Cryopreservation of Patient A-1097

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Introduction

This is the most comprehensive case report produced since that of A-1049 in 1990. This report was produced in conjunction with the paper "Advances in Cryonics Protocols, 1990-2006" and readers are referred to that for further background information on the protocols used in this case. That paper may be found at www.alcor.org/Library/html/protocoladvances.html.

Medical history

Born January 1, 1944, the patient had no history of cardiovascular disease or endocrine disturbance and suffered only from a case of mild asthma. He did not smoke and his alcohol intake was negligible. His mother died in her 70s from Alzheimer's disease and his father from heart disease in his 60s. One of his three sisters had breast cancer.

In August 1988, the patient experienced a tingling sensation in the right arm accompanied by a funny taste. An EEG was performed which indicated an abnormality; subsequent CT scan and stereotactic biopsy confirmed the presence of a diffuse (Grade II) astrocytoma (a type of malignant brain tumor) affecting the left frontotemporal region. The tumor was not surgically resectable, and as it grew the patient developed speech problems, right-sided weakness, and seizures.

Ultimately, treatment consisted of radical radiotherapy and follow-up CT scans appeared normal. However, the long-term prognosis for space occupying lesions (SOL) is poor because tumor growth not only affects neurological function, but elevates intracranial pressure to the point where systemic blood pressure is inadequate to perfuse the brain. As a result, parts of the brain may die, and in time the patient will die from lack of nutrients. After radiation treatment the patient required anticonvulsant medications to control complex partial seizures, the severity of which prevented his return to work. An MRI scan in 1998 detected the presence of residual non-enhancing tumor, consistent with low grade glioma.

During the early months of 2001, the patient's spouse noted deterioration of his handwriting and coordination. MRI in July and CT scan in August 2001 provided evidence of recurrence, which was confirmed on biopsy in October 2001 as glioblastoma multiforme (Grade IV astrocytoma), the most common and aggressive type of brain cancer. Left untreated, all patients die within 3 months and with standard treatment the median survival period is approximately 14 months. Less than 4% of patients who receive treatment survive for more than 5 years. During palliative treatment the patient received an impressive 42 cycles of chemotherapy. He survived for 51 months (4.25 years) following biopsy confirmation of Grade IV glioma.

The patient began oral chemotherapy with the alkalating agent Temozolamide in November 2001. He tolerated the treatment very well and some improvement (reduced brain tumor mass) was first noted on an MRI scan in April 2002. The tumor parameters remained stable for many months afterward, indicating a good response to treatment. Chemotherapy was discontinued in March 2005 for reasons that are not entirely clear in source materials, though it appears that the oncologist suspected that the consistently observed MRI abnormality represented gliosis rather

than residual tumor. The doctor ordered a PET scan to distinguish between residual tumor and gliosis, the results of which were inconclusive, but which appear to have been interpreted (overly) optimistically. Follow-up MRI in May 2005 showed some enhancement of the residual tumor; by August the mass had increased in bulk to involve much of the left temporal lobe and a little of the left basal ganglia. Chemotherapy was resumed in August in light of these changes, but follow-up MRI in November 2005 displayed a marked increase in size and associated mass effect in relation to the known left temporal glioma.

The patient was admitted to the hospital in Canberra, ACT, Australia, on December 25, 2005, after becoming disoriented. He presented with confusion, ataxia, and incomprehensible speech; a CT scan showed the known lesion in the frontotemporal region with associated edema which was clearly progressing. A chest X-ray also confirmed pneumonia for which he was prescribed antibiotics. He was also started on dexamethasone for cerebral edema which resulted in a partial improvement in his sensorium during his hospital stay.

As soon as the patient was improved enough to tolerate air travel, arrangements were made for his immediate transport to the U.S. for hospice care prior to cryopreservation. He was discharged from the hospital on January 9, 2006, and admitted to hospice care on January 10, 2006, under the instructions of his Medical Power of Attorney (MPOA). Alcor was given access to the patient's medical information and hospice staff were instructed to notify Alcor as to the patient's condition, particularly when death appeared imminent.

Agonal Phase

Though, remarkably, the patient required no supplemental oxygen en-route, the flight from Australia to a hospice in the vicinity of the Alcor facility in Scottsdale, Arizona, USA, was hard on the patient, who presented at the hospice on January 10, 2006, exhausted, confused, and upset, though he denied any pain or discomfort. Over the next few days he attempted to communicate with hospice staff but his confused mental state and very soft speaking voice (whisper) prevented interpretation.

Neurological assessment on January 12, 2006, reported no seizure activity since his arrival, but indicated that the patient was bedbound and unable to independently perform any activities of daily living (e.g., ambulation, bathing, dressing, and personal care) except eating. He insisted on feeding himself, though his appetite was poor. Some coughing with fluid intake was noted. His mental status remained alert/oriented but confused with poor communication. The patient was physically weak, with increasing lethargy and lengthy periods of sleep.

Physical assessments continued with no further progression of symptoms until two sudden episodes of vomiting, consisting of green bile and mucus, occurred in the early hours of January 14, 2006. Afterward the patient was unable to tolerate fluids (including liquid food) without aspiration. He refused administration of oxygen from that point on. Later that day oxygen saturation was measured at 68% with a heart rate of 122 and some mottling of the toes was noted. Alcor was notified of changes in the patient's condition at 16:35, and the standby team was deployed. The patient was lethargic and only semi-responsive over the next 24 hours.

Mottling of the knees was noted on January 15, 2007. Oral medications were discontinued, as they were very difficult to administer without fluids. The patient continued to refuse oxygen, and oxygen saturation remained at or below 75%. An assessment at 19:30 on January 16, 2007,

found him to be awake and alert, with eyes open and tracking when his name was called. Though the right side of his body was flaccid, he moved his left upper and lower extremities with frequency and demonstrated increased restlessness throughout the night. Restfulness was restored with medication (lorazepam 1 mg), with apnea observed for 5-10 seconds between shallow respirations.

Care and monitoring continued, with an anomalous oxygen saturation reading of 96% on January 17, 2007. The patient was awake throughout much of the evening with a moist, non-productive cough and crackles in the bases of the lungs. Restlessness in the early morning hours of January 18, 2007, was again countered with administration of lorazepam, with apnea observed for 10-15 seconds between shallow respirations.

An assessment on the morning of January 18, 2007, was carried out in response to audible pulmonary congestion and increased non-productive coughing. The patient was still restless and was administered another dose of lorazepam in conjunction with MSIR 5 mg. Mottling of the knees and cyanosis of the nail beds were noted. Glycopyrrolate 0.4 mg was administered at 09:35 to reduce respiratory secretions. The patient continued to refuse oxygen; oxygen saturation at 12:00 was 65%. Further assessments throughout the afternoon, evening, and night indicated rapid, shallow respirations with periods of apnea up to 10 seconds.

Breathing sounds were dramatically diminished upon assessment at 07:45 on January 19, 2007, and death was determined to be imminent. All vital functions ceased and death was pronounced promptly at 08:37.

Routine medication administration (Appendix A) and vital sign assessment (Appendix B) took place on a daily basis between admission to hospice care and pronouncement of legal death on January 19, 2006.

