CRYONICS

2nd Quarter 2023 | Vol 44, Issue 2 www.alcor.org

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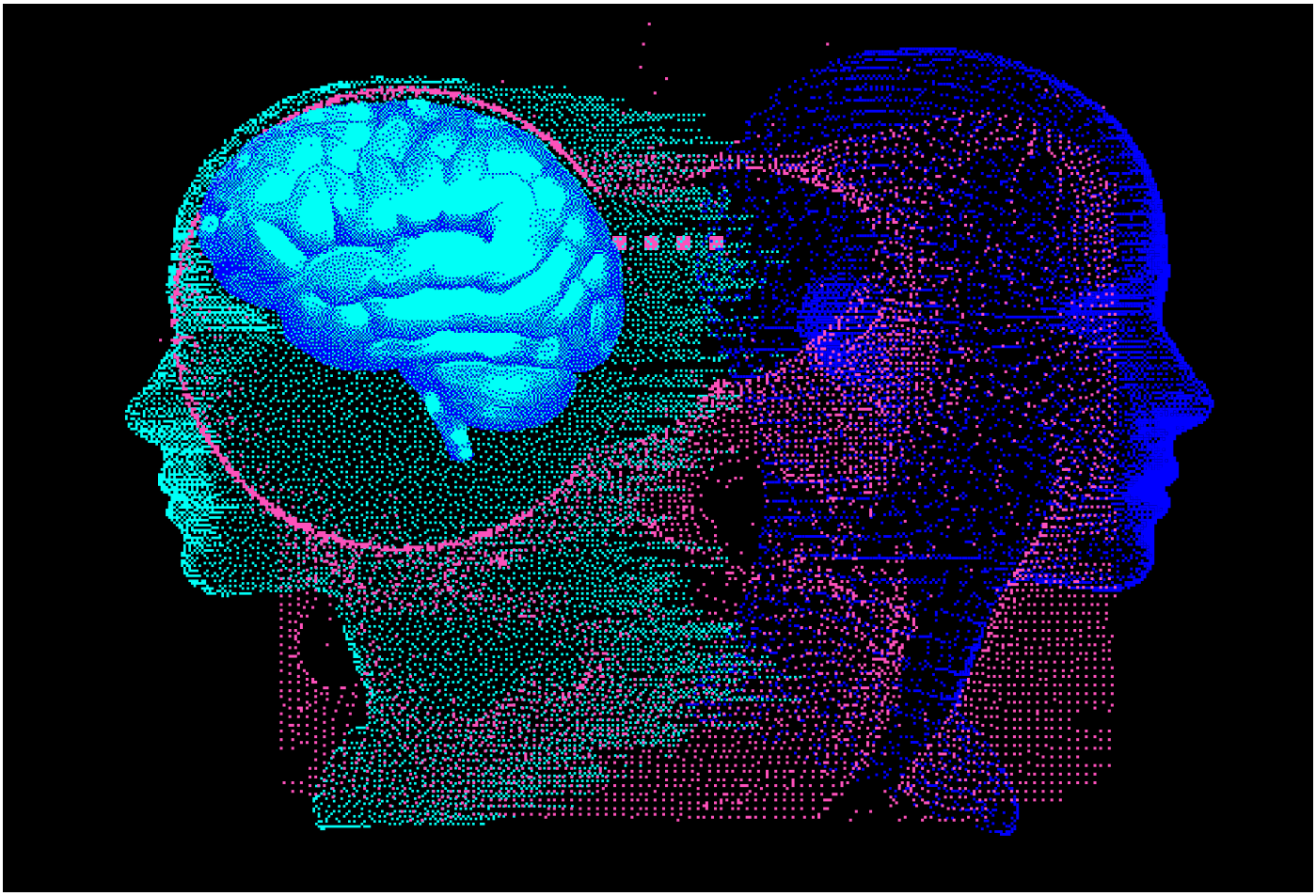

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Cryonics magazine is published quarterly.

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ISSN: 1054-4305

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External Review of Cryonics Organizations. What are the Options?

