 130 lbs. of ice
T+1	08:00	Patient checked into cargo department at airport
T+1	21:39	Arrival of patient at Alcor (NPT = -0.5°C)
T+1	21:57	Start of surgery/cephalic isolation at Alcor
T+1	22:10	Start of burr hole surgery
T+1	22:14	Completion of burr hole surgery
T+1	22:23	Completion of cephalic isolation (neuro case)
T+1	22:24	Weight of cephalon 5.23 kg)
T+1	22:32	Start of open circuit cryoprotection (bladders)
T+1	22:58	Pause at 50% of concentration necessary for vitrification (CNV) achieved
T+1	23:14	Start of sub-zero terminal concentration ramp (off pause)
T+2	02:11	End of cryoprotection (final RI readings: right = 51 Brix, left = 52 Brix)
T+2	02:24	Start of cryogenic cooldown
T+5	18:29	Completion of cryogenic cooldown
T+27	11:50	CT scans made post-cooldown
T+65	13:16	Transfer of patient to long-term maintenance at LN2 temperature

Time Summaries

Event Duration hr:min		days	time	
FIELD STABILIZATION				
00:01	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T-0	13:48	Pronouncement of legal death
00:03	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T-0	13:50	Start of ice bath cooling
00:04	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T-0	13:51	Start of mechanical chest compressions
00:05	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T-0	13:52	Administration of first medication (200 mg propofol)
00:28	From:	T-0	13:52	Administration of first medication (200 mg propofol)
	Till:	T-0	14:20	Administration of final medication (200 cc Decaglycerol)
FIELD SURGERY AND WASHOUT				
00:34	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T-0	14:21	Start of field surgery
01:24	From:	T-0	14:21	Start of field surgery
	Till:	T-0	15:45	End of field surgery
01:59	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T-0	15:46	Start of open circuit washout
00:37	From:	T-0	15:46	Start of open circuit washout
	Till:	T-0	16:23	Completion of closed circuit washout
02:36	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T-0	16:23	Completion of closed circuit washout
CRYOPROTECTANT SURGERY AT ALCOR				
31:52	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T+1	21:39	Arrival of patient at Alcor (NPT = -0.5°C)
00:18	From:	T+1	21:39	Arrival of patient at Alcor (NPT = -0.5°C)
	Till:	T+1	21:57	Start of surgery/cephalic isolation at Alcor
00:26	From:	T+1	21:57	Start of surgery/cephalic isolation at Alcor
	Till:	T+1	22:23	Completion of cephalic isolation (neuro case)
CRYOPROTECTANT PERFUSION AT ALCOR				
00:35	From:	T+1	21:57	Start of surgery/cephalic isolation at Alcor
	Till:	T+1	22:32	Start of open-circuit cryoprotection (bladders)
04:14	From:	T+1	21:57	Start of surgery/cephalic isolation at Alcor
	Till:	T+2	02:11	End of cryoprotection (final RI readings: right = 51 Brix, left = 52 Brix)
32:45	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T+1	22:32	Start of open-circuit cryoprotection (bladders)
00:18	From:	T+1	21:39	Arrival of patient at Alcor (NPT = -0.5°C)
	Till:	T+1	21:57	Start of surgery/cephalic isolation at Alcor
03:39	From:	T+1	22:32	Start of open-circuit cryoprotection (bladders)
	Till:	T+2	02:11	End of cryoprotection (final RI readings: right = 51 Brix, left = 52 Brix)

CRYOGENIC COOLDOWN AT ALCOR				
00:13	From:	T+2	02:11	End of cryoprotection (final RI readings: right = 51 Brix, left = 52 Brix)
	Till:	T+2	02:24	Start of cryogenic cooldown
36:37	From:	T-0	13:47	Cardiac arrest (time estimated)
	Till:	T+2	02:24	Start of cryogenic cooldown
04:45	From:	T+1	21:39	Arrival of patient at Alcor (NPT = -0.5°C)
	Till:	T+2	02:24	Start of cryogenic cooldown

10. Table of Medications Administered

T-0 days

TIME	MEDICATION	DOSE	PURPOSE
13:52 hrs	Propofol	200 mg	Anesthetic; reduces cerebral metabolic demand; reduces the theoretic possibility of increased awareness during aggressive CPS.
13:53 hrs	Sodium citrate	200 mL total (1st dose 60 mL) Note 2	Anticoagulant; prevents blood clot formation.
13:56 hrs	Sodium citrate	200 mL total (2nd dose 60 mL) Note 2	Anticoagulant; prevents blood clot formation.
13:58 hrs	Sodium citrate	200 mL total (3rd dose 40 mL) Note 2	Anticoagulant; prevents blood clot formation.
14:00 hrs	Sodium citrate	200 mL total (4th dose 40 mL) Note 2	Anticoagulant; prevents blood clot formation.
14:00 hrs	Heparin	50,000 IU	Anticoagulant; prevents blood clot formation.
14:01 hrs	Vasopressin	40 IU (1st dose) Note 3	Vasopressor; increases blood pressure during CPS.
14:02 hrs	Minocycline	200 mg	Antibiotic and neuroprotectant
14:03 hrs	SMT (S-methyl-isothiourea)	400 mg Note 4	Neuroprotectant (iNOS inhibitor); protects the brain from ischemic injury; raises blood pressure.
14:03 hrs	Antacid	250 cc total (1st dose 60 IU) Note 5	A buffer used to protect the stomach from acid erosion.
14:03 hrs	Antacid	250 cc total (2nd dose 60 IU) Note 5	A buffer used to protect the stomach from acid erosion.

14:04 hrs	Antacid	250 cc total (3rd dose 40 IU) Note 5	A buffer used to protect the stomach from acid erosion.
14:08 hrs	Decaglycerol/THAM	400 cc total (1st dose 60 cc) Note 6	Decaglycerol inhibits cerebral edema.
~14:09 hrs	Decaglycerol/THAM	400 cc total (2nd dose 60 cc) Note 6	Decaglycerol inhibits cerebral edema.
~14:10 hrs	Decaglycerol/THAM	400 cc total (3rd dose 60 cc) Note 6	Decaglycerol inhibits cerebral edema.
~14:11 hrs	Decaglycerol/THAM	400 cc total (4th dose 20 cc) Note 6	Decaglycerol inhibits cerebral edema.
~14:12 hrs	Antacid	250 cc total (4th dose 60 IU) Note 5	A buffer used to protect the stomach from acid erosion.
~14:12 hrs	Antacid	250 cc total (5th dose 30 IU) Note 5	A buffer used to protect the stomach from acid erosion.
~14:13 hrs	Vital Oxy (w/ saline)	51 mL Note 7	Antioxidants: melatonin, vitamin E (D-alpha tocopherol), PBN (alpha Phenyl t-Butyl Nitrone) and anti-inflammatory carprofen.
~14:16 hrs	Vasopressin	40 IU (2nd dose) Note 3	Vasopressor; increases blood pressure during CPS.
~14:17 hrs	Decaglycerol/THAM	400 cc total (2nd dose 60 cc) Note 6	Decaglycerol inhibits cerebral edema.
~14:18 hrs	Decaglycerol/THAM	400 cc total (3rd dose 60 cc) Note 6	Decaglycerol inhibits cerebral edema.
~14:19 hrs	Decaglycerol/THAM	400 cc total (4th dose 20 cc) Note 6	Decaglycerol inhibits cerebral edema.
~14:20 hrs	Decaglycerol/THAM	400 cc total (4th dose 20 cc) Note 6	Decaglycerol inhibits cerebral edema.
15:50 hrs	Streptokinase	250,000 IU Note 7	A thrombolytic used to break up existing blood clots.