Preparation and deployment

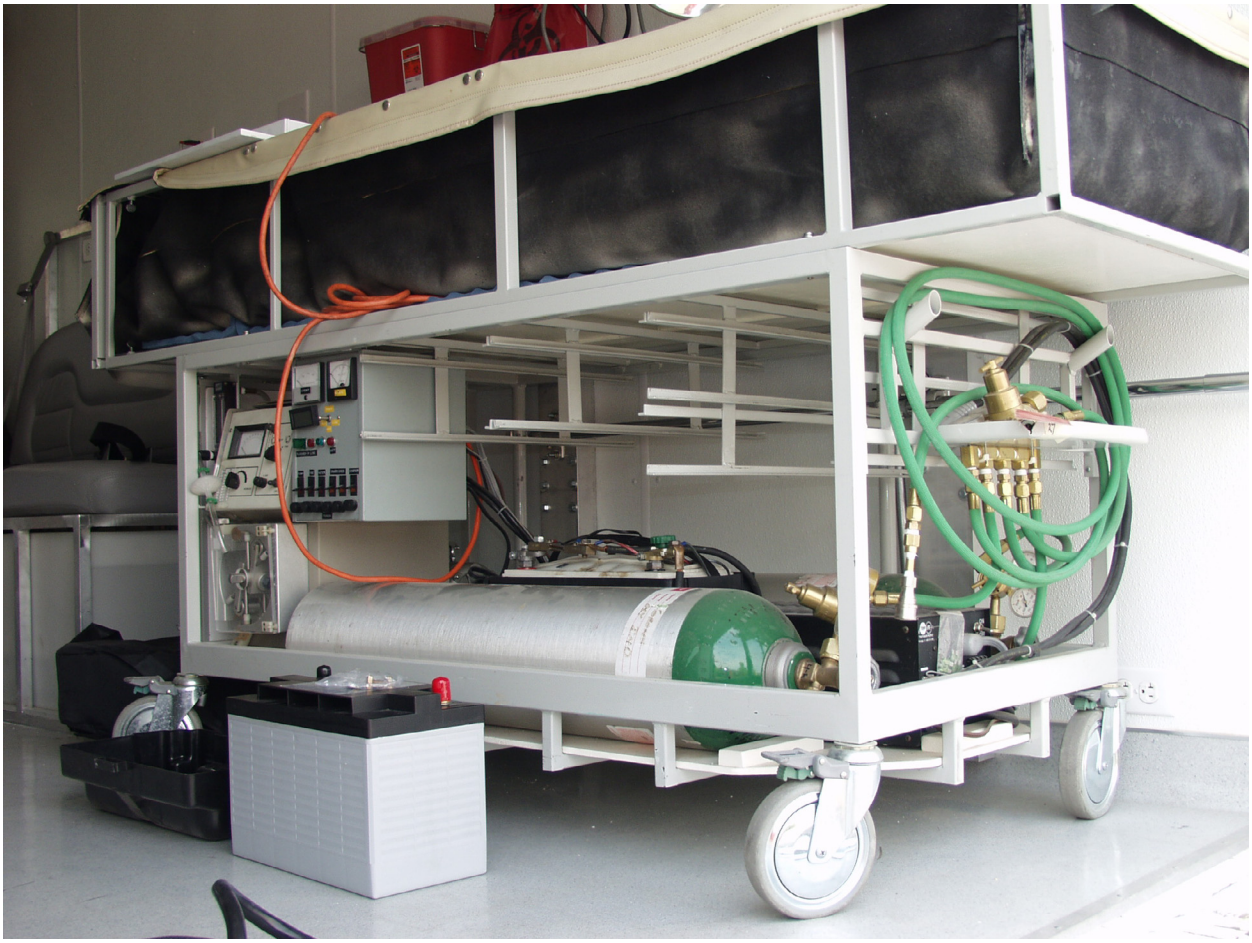
In March 2004, the patient, who lived in Australia at that time, contacted Alcor to inform that his brain cancer had returned. Periodical inquiries regarding his medical condition were made and Alcor was informed that the patient was still receiving treatment. On December 26, 2005, Alcor was informed that the patient was terminal and hospitalized with pneumonia. The patient was receiving palliative care including glucocorticoids to relieve tumor growth induced intracranial pressure. His family requested to have the patient transferred to Arizona but the patient was not well enough to travel.

Once the patient recovered enough to travel, a flight was arranged from Australia to Los Angeles, where he arrived on January 10, accompanied by local medical personnel and his power of attorney. In Los Angeles they were met by an Alcor representative, after which the medical caregivers returned to Australia and the rest flew to Phoenix, Arizona. Although the patient didn't require supplemental oxygen during the trip, the patient was weak, ataxic, and confused.

Because the patient was too ill to arrange for a short-term rental and home hospice at short notice, a hospice was sought close to the Alcor. There was no room available at the Hospice of the Valley that is located less than 5 miles from the Alcor facility so the patient was admitted to a room on the 4th floor at the Hospice of the Valley in Phoenix Baptist Hospital, more than 15 miles from the Alcor facility.

After some transient improvement at the hospice, the patient became increasingly incoherent, and suffered from air hunger. On January 14, the hospital staff notified Alcor that his condition had deteriorated notably and a standby was formally deployed with 24-hour observation of the patient. A fully equipped Alcor vehicle was parked in the main parking lot, close to the ambulance entrance for the emergency room. During this period Alcor ensured that at least three team members were on location, taking turns between being with the patient or staying in the vehicle. Although Alcor was not successful in negotiating to put a central line in place prior to legal pronouncement of death or to use the LUCAS in the patient's room (a typical circumstance), information materials were distributed and a tour of the vehicle was given to hospital staff.

The last 12 hours before pronouncement the patient became unconscious and experienced shallow breathing with oxygen saturations in the low seventies. Stabilization medications were drawn and the standby team members were on high alert. When it became evident that the patient had little time left, the two team members at the bedside called in the remaining team member in the vehicle. At the time of pronouncement, three team members were present with the MARC plus LUCAS, an AMBU compression-decompression cardiopump, airway equipment and adjuncts (i.e., Combitube, endotracheal tubes), the F.A.S.T.1, and ice chests for induction of surface cooling. The medications and CO2SMO were placed on a tray on the MARC and the airway equipment at the head of the MARC.



The MARC.

Stabilization

The patient was pronounced on January 19, 2006, at 08:37. Manual chest compressions were started at 08:39. The first medication, propofol, was given through a peripheral catheter at 08:39 to reduce cerebral metabolism, immediately followed by streptokinase to dissolve existing blood clots, and heparin to prevent new blood clot formation.

After a failed attempt to place the Combitube, a secure airway was established by endotracheal intubation. Positive pressure 100% oxygen ventilations were started at 08:45 using a disposable SUREVENT Emergency Transport Ventilator. Immediately after ventilations were started, the CO2SMO was started to obtain a complete respiratory profile of the patient.

Chest compressions were briefly interrupted to place the F.A.S.T.1 intraosseous infusion device. Ice was placed on the head, neck, groin, axilla, and upper chest to induce hypothermia. With the first three stabilization medications administered, the endotracheal tube and F.A.S.T.1 secured, and ice placed on the patient, the team members left the room to transport the patient to the Alcor vehicle at 08:48. Notes were made on an intermittent basis by one of the team members.

At 08:50 active compression decompression cardiopulmonary support was started using the LUCAS at a rate of 100 compressions per minute, a compression force of 600N, and a compression depth of ~4-5 cm. During transport the antioxidant cocktail VitalOxy was administered using 4x 50 ml syringes. At 08:56 team members exited the hospital with the patient. One team member retrieved the vehicle while the remaining team members stayed with the patient. The patient was loaded into the vehicle using the lift gate.

The transport vehicle.

Cabinets and working space inside the transport vehicle.

Refrigerator and sink inside the transport vehicle.

The MARC secured to the wall inside the transport vehicle (ice machine in rear of photo).

Close-up of ice machine inside the transport vehicle.