By Paul S. Beighley, MD

Why External Review is Needed

It takes little effort when people search the term "cryonics" on the internet to find websites that assert it is a practice founded on quackery or outright fraud. Proponents of cryonics find such opinions offered by critics as characterized by undisguised hostility and outright mischaracterizations. One example is Dr. Tom Hartsfield, a physicist, whose article in Big Think opines that cryonics is "... a grim practice with ghoulish results; at least it makes for some fascinating stories and a bit of dark humor."[1] The Wikipedia entry for cryonics, at the time this article was written, asserts "Cryonics is regarded with skepticism within the mainstream scientific community. It is generally viewed as a pseudoscience, and its practice has been characterized as quackery."[2] Quackwatch, in an article by Stephen Barrett, M.D. references the founder of Skeptic Magazine who infamously (and inaccurately) wrote "To see the flaw in this system, thaw out a can of frozen strawberries. During freezing, the water within each cell expands, crystallizes, and ruptures the cell membranes. When defrosted, all the intracellular goo oozes out, turning your strawberries into runny mush. This is your brain on cryonics."[3] Quackwatch also repeats an assertion by National Council Against Health Fraud president William Jarvis, Ph.D, again which is seen as misleading by the cryonics community, that "Cryonic technology has not been demonstrated to work in laboratory animals. Even if the rest of a person's body could be revived after hundreds of years, the brain could not. Brain cells deteriorate within minutes after death, and any still viable when the body is frozen would be burst by the freezing process. Cryonics might be a suitable subject for scientific research but marketing an unproven method to the public is quackery."[4] Such views have been challenged as not a consensus opinion, misleading and not supported by available research.[5] But despite efforts by informed advocates of cryonics to provide accurate information to counter these narratives, the characterization of cryonics as eccentric wishful thinking at best, and fraud at worst, seems to be shared by many in the fields of medicine, science and academia. The aversion by cryobiologists to openly engage in research for development of technologies specifically designed to advance the goals of cryonics is a well-known and frequently discussed phenomena amongst cryonics advocates.

The credibility issues that cryonics proponents face arise for many reasons not just based on objections that the practice is unsound from a technical standpoint, such as a belief that cryonics is a procedure only available to the wealthy. Distrust might also stem from a lack of established institutional norms and practices that would invite external and transparent reviews, comparable to those employed by mainstream academic, industrial, or medical organizations. There are numerous approaches used by mainstream medical systems, businesses and industry informing how external review could be accomplished and few compelling reasons such scrutiny be avoided. The primary arguments against external reviews by cryonics organizations seem to fall in the following categories: lack of subject matter experts competent to conduct such a review, such a review being intrusive and requiring disclosure of policies and procedures internal to the organization, lack of perceived benefit, desire to retain autonomy and control by cryonic organization leadership, desire to avoid developing external accountability for practices outside of direct organizational control, and financial and administrative costs.

While attempts to promote external review of cryonics organizations are likely to meet with resistance because of the reasons offered above, there are clear benefits in such review that merit strong consideration. These would include: increasing confidence by membership and governing boards, opportunities to bring in new ideas that might assist the organizations in better practices, finding areas for improvement, improving the credibility of cryonics among external critics and helping forestall government regulation of an "unregulated industry" that is not conducting adequate industry supported efforts and perceived as possibly exploitative.[6] Such audits and reviews need not be confined to the evolving technical methods of cryonics organizations, although review processes that address these technical processes from a quality management standpoint are likely to be useful. Audits can look at underlying financial models and assumptions, use of appropriate internal quality standards, hiring practices, and conformance to regulations governing business practices.