Notes:

1. Times with "~" are approximations due to a bodycam not recording during that part of the stabilization.

2. The standard formulation for sodium citrate is 20% w/v. 10 grams of sodium citrate are given to patients who weigh less than 40 kg, and 20 grams are given to patients who weigh over 40 kg. This patient received 20 grams of sodium citrate because the patient's weight was over 40 kg.

3. Vasopressin is given as a fixed dosage of 40 IU, per dose for two doses. The second 40 IU dose is to be administered concurrently with Vital-Oxy, I.V. Vasopressin is to be administered only if the patient's temperature is above 20°C as it is ineffective at cold temperatures.

4. SMT (S-methyl isothiourea) is a fixed-dose and is a powder, (1 vial = 400 mg) dissolved in 10 mL of saline and injected through a 0.2 µ filter. SMT is unstable in solution with a useful life of approximately six hours.

5. An antacid is given in several doses, totaling 250 mL, and inserted through the nasogastric tube in an airway.

6. Decaglycerol/THAM is administered as a custom formulation of 20% w/v decaglycerol and 4.5% w/v THAM (tromethamine) in water (pH = 10.4 and pKa = 8.3).

7. The medications protocol dilutes 70 mL or less, based on body weight, of Vital-Oxy into 150 mL of saline for a total of 220 cc of diluted Vital-Oxy saline. Each mL of Vital-Oxy contains 194 mg Sigma Cremophor EL (or Sigma Kolliphor EL), 155 mg ethanol, 19.4 mg PBN, 3.24 mg carprofen, 1.55 mg melatonin, and 198 IU vitamin E.

8. Streptokinase is not administered with the stabilization medications but is put in the first batch of washout solution. The standard administration of streptokinase is 250,000 IU dissolved in 5 mL of 9% sodium chloride. This medication previously needed to be infused through a 0.2 µ filter. The medication now in use is already sterile filtered and can be reconstituted in the vial.

11. Table of Concentrations (Brix) of nM22 Solution

A-3523 step-ramp, nM22								
Preferred endpoint is over 49.9 Brix from both jugulars for 1/2hr								
2L Bag label number	[nM22], CNV	Molarity of penetrating CPAs*	Brix (calc)	Bag start hh:mm, MST	hrs post pronounc-ement	Bag avg. flow rate, mL/min	Brix, R venous return	Brix, L venous return
2	0.05	0.47	11.81	22:30	32.70	1000.0	0	0
3	0.08	0.78	13.14	22:32	32.73	250.0	0	0
4	0.14	1.29	15.35	22:40	32.87	222.2	0	0
5	0.23	2.15	19.03	22:49	33.02	222.2	10.4	9.8
6	0.50	4.67	29.85	22:58	33.17	166.7	11.5	11.8
7	0.50	4.67	29.85	23:10	33.37	500.0	15.6	14.5
8	1.06	9.91	52.31	23:14	33.43	133.3	16.4	15.8
9	1.06	9.91	52.31	23:29	33.68	285.7	23.3	23.6
10	1.06	9.91	52.31	23:36	33.80	76.9	26.1	26.4
11	1.06	9.91	52.31	0:02	34.23	133.3	42.7	37.7
12	1.06	9.91	52.31	0:17	34.48	90.9	47	43.2
13	1.06	9.91	52.31	0:39	34.85	222.2	50.1	44.9
14	1.06	9.91	52.31	0:48	35.00	95.2	50.7	46.7
15	1.06	9.91	52.31	1:09	35.35	142.9	50.6	48
16	1.06	9.91	52.31	1:23	35.58	69.0	51.4	50.6
17	1.06	9.91	52.31	1:52	36.07	105.3	51.1	49.1
END				2:11	36.38		51.1	51.9
* does not account for concentration of non-penetrating CPAs								

Note: When the bladders with precalculated concentrations of cryoprotectant are made up in the lab, the first bladder in the series contains only the B1 carrier solution with no cryoprotectant and was intended to be used for purging air bubbles. Bladder #2 contains the lowest concentration of cryoprotectant. Limited experience with the bladder system, however, has shown that better edema control is provided when the initial perfusion is done with cryoprotectant. As a result, cryoprotectant perfusion is initiated with Bladder #2. When there is sufficient experience to make this standard protocol, the lab procedure for creating the bladders will be changed so that Bladder #1 will contain cryoprotectant.

12. Discussion

Standby and Stabilization

A detailed medical history is always beneficial prior to performing stabilization procedures. This particular case was unique in that the member was a neuro cryopreservation member, and the initial plan was to perform a neuro Field Cryoprotection (FCP). Upon hearing of three prior heart surgeries, greater discussion should have transpired prior to committing to sternal access. In future, whenever possible under current HIPPA laws, an effort should be made to obtain a full medical history and a discussion between Alcor and the servicing SST organization as to what approach would be most beneficial to the member.

Field Surgery and Blood Substitution

When the initial sternal cutdown began, the patient was found to have undergone three prior open-heart surgeries that had left an abundance of sternal wire closing the sternum. There was a discussion among the SA team leader and the surgical team about abandoning the sternotomy to perform a carotid cutdown. The surgeons were confident they could gain access and the decision was made to proceed with the median sternotomy. The surgery was successful, however, it lasted from 14:22 hrs to 15:47 hrs, for a total surgical time of one hour and 25 minutes. As can be seen in the S-MIX calculation, this extended surgery time was the largest contributor to the total S-MIX score, which offset some of the rapid cooling rates achieved earlier in the procedure.

Near the end of the cryoprotectant ramp the temperature of the arterial perfusate and the temperature of the venous return perfusate was much colder than the nasopharyngeal temperatures (NPT). When perfusion was terminated the returning venous perfusate was at 1°C and the nasopharyngeal temperature was 9°C. This was an observation made during the cooldown.

The surgeon had placed a sterile temperature probe in the thoracic body cavity to record a form of core body temperature. SA has done this on various cases as an extra data set. Nasopharyngeal temperatures during the blood substitution on this case also did not correlate to the core body temperatures taken from the perfusion circuit.