In the vehicle, the remaining medications were given using one biphasic IV set with the exception of the osmotic agent Mannitol, which was found to have crystallized. Another stabilization fluid that was not administered is Maalox. The volume expander hydroxyethyl starch was given by pressure infusion. Table 3 shows a complete list of stabilization medications administered. More ice was added to cover the patient. During transport the MARC oxygen cylinders had to be changed for a larger H cylinder. Upon arriving at Alcor at 09:35, a fresh H cylinder was wheeled to the vehicle on a cart because the cylinder inside the vehicle was mounted to the vehicle.

Surgery

As cardiopulmonary support continued, preparations for a cold carotid flush were begun to rapidly reduce the temperature of the brain. The right and left carotid arteries were raised at 09:45 and 09:56, respectively. 18 gauge catheters were placed in both carotids by direct visualization in order to flush the patient with 10 liters of chilled B1 using a Masterflex tubing pump with a manual pressure gauge at ~120 mm Hg.

While the cold flush procedure was being prepared, a right pharyngeal temperature probe was inserted. The first temperature registered read 16.5 degrees Celsius at 09:58. Air bubbles were observed and removed from the cold flush line before connecting it to the patient. After removing the air bubbles, limited flow of B1 was observed through the line. At that time, another team member stopped mechanical cardiopulmonary support.

The cold flush procedure was started at 10:03 but perfusate flowed back from the right carotid artery. At 10:11 attempts to cool down the brain of the patient this way were abandoned and the patient's temperature had drifted upwards to 20.35 degrees Celsius. Nasopharyngeal temperatures were observed to be identical at that time.

At 10:12 ventilation was stopped and the endotracheal tube and CO2SMO were removed. The patient was lifted from the MARC to the operating table where the patient was prepared for cephalic isolation.

The carotid and jugular vessels were clamped on each side of the trachea and the head was surgically severed. Cephalic isolation was completed at 10:16.

At 10:20, incisions were made for creating the left and right burr holes using a scalpel. During this procedure the patient's head was packed in ice. Skin was separated using a retractor to prepare for the drilling of burr holes in the skull using a craniotome. The first attempt to drill the right burr hole was unsuccessful, and the surgeon moved to create the left burr hole, with success. Another attempt was then made to create the right burr hole, this time with success. Burr holes were completed at 10:26.

At 10:30 the head was placed neck-up in the holding ring of the neuroperfusion box and the carotids cannulated with 16 Fr red Robinson catheters. By this time the perfusion circuit was primed and system pressure was 3 psi (backpressure from the HEX-OX and filters), pump speed 52 ml/min, pressure was 5 mmHg, and temperature of the washout perfusate (B1) was 2.9 degrees Celcius. The right nasopharyngeal probe was reinserted at 10:29 and read 20.7 degrees Celsius.

Flow was established at 10:35. The vertebral arteries were seen to backflush, indicating a complete Circle of Willis, and the vertebral arteries were clamped off at 10:38.

Cryoprotective perfusion

Washout of the patient's blood was started at 10:36. Oxygenation was begun at 10:41 at 6 liters per minute. At 10:46 the arterial temperature read 7.38 degrees Celsius, the left jugular read 11.23 degrees Celsius, and the right jugular read 9.8 degrees Celsius. Thermocouple and sampling lines were placed in the jugular veins at 10:41 and connected to the thermocouple and the process refractometers at 10:43. The head was then tilted from vertical to horizontal position to expose the top of the head in order to observe the burr holes. Crackphone sensors were inserted in the left and right burr holes at 10:45.

At 10:56 the cryoprotectant ramp was started in order to gradually increase the concentration of M22 into the patient. The patient's eyes were taped closed at 11:03. Cooling in the cephalon enclosure started at 11:05. 21 minutes into cryoprotective perfusion the pump tubes tangled and the pump stopped. Adjustments were made and within a minute the pump was started again.

A differential vascular resistance check was done by clamping off and then unclamping the left and right carotid artery respectively. After clamping off the left carotid artery the pressure rose to 72 mm. After clamping off the right carotid artery the pressure rose to 140 mm. At 11:55 foaming was identified in the mixing reservoir, with worse foaming observed at 12:17.

At 12:38 ~50% of target concentration necessary to vitrify was achieved and a rapid increase of the vitrification agent was started. The temperature in the cephalic enclosure read 5 degrees Celsius. At 12:39 the 0.2 micron filters were switched. Chiller temperature was set to –5 degrees Celsius at 12:45.

At 13:33 the cryoprotectant ramp was stopped. The cooling of the cephalon enclosure was stopped at 14:14. Cryoprotective perfusion was terminated at 14:15. Depth of the burr hole crackphone wires were adjusted and secured with surgical staples. A left nasal pharyngeal thermocouple was inserted and secured with surgical staples. The "Sullivan Screw" was installed to enable lifting the cephalon. After removing the left and right cannulae, the head was removed from the enclosure and moved to a modified LR40 dewar.

Visual observation of the brain shows extreme shrinkage, one observer calling it "fist sized." Retraction from the skull was greater than 1 inch.

After installing the top on the LR40 and hooking up the temperature probes, cryogenic cool down was started at 14:38. When a temperature of –110 degrees Celsius was reached, the cooling rate was reduced to minimize fracturing. The crackphone was started at 22:50 in the evening. After reaching temperatures close to –196 degrees Celsius, the head was immersed for the final temperature drop. After reaching liquid nitrogen temperatures, the cephalon was moved to a Bigfoot dewar for long term care.

The computers used to control cryogenic cool down.

Discussion

The quality of a cryonics case can be evaluated from at least two distinct perspectives. One perspective is to establish how well the objectives of stabilization and cryopreservation were achieved in a particular case. This information is important to guide research and assist future resuscitation attempts. An obvious limitation of this approach is that an evaluation like this fails to distinguish between the performance of a cryonics organization and the "fixed" constraints within which a cryonics organization must operate. For example, everything else the same, a patient who is pronounced close to a cryonics facility will benefit from a shorter (cold) ischemic period, which in turn may facilitate better cryoprotectant perfusion. Clearly, for quality assurance purposes, the question is what could have been *reasonably* expected from a cryonics organization taking into account the constraints the cryonics organization had to work with.

In this case the patient greatly benefited from relocating to a hospice close to the facility. Considering the fact that Alcor was informed well in advance about this member, a reasonable question is whether a hospice even closer to the facility, let alone a home hospice, could have been secured in collaboration with the patient or his medical representatives at an earlier stage of preparation, which in turn raises the more general question of when preparations for the case began. Clearly, this is ultimately an issue between the cryonics organization and the member or his representatives.

The distance between the Alcor facility and this specific hospice raises the question of whether the patient could have benefited from rapid surgery, washout, and blood substitution *in the vehicle* prior to transport to the facility. Considering the superior cooling rates that can be achieved by using intravascular cooling instead, or in conjunction with, external cooling, the answer to this questions seems to be affirmative *in theory*. The practical question, however, is how well prepared and skilled a cryonics organization should be to allow for slightly longer transport times if the trade-off will be in favor of more rapid cooling during the earlier stages of stabilization.

One major advantage of the recent progress cryonics organizations have made in developing liquid ventilation for field use is that the advantages of rapid cooling after pronouncement can be secured without risking time-consuming surgical complications and other delays in transport time. Liquid ventilation not only eliminates the need for surgery to achieve rapid core cooling, the procedure itself is compatible with driving the vehicle, a feature that doesn't necessarily apply to perfusion. For example, the sloshing of the washout solution in the reservoir during driving will present an additional challenge for the perfusionist and may consistently trigger the low- and high-volume alarms in the perfusion circuit.