Accreditation as a Solution

One approach that might be applicable to the development of an external review process comes from established norms used by institutions providing medical services that invite review through accreditation. As cryonics organizations refer to members in stasis as "patients," adapting approaches employed by medical service providers makes eminent sense and is consistent with the philosophy cryonics advocates as a technology intended to preserve life/health. If common policies and procedures can be

agreed on, cryonics organizations might support development of an accreditation system. Such a system relies on external accreditation organizations, and there exist accreditation bodies supporting review of numerous endeavors besides clinical care systems, such as financial organizations and manufacturing. Yet in all these cases, the goals are similar, to provide peer evaluation to document competence, conformance with applicable standards, and evaluation on the ability to carry out specific tasks. Accreditation evaluation is conducted mainly by expert volunteers and depends on documentary evidence presented by the organization itself. The main goals of accreditation are quality enhancement and performance improvement as well as quality assurance.[7]

As a prime example, the Hospital Accreditation program was formally established in the United States in 1951 by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). Its use spread to Canada and Australia during the 1960s and eventually extended throughout the world in the 1990s.[8] While there is robust criticism of JCAHO in terms of methodology and results[9] it is nevertheless considered highly reputable by most hospitals and the public, a gold standard which accredits and certifies over 22,000 health care organizations and programs in the United States.[10] This accreditation has been defined as "A self-assessment and external peer assessment process used by healthcare organizations to accurately assess their level of performance in relation to established standards and to implement ways to continuously improve."[11] These reviews offer external experts to come in at the request of the system to review technical and administrative processes. During the evaluation by accreditors, attention is paid to the determination of conformance with published evidenced based medicine and practices. Critically, accreditation is not solely focused on technical practices; in addition, there is a review of administrative structure, leadership, governance and other issues that would be likely to have an impact on the application of medical procedures and care.

Another stand out example worthy of consideration as having some methodologies applicable to cryonics review is that of the International Laboratory Accreditation Cooperation (ILAC), an international organization for accreditation bodies involved in the accreditation of medical testing organizations, and which defines accreditation as "the independent evaluation of conformity assessment bodies against recognized standards to carry out specific activities to ensure their impartiality and competence."[12] By applying these standards the ILAC affirms that the government, procurers, and consumers can have confidence in the calibration and test results, inspection reports and certifications provided."

In her extensive review[13] Knapp describes the challenges in exploring the feasibility of developing certification or accreditation standards in organizations that do not have existing structure for the same. She points out these efforts apply across such areas as "healthcare, real estate, and finance – certification and accreditation are attempts at self-regulation, an active strategy to fend off the possibility of federal and state regulation." Implementing these programs is not trivial. Challenges include determining if such an approach is even necessary through a thorough needs assessment, defining the specific elements of how standards are to be defined and evaluated, establishing how such efforts will be managed, evaluating funding requirements, and involving individuals with the necessary expertise. Despite acknowledging these challenges as significant, she also lays out a framework to approach resolving them and asserts it is achievable if there is adequate leadership and commitment from the organization. Her conclusion is that accreditation offers numerous benefits; recognizing program performance and outcomes, increasing confidence in the performance of the organization, defining content and scope of practice by participants in the organization, increasing credibility and ensuring consistency in outcomes.

Ultimately credible accreditation bodies, be it for the purpose of reviewing medical or nonclinical industry, attempt to ensure that conformity to accepted standards are maintained. They publish well established evaluative standards and have transparent policies in how assessments will be conducted. Not confined to the United States, large scale accreditation bodies, that are recognized as competent and capable, will sign regional and international agreements. These accreditation bodies then can assess and accredit conformity assessment bodies internationally to the relevant standards.[14]

Admittedly though, while a mainstream and accepted practice, there remains limited and contested evidence supporting the effectiveness of accreditation programs. Critics point out that inspections related to accreditation can be highly stressful, viewed as intrusive, and come at a cost. These critics of accreditation have further argued it can impose extensive bureaucratic burdens and restrain innovation.[15] There is some concern that accreditation organizations may begin to evolve requirements which are primarily focused on justifying their repeated audits rather than on improving systems. Nevertheless, the review by external experts who can provide new ideas and honest criticism, leads medical groups who receive accreditation to view it with a sense of pride and employ it as a marketing tool.