The decrease in delivery and return of perfusate in the patient circuit would suggest a decrease in the nasopharyngeal temperatures (NPT). However, the NPTs took longer to decrease than would be expected. The initial drop in NPT would suggest water incursion into the nasopharynx. If this were true, it would be probable that the pooling water did not decrease in temperature as quickly as the surrounding tissue and provided a skewed reading of what the purposed brain temperature would read.

Transport to Alcor

Air transportation has been increasingly difficult during and following Covid due to flight delays and operating hour restrictions at airline cargo. Airline cargo requires a minimum of two hours, and recommends three hours, prior to departure for cargo to be checked in. This limits early morning flights as many cargo departments do not open until 07:00 hrs. Given enough time and sufficient funding over the minimum, discussions about potential private air transport would be beneficial, especially for members at greater distances, but of course in the end it will be a fiscal decision.

Cryoprotectant Surgery and Perfusion at Alcor

It is protocol to record the patient's nasopharyngeal temperature (NPT) when the patient is first brought into the operating room at Alcor. On this case, the datalogger probe was reading -0.5°C, and this was confirmed by reviewing the video tape of the case. However, this temperature reading is not considered accurate as the datalogger was malfunctioning because the patient was only on water ice. Plus, there is no data file to confirm this as the logger had stopped recording before the patient reached Alcor.

This case took place during the same time as another SA case was underway. Alcor personnel were expecting multiple patients to be brought to the OR in a short period of time. The OR-based perfusion system is complex and takes a lot of time and effort to break down, clean and prepare for another case. Because this patient was due to arrive at Alcor at over 33 hours from pronouncement, this length of time typically results in a straight freeze procedure. However, the decision was made to not abandon the patient to straight freeze but to use the step ramp bladder protocol which has an aggressive cryoprotectant ramp profile.

The amount of dehydration or edema in the brain, obtained by measuring the weight of the cephalon both before and after cryoprotectant perfusion, is an indication of how well the brain was vitrified. The cephalon was weighed before perfusion, but not afterward. This was an oversight that resulted from the OR not having been used for an extended time due to Covid. This error was pointed out during the debrief meeting to remind everyone of the importance of this measurement.

At least twice during the cryoprotectant perfusion, air bubbles accumulated in the refractometer sampling lines which resulted in inaccurate refractive index (RI) readings. The air bubbles were cleared, and the problem corrected. This is not a new problem. These bubbles are the result of the RI sampling lines flowing faster than the effluent. Perfusion personnel need to check the RI sampling lines at regular intervals during the perfusion to prevent this. Alcor's R&D personnel are aware of this problem and are working on a solution.

Between the 30-minute countdown for equilibration and the termination of the cryoprotectant ramp, it was noted that the patient's skin was darkening and both corneas had collapsed. These are both normal results of contact with the vitrification solution.

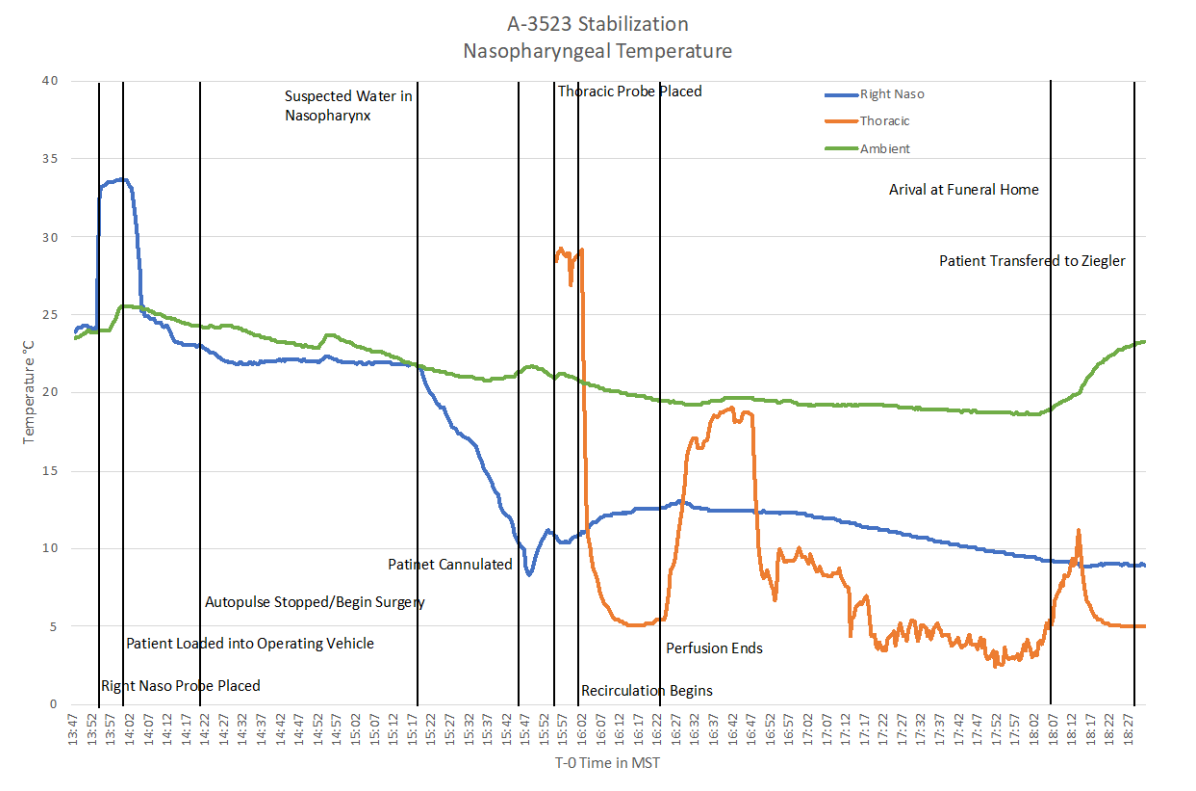
The CT scans show extensive areas of frozen blood, which is remarkable given the fact that the patient got a prompt washout. While surgical delays were more extensive than usual, more plausible causes include age or dementia-related compromise of the vasculature, a compromised Circle of Willis, pre-mortem/iatrogenic causes, or the extended transport time on water ice to Alcor.