There has been considerable debate in cryonics what the "ideal" number of team members should be during standby and stabilization. This question will not be addressed in great detail in this article but some general guidelines seem reasonable:

(1) If a standby is performed in shifts, the total number of team members should be bigger than the number required for a case to accommodate a scenario where one or more of the team members are not available or too exhausted or stressed to participate.

(2) The total number of team members during a case should be able to perform the most fundamental interventions (cardiopulmonary support, induction of hypothermia, and medications) in *parallel* instead of in sequence.

(3) There should be at least one team member who has a *global* view of the case. Typically this is the scribe who is assigned to note taking. It may also be desirable if the *team leader* is not too involved in the case to ensure that interventions are adequately performed and properly prioritized.

(4) If more sophisticated monitoring modalities are considered, the number of team members should be increased accordingly. Although one can argue that interventions are far more important than collecting data *about* them, one of the most interesting things to know during stabilization is how a patient responds to interventions compared to a baseline that was established immediately after pronouncement.

In the present case, the team was hard pressed to start all interventions in parallel, let alone to assign a specific individual as a full time scribe or hands-off team leader. The problem of inadequate data collection in this case was further aggravated by the lack of formal stabilization data collection sheets and the absence of voice recordings.

Good data collection sheets not only improve the quantity and quality of data collected during a case, but also serve as a checklist to assist deployment of equipment prior to legal pronouncement and to alert the scribe to errors and omissions during a case. Especially during

the first 10 minutes of stabilization, many interventions happen simultaneously. It is a non-trivial challenge to design a set of practical data collection sheets that will enable team members to document all these procedures comprehensively. Alcor is currently working on comprehensive data collection sheets for all parts of the procedures.

The challenge of documenting all the aspects of case is one reason why some cases have benefited greatly from the use of time stamped voice recorders. If these voice recorders are used with clip on microphones, team members can perform procedures and record them at the same time. If permitted, voice recorders can further improve the quantity and quality of data collection, fill in gaps and complement written notes, and help to generate an accurate and consistent timeline of events.

One minor problem concerning preparation for this case was availability of enough pressurized oxygen cylinders in the vehicle. Because the expected running time of the LUCAS and respirator could have been conservatively estimated in this case, an ample supply of oxygen should have been made available during preparation for this case. Because the hospice was not unusually far from the Alcor facility, it's not clear why the vehicle was not equipped to run these devices for at least such a period of time.

This case benefited greatly from rapid intervention after legal pronouncement of death. Starting vigorous chest compressions is a multi-faceted intervention because without it the other two treatment modalities are impossible (medication circulation) or less effective (induction of hypothermia). In this case, mechanical cardiopulmonary support was not allowed in the hospital, which raises the question of whether (intermittent) manual chest compressions should be used as a bridge to mechanical cardiopulmonary support or whether every effort should be made to get the patient as quickly as possible to a location where uninterrupted mechanical chest compressions are allowed.

The Combitube takes advantage of the fact that the most "natural" angle to place an airway is the esophagus. It is therefore the obvious choice for team members without extensive experience in endotracheal intubation. Sometimes the Combitube is used as backup when endotracheal intubation fails. In recent cases, the Combitube has been the default choice. Because the Combitube is a dual-lumen tube, a fluid such as Maalox can be administered through the non-airway tube. This has a clear advantage over placing an endotracheal tube *and* a gastric tube. A major risk, however, is that Maalox will be poured down the lungs in the event the Combitube is unknowingly placed in the trachea. In this case placement of the Combitube failed and an endotracheal tube needed to be placed. Despite the advantages of Maalox administration through the Combitube, more thought needs to be given to using the Combitube as the default choice.

Vasoactive medications are an essential part of cardiopulmonary support and should be given immediately after start of chest compressions to support blood pressure. If chest compressions have started but administration of all the stabilization medications needs to be completed at a later time, at least one vasoactive medication should be included with the medications that are administered prior to transporting the patient. A vasoactive medication like epinephrine is supplied as a 30 ml vial because this medication has a short half life and intermittent administration of repeated doses is desirable during prolonged cardiopulmonary support.

Because intracranial pressure is a common diagnosis in terminal brain cancer patients, it was very unfortunate that Mannitol, an osmotic fluid that is routinely given to treat intracranial

pressure, was not administered because it was found to have crystallized. Mannitol comes out of solution when refrigerated. For this reason, Mannitol is an important exception to the rule that medications need to be refrigerated or chilled in ice before administration. To remedy this situation, Alcor supplies warming pads with their medication kits to dissolve the crystals.

Pauses between administration of the medications are recommended in situations where chest compressions have not been started or circulation is poor and intermittent. If (mechanical) chest compressions are given without interruption, no pauses are required between the medications.

Although the medications are displayed as a chronological list, the large volume fluids do not need to be delayed until all the small volume medications have been administered. If circumstances permit, the large volume bags and bottles can be hung on the IV pole of the portable ice bath to facilitate rapid priming of the lines and administration. As soon as IV access is obtained the drips can be started and the small medications can be injected through the injection port in the line or a series of stopcocks. In case the patient is extremely dehydrated, or has died from hypovolemic shock, rapid infusion of volume expanders is critical to secure adequate mean arterial pressure.

Placement of the F.A.S.T.1 was smooth and successful and validates the recent transition from peripheral IV access to sternal intraosseous infusion. Because large volumes can be infused through the F.A.S.T.1, placement should be considered even if there is already a small bore peripheral IV in place. An additional advantage is that the peripheral catheter can be used for phlebotomy or administration of medications that are not compatible such as epinephrine and THAM. Notwithstanding the increasing popularity of intraosseous infusion in emergency medicine, some concerns have been raised about using this technology in the context of an aggressive vasopressor protocol. More detailed study how vasoconstriction affects blood flow to (peripheral) bone marrow is desirable.

A related concern is the placement of the sensor for oxygen saturation (SpO2) of the blood. In this case the CO2SMO sensor was placed on the left index finger. One potential limitation of pulse oximetry during stabilization is that hypothermia and vasopressor-induced peripheral vasoconstriction limits blood flow to the location of the sensor and can produce unreliable oxygen saturation measurements.

A more serious concern is generating adequate cerebral perfusion in patients whose pathogenesis includes increased cranial pressure. If a patient's systemic blood pressure is insufficient to counter intracranial pressure, the brain cannot be perfused with oxygen and nutrients, cerebroprotective medications cannot be delivered to the right areas, and the cooling rate of the brain is reduced. Although relatively high oxygen saturation levels were recorded in the case, it is not clear how well the brain was being oxygenated.

One solution, which would also address the limitations of peripheral pulse oximetry, would be to take routine cerebral oximetry measurements in cryonics patients. Global cerebral oxygen saturation can be measured by a method called jugular venous bulb oximetry, in which a catheter is placed in the internal jugular vein towards the base of the brain to measure mixed venous cerebral oxygenation. Although this is a reliable method to look at trends in cerebral oxygenation, it is too invasive for a typical cryonics context.

It still might be worthwhile to do a renewed study into non-invasive cerebral oximetry monitoring. One FDA approved device that is available for non-invasive monitoring of regional cerebral oxygenation is the INVOS cerebral oximeter. The INVOS cerebral oximeter operates by the same principles as pulse oximetry by using infrared light to determine oxygen saturation levels. The sensors can be conveniently placed on both sides of the patient's forehead.

One limitation of the INVOS is that this technology can only look at oxygen saturations in the cerebral cortex. This may limit its use in situations such as brain tumors and reperfusion after cardiac arrest in which regional cerebral blood flow variations can be expected. And like pulse oximetry, it does only look at oxygen saturation of hemoglobin without adjusting this for the absolute number of red blood cells in the patient's blood.