Quality Management Systems and Standards

Apart from accreditation, another consideration for cryonics organizations might be to look at reviews of quality management systems (QMS) that they currently already have in place, but which have not been formally evaluated. A QMS is defined as a structured and formalized system that consists of policies, processes and procedures used by an organization to provide services and operate in a way that meets the needs and expectations of stakeholders. It is fundamentally about managing customer satisfaction although it can also be used to manage risk areas of the business. These systems maintain consistent operations, management and results in the provision of services, and give assurance to interested

parties, improving reputation. One well established route that some industries employ in establishing standards is through the American National Standards Institute[16] (ANSI) process. ANSI describes the development of standards as; "… intended to verify that the principles of openness and due process have been followed in the approval procedure and that a consensus of those directly and materially affected by the standards has been achieved. ANSI coordination is intended to assist the voluntary system to ensure that national standards needs are identified and met with a set of standards that are without conflict or unnecessary duplication in their requirements." ANSI is the U.S. member of non-treaty international standards organizations such the International Organization for Standardization, which is an independent body whose membership consists of standardization bodies. As of 2022 there are 167 members each representing their specific country and with each country having one member. While originally focusing on mechanical engineering, as of April 2022 there have been over 24,261 standards created covering a wide variety of products and services. It should be clear that whichever method is chosen to establish institutional standards, adequate training of those to be involved in the process will be necessary as well as possible consultation with experts in the field. Further, a decision would be needed as to whether actual membership and participation with ANSI as a formal member is necessary, rather than making use of their process which involves submission of the standards of development and procedures to ANSI for review and accreditation.

A key standard used to develop and evaluate QMS systems is defined by ISO 9001. The essential steps envisioned by ISO in a QMS are first to define what is to be done through appropriate manuals and procedures, next is to ensure these manuals are followed, next is to demonstrate the effectiveness of the procedures, next must be to monitor and verify the compliance of use of the requirements and assessing effectiveness, finally are improvement efforts on policies and procedures with monitoring of the results of these improvement efforts. In ISO 9001 there are seven quality management principles which serve as a framework for the standard and are customer focus, leadership, engagement of people, process approach, continual improvement, evidence-based decision making and relationship management. Certification in ISO 9001 is accomplished by Certification Bodies which are independent from ISO but accredited through them. There are consultants and firms which are hired by businesses to develop and implement QMS that are consistent with ISO 9001. The advantage of this approach is organizations can work towards this goal independently. Further, many practices such as those Alcor currently employs meet the likely requirements of such a certification. It may be that cryonics organizations would need to create a certification program from scratch to avoid the potentially high costs of a program such as ISO. It is also unknown if ISO would be a willing partner to work with cryonics organizations given the reputational issues described earlier.

If there is an interest by cryonics organizations in a more limited approach with a focus on technical standards it would require reputable, established cryonics organizations, and independent experts, if possible, to meet and agree on best practices, as a group effort. Unfortunately, since resuscitation after suspension has not yet been proven to be technically feasible, these standards would be accurately described as evidence informed rather than evidence based, as the ultimate proof would be demonstrated success. But as a lesser standard, evidence informed practices or best practices, could possibly be agreed on, even including some degree of variation in fundamental technical practices, between cryonics providers. Objective measures such as keeping brain tissue viable by contemporary criteria prior to suspension, maintenance of blood gases in a healthy range during stabilization, CT scans demonstrating a lack of structural damage or ice formation might be some examples. The follow up effort would be to agree on research protocols that would then be able to verify which standards are best and as they are changed over time achieve common consensus. While the development of a set of common standards sounds more than reasonable in theory, overcoming organizational inertia, rivalries, and interpersonal conflicts could make what should be a dispassionate process impossible. Fortunately, dispassionate mechanisms for creation of standards by organizations have been elaborated. Once such process is as follows[17]: First, identify the need. Second, create a technical committee involving experts and stakeholders to create the specific standards. Third, the technical committee creates a draft outline of the standard that is agreed upon by consensus. Fourth, there is a period of public review with a goal of getting feedback from a wide audience of stakeholders to improve quality and the standards over relevant areas and perspectives. After feedback has been gathered, the technical committee makes changes as necessary and if a majority agree the final standards are published.

Is Credentialing an Option?