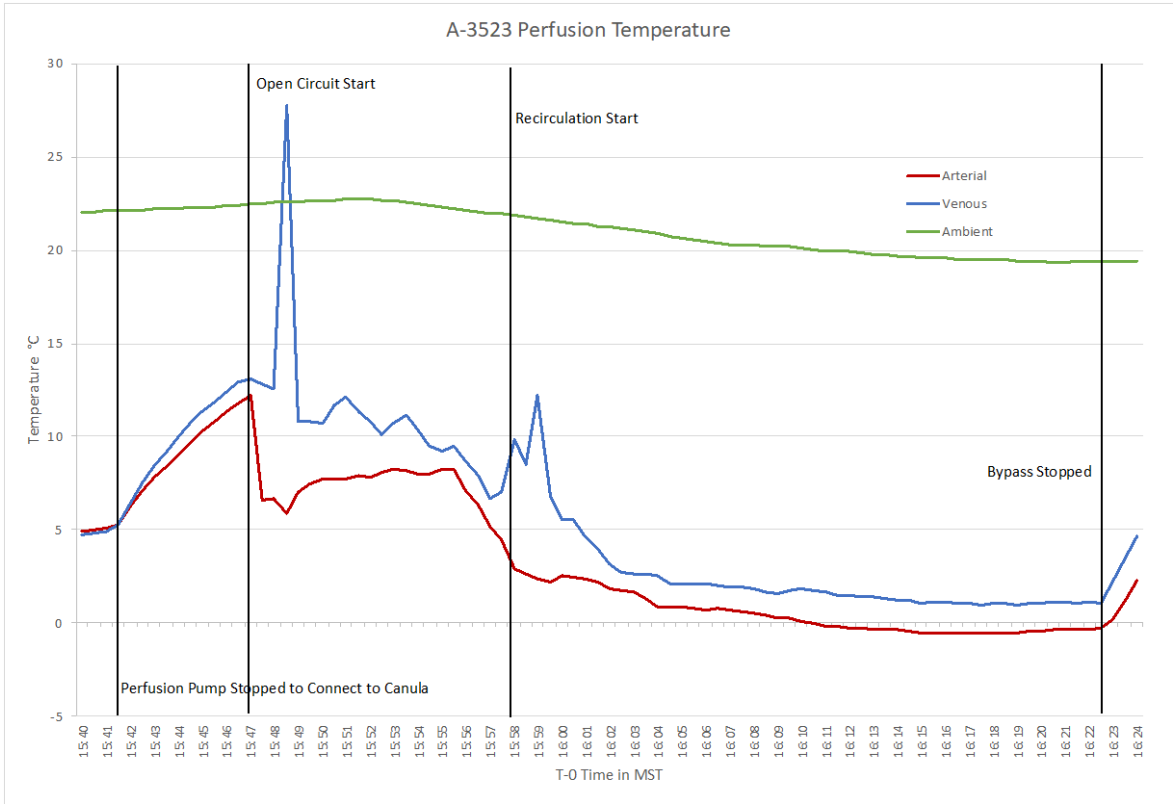
Given that this patient already received washout with MHP-2, it is sensible policy not to do another washout during cryoprotection. In fact, future FCP protocol at Alcor is to omit bags with 0% CPA washout solution.

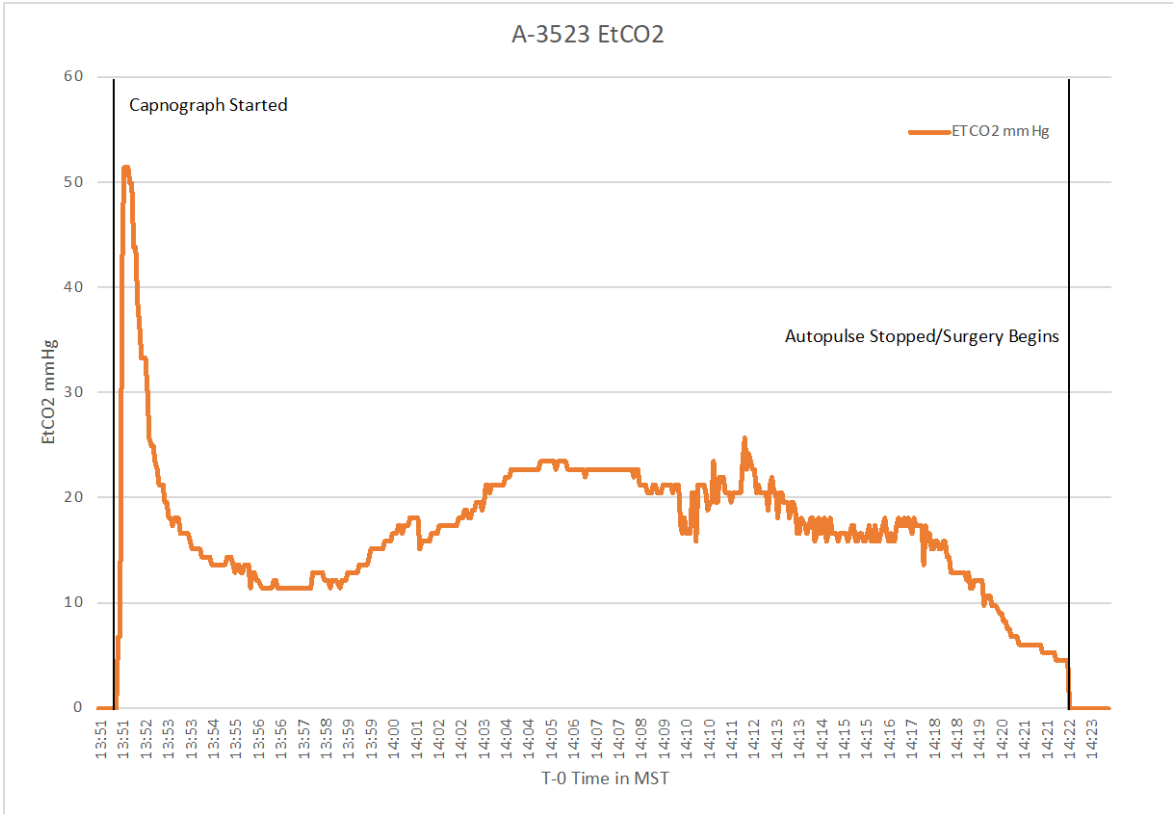
To further mitigate the effects of cold ischemia-induced perfusion impairment, the decision was made to start with bag 4 of the FCP protocol, which yields a starting concentration of 14% CNV, or 1.29M of penetrating CPA. Given the consensus in cryobiology to not exceed 3x intracellular molarity for each step, this starting concentration may have been too aggressive.