These limitations notwithstanding, a device like the INVOS can provide team members with pertinent data about the organ cryonics is most concerned with: the brain. Opinions about non-invasive cerebral oximetry have ranged from it being a life saving piece of equipment in extracorporeal perfusion to nothing more than a random number generator in a critical care context. Additional research into cerebral oximetry in cryonics is desirable.

Considering the fact that this patient benefited from rapid intervention and a relatively short stabilization time, it is unfortunate that the i-STAT was not used to do "bedside" blood gas analysis during stabilization. The objective of stabilization in cryonics is to keep the patient's brain viable by contemporary medical criteria. The question whether this objective was achieved cannot be answered by only checking off a list of interventions that were done during stabilization. For landmark cases, such as the Arlene Frances Fried and James Galagher cryopreservations, additional blood gas measurements were available to answer this question.

Although the i-STAT itself is relatively easy to use, routine use of the I-STAT will require a practical protocol for venous access, phlebotomy technique, and on-site evaluation of measurements and intervention options. Routine use of this equipment during stabilization will also require a re-evaluation of the number of individuals needed for a stabilization because intermittent collection and on-site analysis of blood is not compatible with many other tasks.

Rapid cooling of the brain is a very important intervention to mitigate injury during cerebral ischemia. In this light it is understandable that an attempt was made in the operating room to increase selective cooling of the brain. Flushing the carotid arteries or jugular veins in the absence of circulation can achieve better cerebral cooling rates than systemic or focal external cooling.

In this case the attempt was counterproductive because the procedure had not only never been practiced or validated before, the 18 gauge catheter that was used was too small in diameter as well. As a result, the opposite was achieved of what was intended: the patient's brain temperature drifted upwards again instead of going down. This incident emphasizes the risk of introducing new procedures during a real case.

In this case the question can be asked if this intervention would have conferred much additional benefit even if it were successfully implemented. Because the patient was a neuropatient, prompt cephalic isolation and circulation of the chilled washout solution through the brain might have achieved equally potent results without the challenges associated with a carotid or jugular flush.

When cyclic lung lavage becomes available in cryonics, the need for such interventions may become even more redundant.

Isolated head perfusion offers several advantages. The most obvious advantage is that cephalic isolation prior to cryoprotective perfusion reduces the time between start of surgery and start of washout and cryoprotectant perfusion. This is especially beneficial in cases where the patient presents at relatively high core temperatures. A related advantage is that only cannulating the brain does not require the additional step of clamping off the descending aorta and the extremities.

Because most of the cross-section of the stump is available for venous drainage, isolated head perfusion should present fewer pressure related complications during perfusion. Typically central venous pressure tends to rise during the final stages of cryoprotectant perfusion, shunting a portion of the perfusate through the (normally) higher resistance bridging veins. As a consequence, less burr-hole drainage and facial edema has been observed in isolated head perfusion.

Preparation of organ preservation solutions and cryoprotectant agents is generally done by the cryonics organizations themselves. Changing formulations and concentrations of perfusate may further complicate matters. The carrier solution of M22, B1, should contain 1.0 mM of calcium chloride and 2.0 mM of magnesium chloride. Because calcium chloride and magnesium chloride are hygroscopic, these components are typically obtained in a hydrated form or as liquid medical parentals for injection.

The formula used during this case, however, only contained 0.25 mM of calcium chloride and 1 mM of magnesium chloride. If 10% dihydrate calcium chloride and 20% hexahydrate magnesium chloride for injection are used in the right molar concentrations, the correct volumes should be 1.47 ml/L and 2.035 ml/L respectively. As a result, the amounts of calcium chloride and magnesium chloride in the carrier solution were much lower than should have been in this case.

Because B1 is a cryonics modification of the published carrier solution of M22 (LM5 + proprietary additive), the osmolality for B1 is currently unknown. This not only restricts the number of important quality controls that can be done on the perfusate, there is the additional risk that the solution is prepared in undesirable osmolality. In light of the lower concentrations of calcium chloride and magnesium chloride, there is a risk of introducing a hypo-osmolar carrier solution which could aggravate ischemia-induced cerebral edema during the early stages of washout and perfusion.

Concerns have been raised about the composition of M22 and its limited ability to cross the blood brain barrier (BBB). It is believed that none of the polymers in M22, such as the two ice blockers and polyvinylpyrrolidone K12, cross the BBB. This raises the question of how M22 works as a vitrification agent inside the brain. It is currently believed that the polymers in M22 contribute to vitrification of the brain by dehydration. M22 draws water outside of the BBB into the vascular space and raises the concentration of salts and proteins in brain tissue as result. The increased concentration of salts and proteins and the permeable cryoprotectants in turn prevent ice formation.

It is important to stress that this mechanism is not necessarily active in all human cryopreservation patients. For example, some patients have a compromised BBB as a result of ischemic injury. As a consequence the polymers in M22 cannot just be substituted for inexpensive polymers to induce hydration. Another reason why this cannot be done is because these components are necessary to prevent ice formation in the blood vessels.

An obvious concern about the mechanisms by which M22 confers vitrification of the brain is that the mechanisms by which it prevents ice formation are exactly the mechanism that have traditionally been put forward as one explanation why extracellular freezing is undesirable; a toxic concentration of salts. Which raises the question of whether the reduced toxicity benefits of M22 as observed in kidneys is equally available to human cryopreservation patients.

A related concern is the significant amount of brain shrinking observed in vitrification cases, and this case in particular. Historically, Alcor has used a cryoprotective "ramp" in perfusing patients with glycerol to avoid osmotic injury associated with the limited permeability of glycerol. Because water leaves the cells around 1000 times as fast as glycerol diffuses into the cells, initial high concentrations of glycerol can produce significant cellular injury. Although the use of a cryoprotective ramp is effective to mitigate this problem, cold ischemia and toxicity do not allow for time-consuming careful equilibration of brain tissue.

This raises the question of how much brain shrinking is compatible with good structural preservation and viability of the brain. To answer this question it is useful to look at the literature about clinical mannitol-induced shrinking of the brain and the molecular biology of volume change induced apoptosis.

In a canine study that compared single and multiple mannitol infusions ranging from clinical to "massive," a massive single infusion of mannitol produced a brain volume decrease from 98% percent of cranial capacity to 77%, resulting in death of the dogs, possibly from cerebral dehydration[1].

Is the (extreme) brain shrinking and dehydration as observed in this patient compatible with viability of the brain? One of the upstream events in apoptosis is normotonic cell shrinkage, or 'apoptotic cell volume decrease' (AVD)[2]. Recent research indicates that cell shrinkage may not only be a part of the apoptotic program, but that sustained cell shrinkage *per se* may be a sufficient condition for apoptosis in certain cell types[3,4,5].

Because completion of apoptosis requires energy, DNA transcription and protein synthesis, brain shrinkage-induced apoptosis should not be a serious concern during cryoprotection. Cryoprotection generally happens at (ultra) profound hypothermia, or during the final stage, at subzero temperatures. Even if the patient is being oxygenated during a part of this procedure, the significant reduction in metabolic rate should protect the patient from controlled cell death.

A more practical concern is how cryoprotection induced cell volume changes can damage the cell and cell contents. For example, if a portion of the water in the brain is osmotically inactive because it is bound to protein surfaces what is the effect of having a large osmotic gradient between the intracellular and extracellular space? Aside from rapid water efflux, dehydration, intracellular solute concentration, and increased proximity of intracellular proteins, there may be a maximum volume reduction that cells can sustain beyond which the interaction between the shrunken cell membrane and cytoskeleton will damage the cell.