Another option would be for cryonics organizations to create a credentialing system. Credentialing is a core process of organizations that provide medical service. However, unlike accreditation, credentialing is a process applying to individuals, not organizations, and is intended to establish whether an individual possesses adequate qualifications to perform the technical requirements of the position they hold. Certification programs that provide credentialing to individuals can flow from the emerging training and expertise necessary for competent practice. It is possible that developing certification requirements can be the precursor to accreditation programs.[18] Cryonics organizations do seek out individuals with medical backgrounds who have been credentialed to engage in medical procedures relevant to those used in standby and preservation. It remains an open question if a credentialing program would be worthwhile in the current environment of limited numbers of procedures performed and available resources, and who would be involved in offering some certification or credentials. Medical systems

do provide "privileging" requirements for clinicians employed that might be adapted for this purpose by individual cryonics organizations, but it would be a significant outlay of time and resources as such requirements involve a delineation of scope of practice, demonstration of proficiency of these practices, and ongoing education and training to maintain proficiency.

Financial Audits

The National Council of Nonprofits, referring to charitable nonprofits in a statement that might likewise apply to nonprofit cryonics organizations, notes that even if the state or federal legal requirements for audits do not apply to a particular nonprofit, there are a number of reasons why a nonprofit may decide to conduct an independent audit. One primary reason is to demonstrate the organization's commitment to financial transparency. Publishing an independent audit report on a nonprofit's website or providing the report to those who request it are examples of transparent practices that donors and the public have come to expect from nonprofits.[19] In the case of cryonics organizations, most members are realistic in understanding the severe limitations and hurdles that must be overcome if recovery is someday to be possible. With this in mind, maintaining individuals in suspension at necessary temperatures without any significant fluctuation for a period of perhaps centuries presents not only unique technical challenges but also requires maintaining an administrative and financial structure that will support the ongoing effort. As such, it would be worth consideration of obtaining external audits to look specifically at financial aspects of the assumptions underlying planning employed by cryonics organizations and make these publicly available. Such audits need to have a scope beyond the immediate short and mid-term and look at extremely long time frames and determine if the financial assumptions being made are realistic. In addition, because larger and well-funded organizations may step in once cryonics technological issues are overcome, there needs to be a business plan to consider what might be done if new memberships were to cease and members in suspension needed to be maintained without additional financial donations or support beyond trusts or similar mechanisms.

Institutional Review Board

IRB stands for Institutional Review Board and involvement of these bodies is required in the US for medical research involving human beings. A primary purpose of these Boards is to review research protocols ensuring they are ethical and reasonable in scope. The interesting wrinkle here is that IRBs are required for research on living experimental subjects, legally our members undergoing cryopreservation are not living, yet we view them as being alive. If an IRB like system was used by a cryonics organization in providing oversight, there could be a shared IRB or each organization could develop their own. The primary costs are the time spent by the members meeting, need for administrative support to create documentation, and need for experts, although from diverse backgrounds and not strictly scientific, members.

Disclosures

In addition to attempting to increase credibility through institutional measures, consideration might be given by cryonics organizations that publish magazines or offer conferences to adopt additional practices employed by mainstream medical conferences and journals.[20] This might include appropriate conflict of interest and financial disclosure statements by individuals writing or presenting information which are scientific or technical in nature. At medical conferences, all presenters should provide a conflict of interest statement as part of their presentation. It is accepted practice in medical journals that conflict of interest disclosures are declared at the end of the published articles and include both a Declaration of Conflicting Interests section and a Funding section. Further, consideration for published articles in this category (and perhaps those presented at conferences as well) should include peer review. If peer review is not a part of the publication requirements, then that should be stated somewhere in the periodical. Volunteers willing to conduct peer review do not have to belong to the cryonics organization they are reviewing articles for and might be pooled by different organizations.