13. Cryoprotection and Temperature Graphs

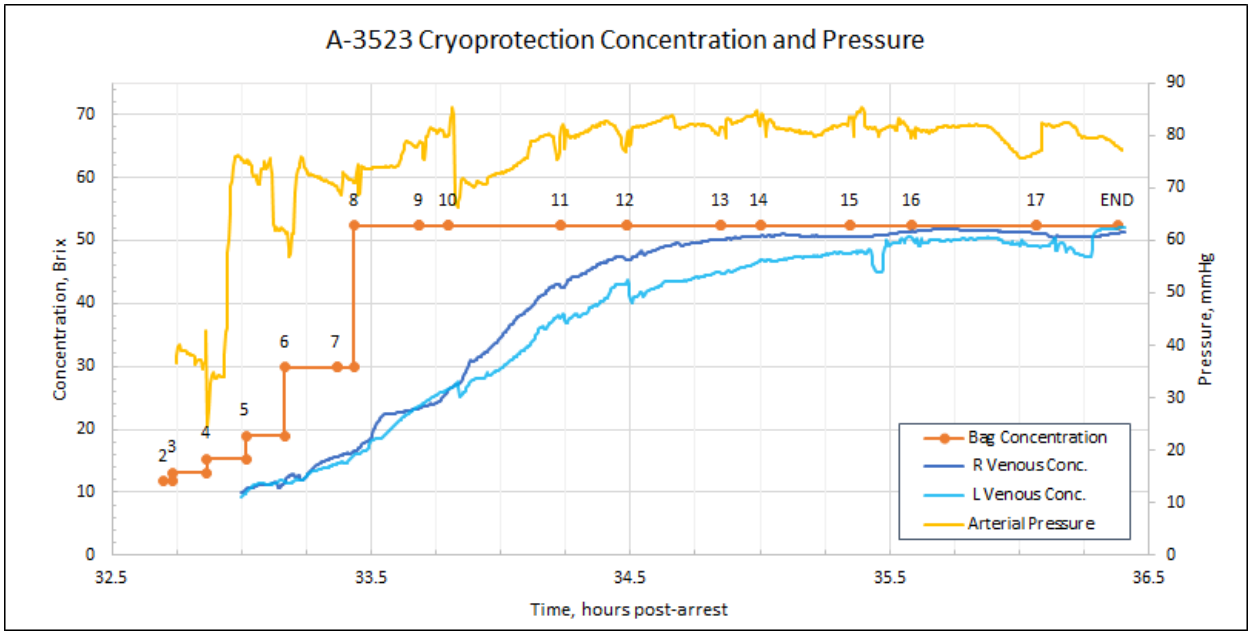
Graphs provided by SA:



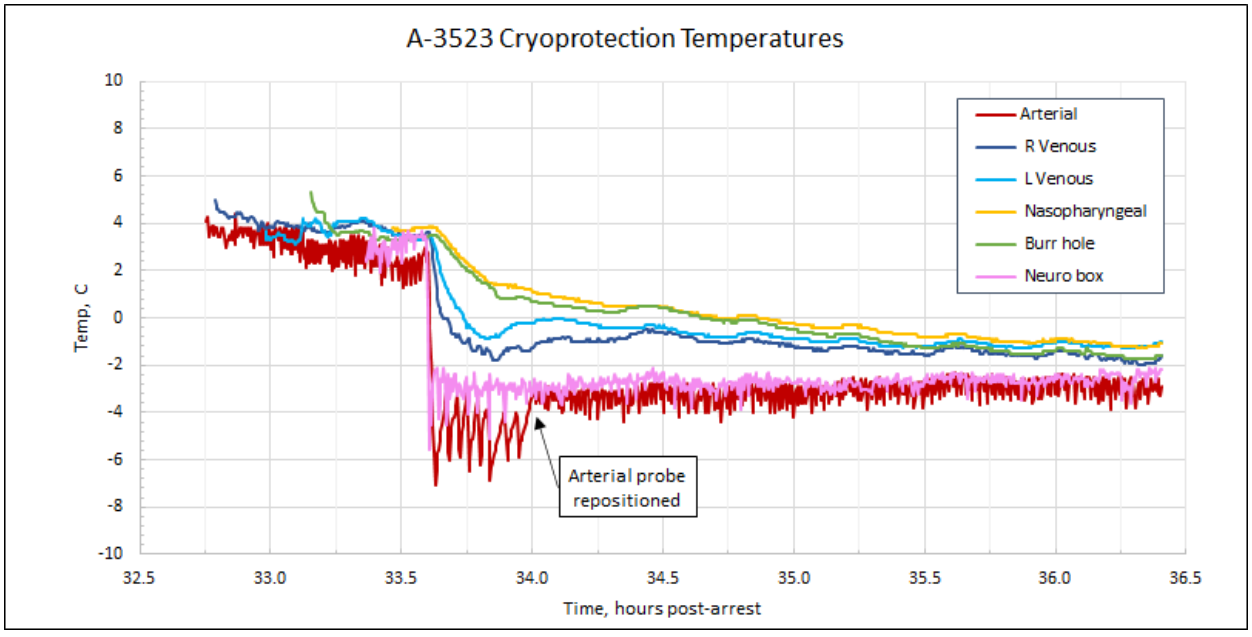

A-3523 Perfusion Temperature
Open Circuit Start
Recirculation Start
Arterial
Venous
Ambient
Bypass Stopped
Perfusion Pump Stopped to Connect to Canula
Temperature °C
T-0 Time in MST

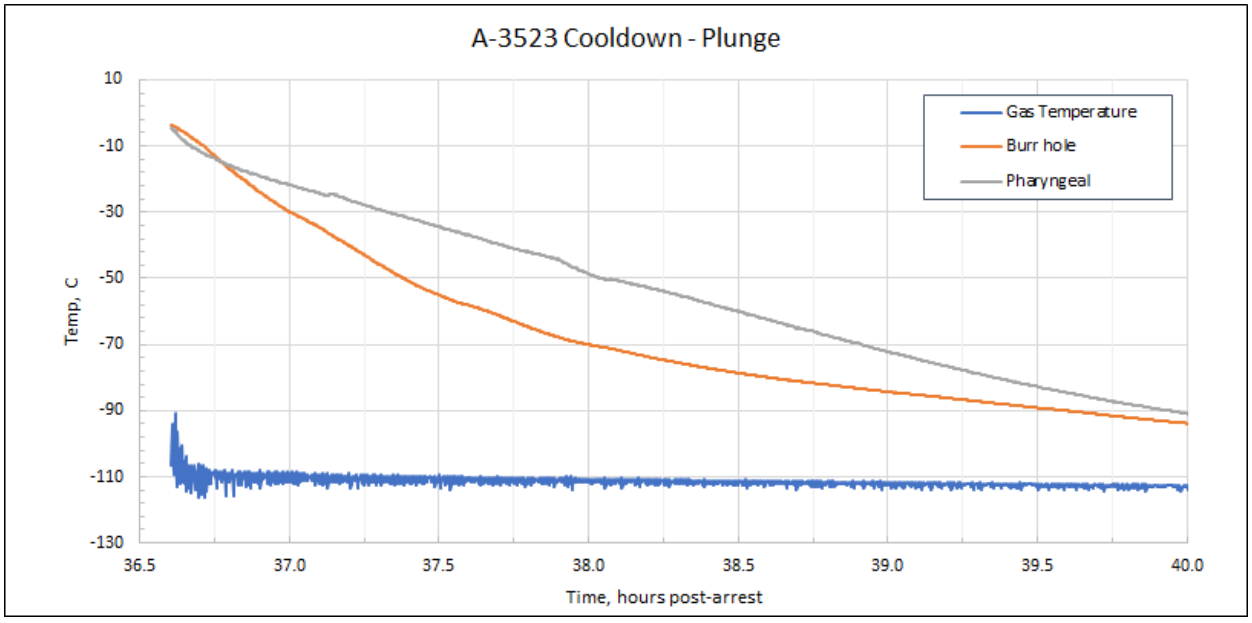

A-3523 EtCO2
Capnograph Started
ETCO2 mm Hg
Autopulse Stopped/Surgery Begins
EtCO2 mmHg
T-0 Time in MST