A related concern is how extreme brain shrinkage will affect optimal distribution of the vitrification agent. What is the effect of brain shrinking on the hemisphere and layer connecting bridges, inter-endothelial junctions, and intra-cellular connections in the brain? Even allowing for a hypothetical zero toxicity, the presence of the non-penetrating polymers in M22 limit the degree to which volume for volume equilibration can be achieved in the brain.

The issue of dehydration raises a more general issue about vitrification agents in human cryopreservation. Are vitrification agents that are optimal for healthy (non-ischemic) brains optimal for ischemic brains as well? Does (ramped) cryoprotective perfusion reverse ischemia-induced perfusion impairment of the brain? Can the carrier solution be modified to improve cryoprotective perfusion of ischemic patients? Should ramping be more aggressive in ischemic / edematous patients? And, last but not least, what methods are available to cryonics organizations to determine whether a patient's brain has resisted ice formation after cryogenic cooldown? These questions indicate a number of valuable research directions for existing cryonics organizations and associated laboratories.

Appendix A: Medications administered during agonal phase

	01/10	01/11	01/12	01/13	01/14	01/15	01/16	01/17	01/18
Dilantin 300 mg PO QHS	2100	2100	2100						
Dilantin 400 mg PO QHS				2100	2100	D/C			
Advair Diskus 250/50 2 puffs BID	2100	0900 2100	0900 2100	0900 2100	0900 2100	0900 D/C			
Augmentin 875 mg PO BID	2100	0900 2100	0900 2100	0900 2100	0900 2100	0900 D/C			
Coenzyme Q10 1 tab PO QD		0900	0900	0900	0900	0900 D/C			
Decadron 4 mg PO TID	2200	0600 1400 2200	0600 1400 2200	0600 1400 2200	0600 1400 2200	0600 D/C			
Depakote 500 mg PO BID	2100	0900 2100	0900 2100	0900 2100	0900 2100	0900 D/C			
Thiamine 100 mg PO QD		0900	0900	0900	0900	0900 D/C			
Dulcolax supp. 1 PR QD PRN					0400 0800				
Sorbitol 30 cc PO QOD-BID PRN					1310				
Colace 100 mg PO QD					0900	0900 D/C			
Lorazepam 1 mg PO PRN					1945	1200 1545	2300		0245 0800
Prochlorperazine 25 mg PR 8 hr PRN					0350 1515				
MSIR 5 mg PO Q 1hr PRN									0800
Glycopyrrolate 0.4 mg IM Q 4hr PRN									0935

Appendix B: Vital signs taken during agonal phase

	01/11	**01/12**	**01/13**	**01/14**	**01/14**	**01/14**	**01/14**
Time	0900	0900	0900	0900	1630	1800	2200
BP	113/79	103/65	130/77	99/60	--	108/71	--
Heart Rate	86	69	69	90	122	--	124
Resp. Rate	16	16	15	20	--	--	--
Temperature	97.9°F	97.2°F	98.5°F	97.0°F	--	--	--
SaO_2	97%	96%	93%	98%	68%	--	72%

	01/15	**01/15**	**01/16**	**01/17**	**01/18**	**01/18**	**01/19**
Time	0900	1830	0900	0900	0900	1200	0745
BP	95/68	115/78	111/76	114/82	123/81	118/83	--
Heart Rate	106	105	104	122	117	125	--
Resp. Rate	23	32	16	28	24	18	--
Temperature	97.3°F	98.1°F	96.2°F	96.3°F	98.0°F	--	101.4°F
SaO_2	74%	75%	75%	96%*	73%	65%	84%

*An anomalous oxygen saturation reading.

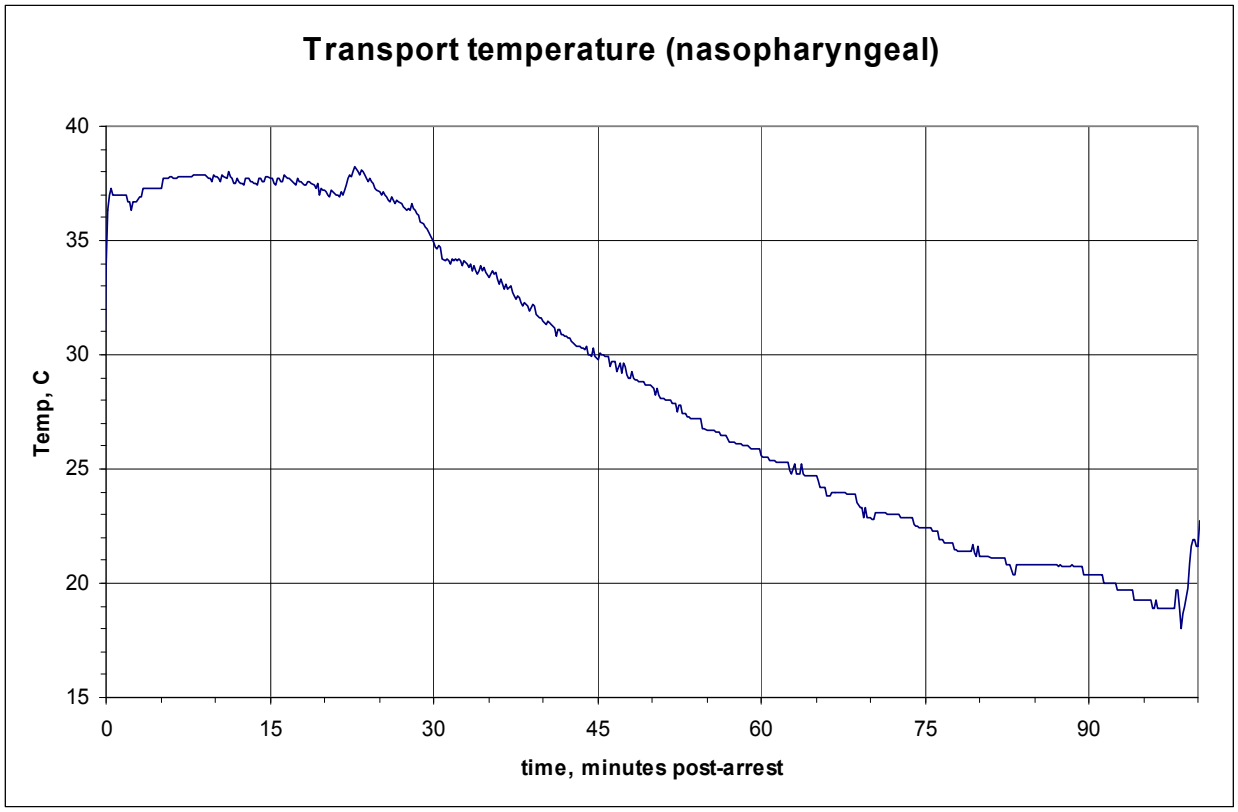
Appendix C: Detailed timeline, A-1097, January 19, 2006