In summary, advancing the reputation and acceptability of cryonics into the mainstream requires adoption of practices that are considered as offering transparency and external review. Such review offers additional benefits beyond reputational, offering members of cryonics organizations reassurance that policies and procedures meet reasonable standards. While there are costs, both financial and intangible, to creating review structures the benefits have the potential of being profound. ■

Dr. Paul Beighley is a board-certified psychiatrist with a background in computer science. He has had successful careers in the US Military, private practice, and as a Foreign Service Officer for the US Department of State. Most recently, he served as Medical Director for the Hawaii Department of Health on the Island of Hawaii (the "Big Island"). Dr. Beighley's current interests include cryonics reintegration, supporting journalists who have experienced trauma, and providing assistance to embassy employees and their families living and working abroad. He is based on the Big Island of Hawaii and remains dedicated to his work in psychiatry and his ongoing research in these areas of interest.

The author declares that he has no relevant or material financial interests that relate to the information presented in this paper.

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Alcor A-2705 Case Report: A Cryopreservation with Dignity

Summary

All 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 pronouncement of legal death, T-X represents occurrences before T-0, and T+X represents occurrences following T-0.

A-2705 was a 67-year-old male with neuro cryopreservation arrangements who used the death with dignity laws in his state to legally terminate his life. Per the death certificate the cause of death was cardiac arrest subsequent to liver cancer and a gastrointestinal tumor. Cardiac arrest took place at about 17:15 hrs and the member was pronounced legally deceased in August of 2020 in the state of Washington at 17:17 hrs on T-0 days.

A Field Cryoprotection (FCP) was performed before the patient was transported to Alcor. Dry ice cooldown was initiated in the field at 22:54 hrs on T-0 days. Cryogenic cooldown was initiated at Alcor at 15:05 hrs on T+1 days and terminated at 18:33 hrs on T+5 days. CT scans at LN2 temperature were obtained at 11:00 hrs on T+8 days. The patient was transferred to long-term maintenance at LN2 temperature at 15:38 hrs on T+56 days.

Patient Assessment

This report was finalized in 2023 but the case took place in 2020; some details are no longer available.

T-123 days

The member notified Alcor by email of a diagnosis of terminal cancer and that physicians had given the member approximately 6 months to live. The member intended to utilize Washington state's Death with Dignity Act (DWD) to choose the legal date and time of death.

Deployment

T-121 days

Alcor's Medical Response Director (MRD) placed the member on the Watch List for monthly follow-up to continue to track the progression of the disease process. Planning the logistics of the case and a potential timeframe for the DWD date was initiated.

T-88 days

The member reported that the most recent MRI scans were not encouraging and that the predicted DWD date would be in mid-September.

T-44 days

Again, the member reported that the most recent MRI scans were disappointing. The oncologists had taken the member off the clinical trial drug formerly taken and had started a different drug. The member planned to terminate the cancer treatments and enroll for in-home hospice care. The projected DWD date was moved forward to the first week of September.

T-32 days

After extensive discussions which included Alcor's MRD, Readiness Coordinator (RC), Scientific Advisor (SA), and both strategic partners (International Cryomedicine Experts (ICE) and Suspended Animation (SA)), it was decided that this member would have a Field Cryoprotection (FCP) following stabilization in the field. As ICE personnel had previous experience with the FCP procedures this was an ICE directed case with SA assisting and being trained in the FCP procedures. Both organizations would be deployed on this case for standby, stabilization and transport (SST).

T-10 days

The member's family secured a private nurse for pronouncement of legal death. She was available 24/7 and there were no limitations on her availability or potential problems. The member's health was deteriorating rapidly; the member and family were ready to finalize the DWD date. Alcor's entire team was put on alert.

Standby

T-5 days

After coordinating with the nurse who would pronounce, about her availability, the member and family chose the date when the DWD medications would be taken by the member. The SST team members deployed and over the next four days, set up equipment and made preparations. A funeral home near the member's home had already been contracted to provide the

death certificate, transit permit and air transport of the patient back to Alcor.

T-0 days

The entire SST team had arrived at the member's home by 09:18 hrs. All equipment and stabilization medications were in place and ready for use. It was agreed in advance that all SST team members would remain outside the member's room while the family assisted the member with taking his DWD medications. It was further agreed that team members would wait to initiate stabilization procedures until the family informed the team that the member had been pronounced legally deceased by the nurse.