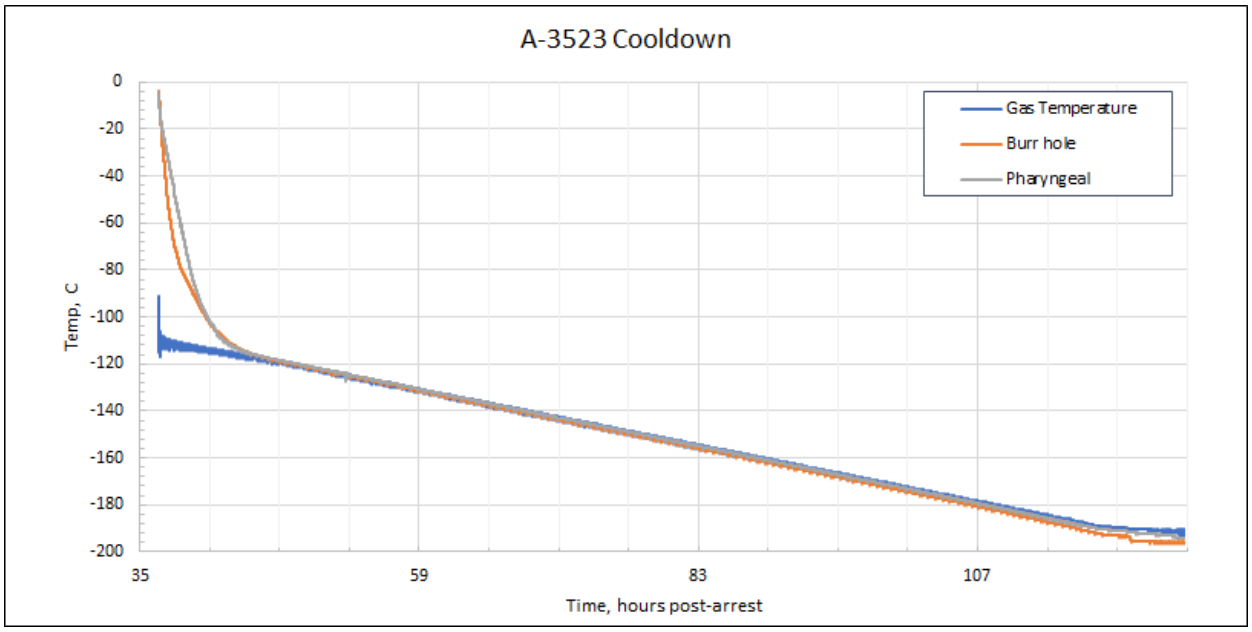
Graphs provided by Alcor:



Note: The cryoprotection graph is a hybrid of field cryoprotection data and data gathered in the operating room.




A-3523 Cooldown - Plunge
Gas Temperature
Burr hole
Pharyngeal
Temp, C
10
-10
-30
-50
-70
-90
-110
-130
36.5
37.0
37.5
38.0
38.5
39.0
39.5
40.0
Time, hours post-arrest


A-3523 Cooldown
Gas Temperature
Burr hole
Pharyngeal
Temp, C
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
35
59
83
107
Time, hours post-arrest