08:37 Patient pronounced
08:39 Start of manual CPS
08:39 Administration of propofol
08:40 Administration of heparin
08:40 Administration of streptokinase
08:40 Placement of endotracheal tube
08:45 Start of ventilation (Surevent, 100% oxygen)
08:45 Placement of sternal intraosseous infusing device (F.A.S.T. 1)
08:45 Start of CO2SMO
08:48 Team leaves the room
08:50 Start of mechanical CPS (Lucas)
08:56 Exit from hospital
08:56 Administration of hetastarch by pressure infusion
09:35 Patient arrives at Alcor
09:45 Right carotid artery raised
09:55 Start of "cold flush" preparation
09:56 Both carotids raised
09:58 Bubble in flush line
09:58 Right pharyngeal temperature probe inserted
09:58 Nasal temperature 16.5C
09:58 Attempt to flush bubbles out of cold flush line
10:00 Limited flow started
10:00 Stop mechanical CPS (Lucas)
10:01 Insertion of needle for cold flush into right carotid
10:02 Nasal temperature 19.0C
10:03 Perfusate is flowing back out of veins
10:05 Infused patient with 1 liter of perfusate
10:08 Nasal temperature is 20.35C
10:10 Pharyngeal and tympanic temperature readings are identical
10:11 Cold flush attempt abandoned
10:12 Thumper, perfusate, monitoring equipment, and ice bath removed
10:12 Ventilation stopped.
10:12 Endotracheal tube removed
10:14 Cephalic isolation
10:16 Remaining tissue severed
10:20 Cephalic enclosure ready
10:20 Incisions made for burr holes
10:20 Skin separated
10:20 Ice around the patient's head
10:21 Start drilling right burr hole (doesn't go through)
10:23 Left burr hole drilled
10:25 Second right burr hole drilled
10:26 Perfusion circuit: system pressure 3 psi, pump speed 52 (circuit open).
Pressure 5 ml, temperature 2.9C
10:29 Right nasopharyngeal reinserted
10:20 Nasopharyngeal temperature 20.7C
10:30 Cephalon moved to enclosure

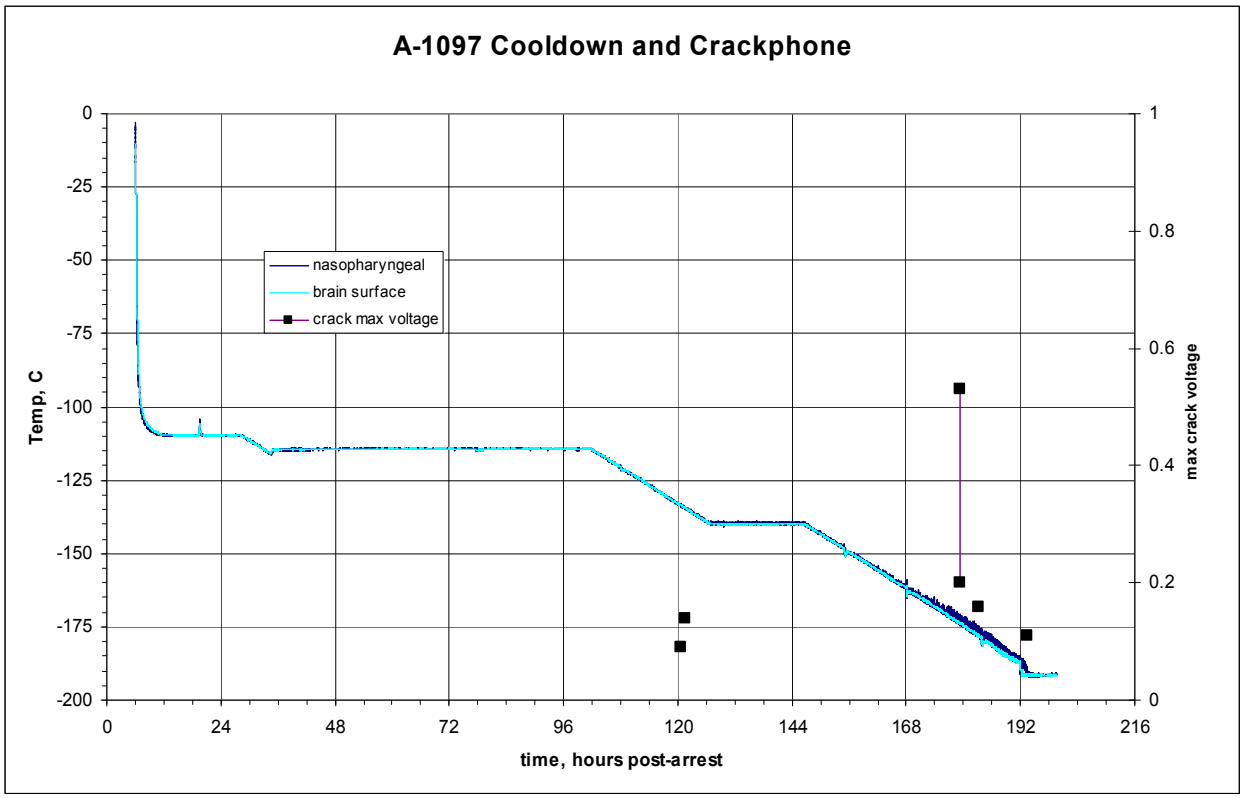
10:30 Cephalon clamped in
10:31 Cephalon inverted in clamps
10:33 Tube inserted in right jugular and tied
10:36 Tube inserted in left jugular and tied
10:36 Start washout
10:39 First sample of right jugular
10:41 Start oxygenating of perfusate at 6L/min
10:43 Left jugular temperature 11.22C
10:44 Reservoir refilled
10:44 Start of cryoprotective ramp
10:46 Arterial temperature 7.38C. Left jugular temperature 11.23C.
Right jugular temperature: 9.8.
10:56 Start of closed circuit cryoprotection
10:51 Rotating head parallel to the floor (face up)
10:51 Clamping off bypass.
10:53 Blue crackphone attached to LEFT burr hole
10:55 Green crackphone attached RIGHT burr hole
10:55 Adding concentrate to mixing reservoir
10:56 Start closed circuit perfusion
10:58 Left thermo couple inserted
11:03 Patient's eyes taped
11:05 Start cooling in patient enclosure
11:17 Pump tubes tangle. Pump stops
11:18 Pump starts again
11:21 Differential vascular resistance check started
11:22 Clamping off right carotid artery. Pressure rises to 140mm
11:23 Opening up right carotid artery again.
11:24 Closing left carotid artery. Pressure rises to 72mm.
11:25 Opening up left carotid artery again.
11:48 Right jugular sample
11:52 Left jugular sample
11:55 Foaming identified in mixing reservoir
12:17 Foaming worsening
12:23 Arterial sample
12:29 Right jugular sample
12:33 Left jugular sample
12:37 Arterial sample
12:38 Cryoprotectant concentration at ~50%
12:38 Rapid increase of cryoprotectant concentration
12:38 Patient enclosure temperature -5C
12:39 Switching filters
12:45 Chiller temperature set to -5C
12:45 Left jugular sample
12:47 Right jugular sample
13:00 Left jugular sample
13:05 Vascular resistance test
13:05 Ramp shut off
13:07 Closing off right side
13:08 Opening right side again

13:09 Clamping off left side
13:01 Opening left side again
13:11 Repeating vascular resistance test
13:12 Closing off right side
13:12 Opening right side again
13:12 Ramp on again
13:15 Right jugular sample
13:19 Right jugular sample
13:29 Arterial sample
13:30 Right jugular sample
13:33 Ramp stopped. End of cryoprotectant concentration increases.
13:46 Left jugular sample
13:48 Right jugular sample
13:50 Arterial sample
14:00 Left jugular sample
14:02 Arterial sample
14:14 Left jugular sample
14:14 Cooling patient enclosure stopped
14:15 Right jugular sample
14:15 End of cryoprotective perfusion
14:15 Depth of right burr hole crackphone adjusted
14:16 Depth of right burr hole crackphone adjusted
14:20 Adjusting and securing crackphone wires with surgical staples
14:21 Visual observation of the brain: extremely shrunken (fist sized).
Retraction greater than 1 inch.
14:22 Left nasal pharyngeal thermocouple inserted and secured with surgical stapler
14:26 “Sullivan screw” installed
14:29 Removal of left and right jugular tubes
14:30 Head removed from enclosure to intermediate cool down container
14:34 Hooking up temperature probes
14:35 Top installed on intermediary cooling container
14:38 Start of cooldown to -110C
22:50 Start of crackphone recordings