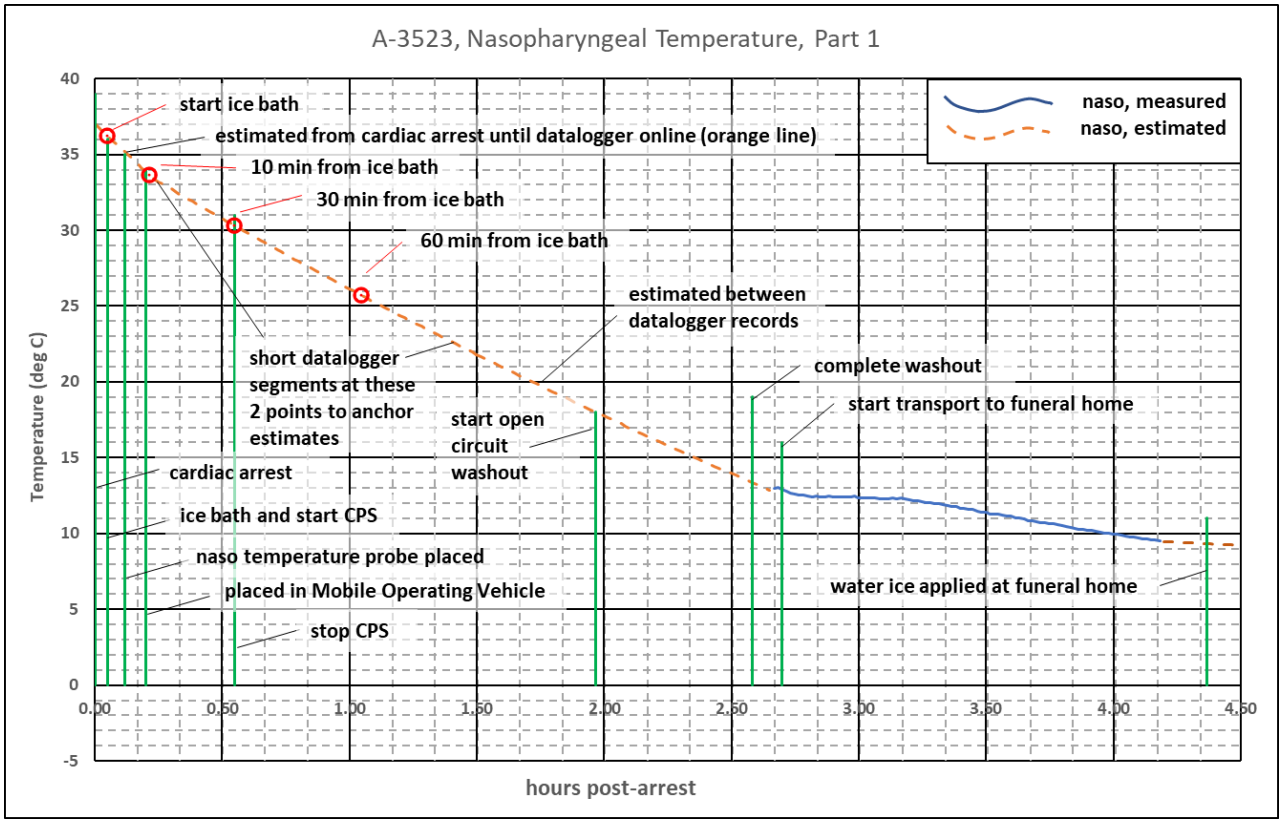
14. S-MIX Data

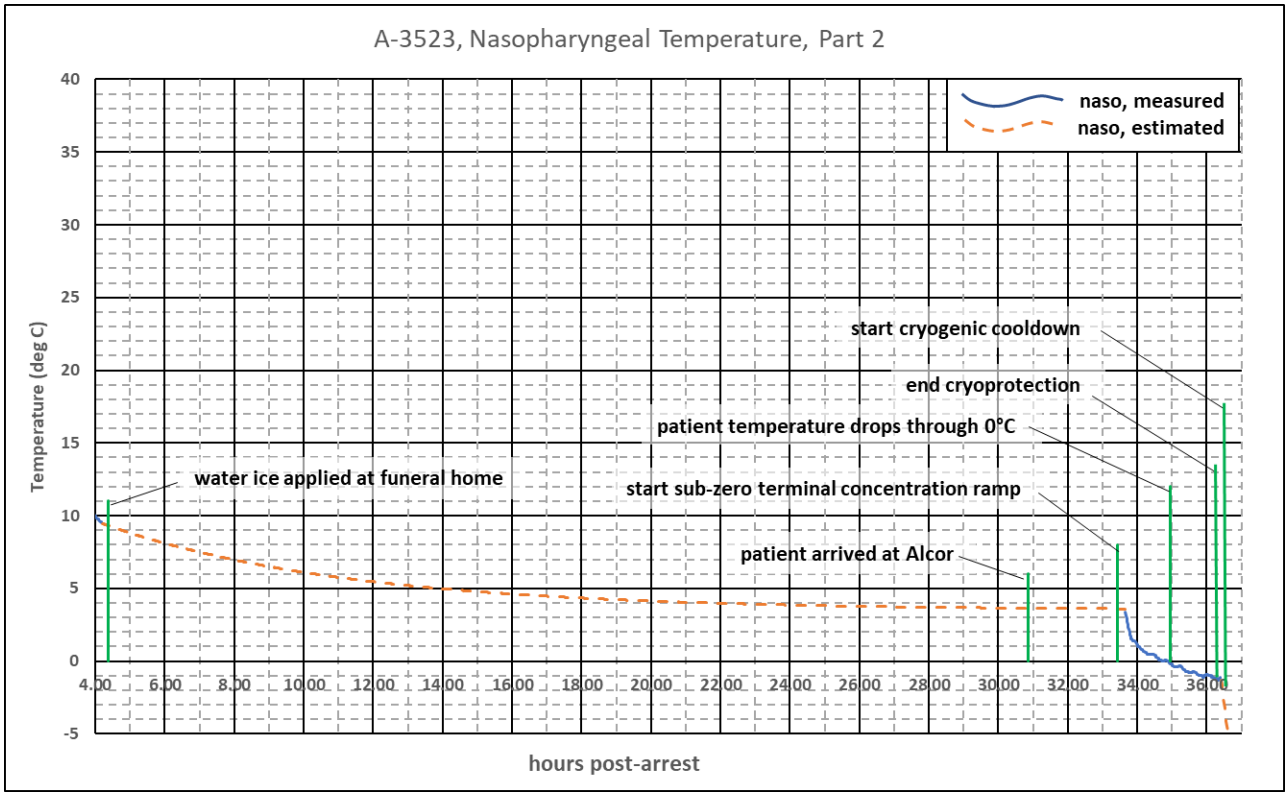
The Standardized Measure of Ischemic Exposure (S-MIX) expresses the total ischemic exposure prior to the start of cryogenic cooling as the equivalent duration of normothermic ischemia. An S-MIX of 00:00 (hh:mm) is the ideal case of no ischemic damage. The higher the S-MIX time, the more damage. Factors that improve the S-MIX, and that are quantitatively accounted for in the below table are: shorter times at higher temperatures, ventilation during cardiopulmonary support (CPS), and oxygenation during blood washout. The duration from cardiac arrest to 0 C is 34.57. As shown below, and due to lowering of the body temperature, S-MIX duration is shorter, at 04:25.

event	segment #	days (T+X)	time (MST) duration	post-arrest	Tnaso (deg C)	CPS w/ ventil.	washout oxygen.	S-MIX (hh:mm)
cardiac arrest		T-0	13:48	00:00	37.0			
	seg 1		00:03	00:03	-0.8	no	no	00:03
ice bath & mechanical CPS with ventilation		T-0	13:51	00:03	36.2			
	seg 2		00:04	00:04	-1.0	yes	no	00:02
right naso temperature probe placed		T-0	13:55	00:07	35.2			
	seg 3		00:05	00:05	-1.3	yes	no	00:02
placed in mobile operating vehicle		T-0	14:00	00:12	33.9			
	seg 4		00:21	00:21	-3.6	yes	no	00:07
CPS stopped		T-0	14:21	00:33	30.3			
	seg 5		01:25	01:25	-12.3	no	no	00:35
start open-circuit, oxygenated washout		T-0	15:46	01:58	18.0			
	seg 6		00:37	00:37	-4.7	no	yes	00:00
complete washout		T-0	16:23	02:35	13.3			
	seg 7		00:07	00:07	-0.4	no	no	00:01
start transport to funeral home		T-0	16:30	02:42	12.9			
	seg 8		01:40	01:40	-3.6	no	no	00:17
patient in Ziegler in water ice in 3.8 C cooler at		T-0	18:10	04:22	9.3			
	seg 9		26:29	26:29	-5.7	no	no	02:54
patient arrival at Alcor		T+1	20:39	30:51	3.6			
	seg 10		02:35	02:35	0.0	no	no	00:15
start sub-zero terminal contration ramp		T+1	23:14	33:26	3.6			
	seg 11		01:31	01:31	-3.8	no	no	00:08
patient passes thru 0 C		T+2	00:45	34:57	-0.2			
totals:			34:57	34:57	-37.2			04:25

The below plots show events related to the S-MIX calculation. There are several time segments that are estimated when datalogger data is unavailable. From 00:00 (cardiac arrest) to 00:12, the nasal probes are not yet in place and so this estimated segment starts at normal body temperature and ends when the probes are inserted. At 00:12 there are a few good datalogger points, not visible in the plot. From 00:12 to 01:25, the datalogger data indicates that cold water touched the nasopharyngeal probes, invalidating that data, and so the temperature is estimated. At 01:25, there are a few good datalogger points not visible in the plot. From 01:25 to 2:40, again the datalogger data is invalidated due to cold water contact with the probe. From 4:10 to 32:40 there is missing datalogger data and so this segment is estimated. The red dots can be used to construct a metric for how fast the patient is initially cooled (see the Patient Cooling Rate table

below). This is a critical period since body temperature is highest and ischemic damage most rapid.