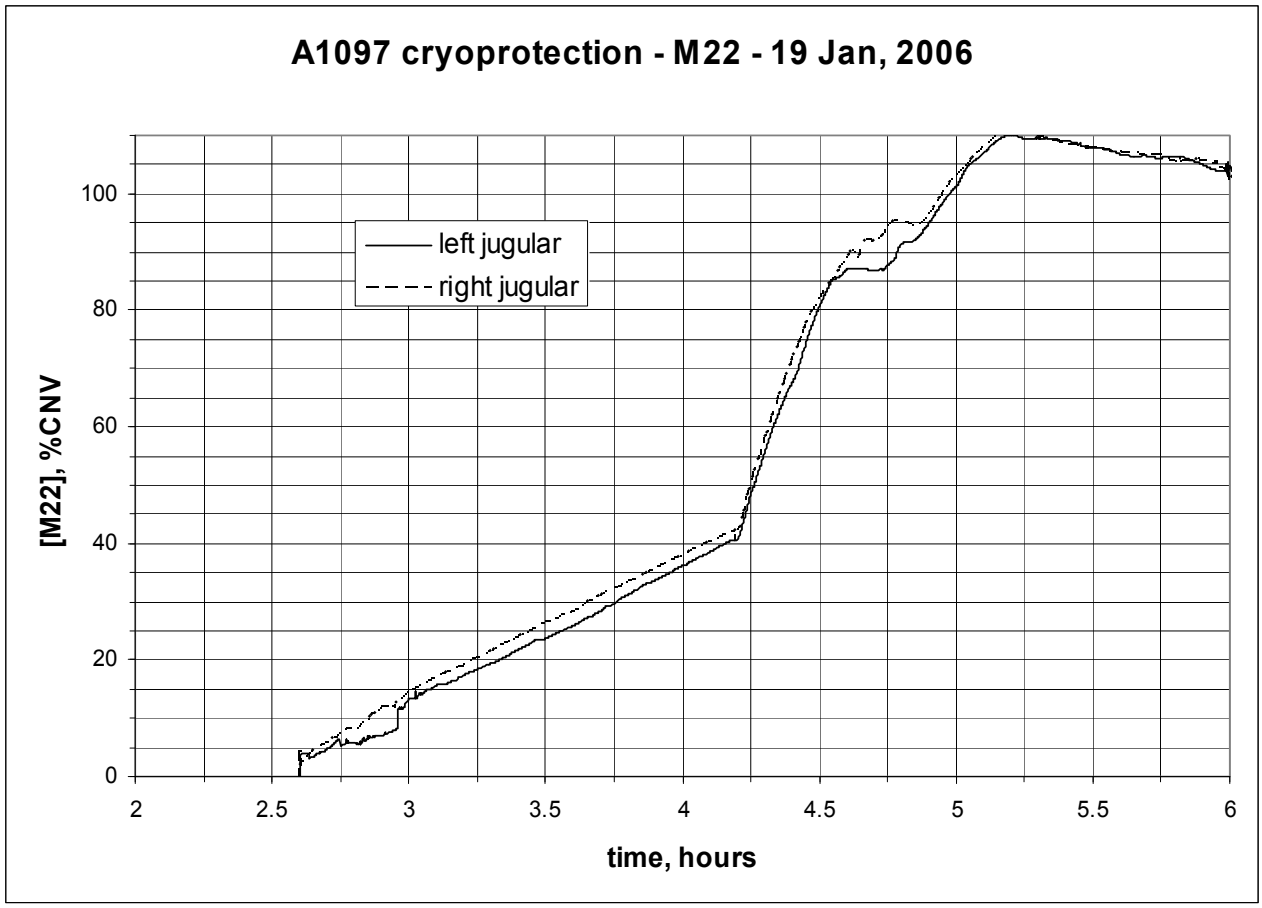
Appendix D: Transport temperature (nasopharyngeal)



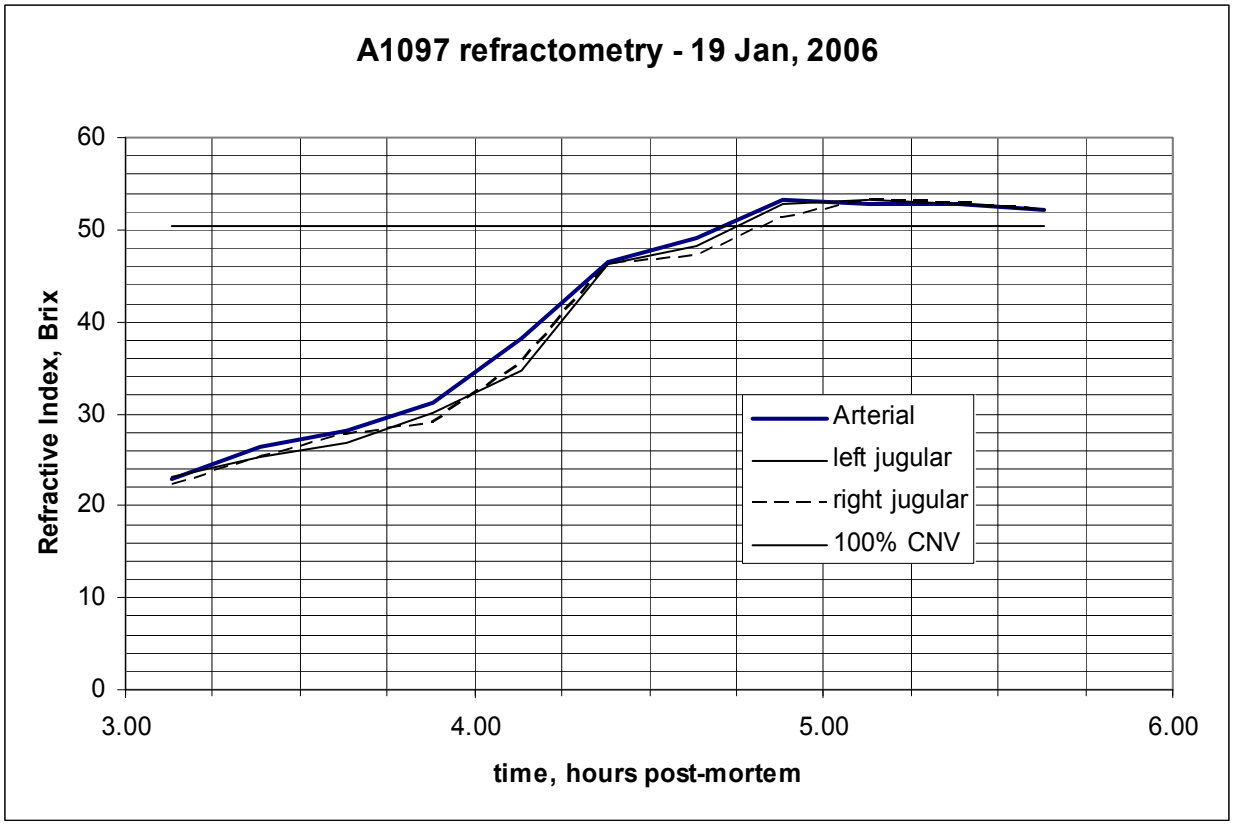
Appendix E: Cooldown temperature and cracking events registered by crackphone



Appendix F: Concentration of M22 during perfusion



Appendix G: Refractometry data



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