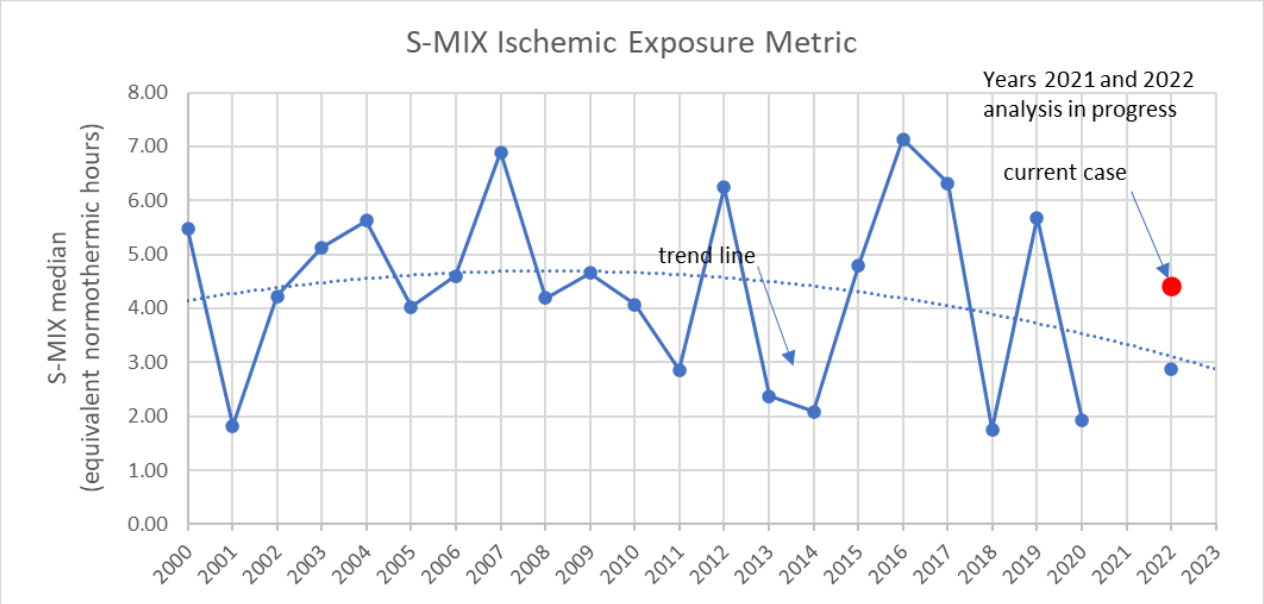




The below table provides cooling data for 10, 30, and 60 minutes after the team first applies water ice.

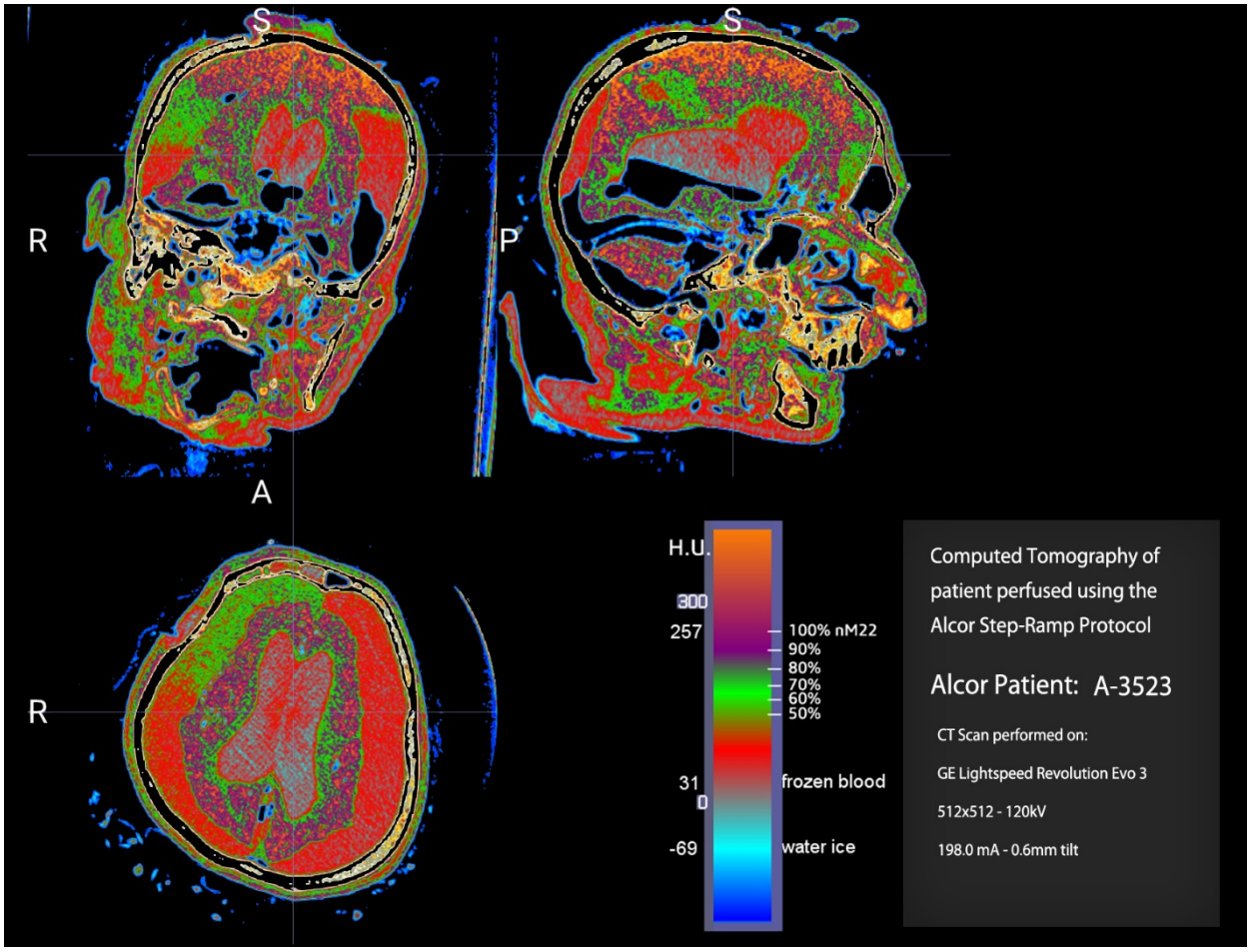
Patient Cooling Rate			(patient weight 73 kg; 160 lb)	
Note: time = 0 at start of ice bath	0 min elapsed	10 min elapsed	30 min elapsed	60 min elapsed
Naso temperature (°C)	36.2	33.6	30.3	25.7
Temperature drop (°C) from t = 0	0.0	-2.6	-5.9	-10.5
Cooling rate (°C/min) from t = 0	N/A	-0.26	-0.20	-0.18

The following plot shows the trend of S-MIX achieved since 2000.



15. CT Scans

Cryoprotectant Distribution (Post-cryopreservation CT scan)



The post-cryogenic cooldown CT scan was obtained on T+27 days; the patient was at liquid nitrogen temperature (-196°C).

The CT scan shows a heterogeneous distribution of cryoprotectant agent (CPA) with low concentrations of CPA in the core of the brain, including frozen blood or frozen washout solution.