The member took the DWD medications at 10:02 hrs. Per the family, the member lost consciousness at 10:34 hrs, and at 11:49 hrs vital signs (respiration, pulse, oxygen) remained stable. The vital signs were still stable at 13:44 hrs. At 15:03 hrs the heart rate had dropped into the mid-'40s and respirations were labored. At 15:56 hrs the blood pressure was 104/62. The member went into cardiac arrest at approximately 17:15 hrs and was pronounced legally deceased by the nurse at 17:17 hrs.

Stabilization

As there were five team members several steps in the stabilization procedure could be accomplished at the same time. At 17:20 hrs the rectal occlusion device was placed as the patient was rolled onto the Megamover and then moved into a body bag on a bed of crushed ice to start external cooling. The King airway was placed, and approximately 60 lbs. of crushed ice was placed over the patient in the body bag.

The SAVe ventilator was started to ventilate the patient and the $ETCO_2$ capnograph device were started at 17:20 hrs to monitor the effectiveness of cardiopulmonary support. The first $ETCO_2$ reading was 26. The first intraosseous (IO) device was placed in the tuberosity of the right lower leg to access the patient's vasculature for the administration of medications and a nasopharyngeal probe was placed in one of the patient's nares (which nare was not noted). At 17:21 hrs the ROSC-U chest compression device was placed on the patient to initiate mechanical chest compressions to optimize cooling and to circulate the stabilization medications when administered.

The nasopharyngeal probe was secured to the patient's face to prevent it from being dislodged and the tubing for the surface conduction cooling device (SCCD) was placed around and over the patient to circulate cooled water to optimize external cooling. Approximately 3 to 4 gallons of water were added to the body bag. At 17:23 hrs the first stabilization medication was administered (see the below Table of Medications Administered for the names of the medications, the times of administration, and the dosages). The cooling mask for the SCCD was placed on the patient's face to further increase the effectiveness of the cooling and the pump was started but there was no flow. Two more gallons of water were added to start the flow, but it was intermittent and there was no room in the body bag for more water.

The $ETCO_2$ reading was 27 at 17:25 hrs and the nasogastric tube was tied off to prevent the backflow of the antacid. A second IO was placed in the tuberosity of the left lower leg at 17:28 hrs to increase the efficiency of the administration of medications. At 17:43 hrs a Zoll impedance threshold device was added to the airway to increase venous return to the heart and therefore increase cardiac output during cardiopulmonary support. At 17:46 hrs the $ETCO_2$ reading was 10 (see Discussion section). At 17:48 hrs all the stabilization medications had been administered.

Air was escaping from the patient's mouth at 17:48 hrs. The ventilator was turned off and approximately 30 cc of air was added to the lumen of the airway. The automated ventilator was turned on at 17:51 hrs.

Field Surgery and Washout

The patient was moved into a vehicle to be transported to the funeral home and arrived at the funeral home at 18:18 hrs. The patient was placed on the operating table at 18:22 hrs and cannulation surgery was started at 18:40 hrs. The left carotid artery was isolated at 18:56 hrs. Cardiopulmonary support, ventilation and the face mask were all terminated at 18:57 hrs to facilitate cannulation of the arteries. The nasopharyngeal temperature (NPT) was 22.6°C at 19:03 hrs. By 19:06 hrs bilateral burr holes had been established in the patient's skull.

The right carotid artery was isolated at 19:12 hrs and the cephalic isolation was initiated at 19:15 hrs using a mallet and osteotome. The cephalic isolation was completed at 19:21 hrs. The lines for perfusate bladder #1 were primed at 19:27 hrs. An 18 French (Fr) catheter was used at 19:32 hrs to cannulate the left carotid artery.

Concurrently, open circuit cryoprotectant perfusion using the gravity feed field system (see Discussion section) was initiated through the left carotid artery at 19:32 hrs. An 18 Fr catheter was used at 19:32 hrs to cannulate the right carotid artery.

Open circuit cryoprotectant perfusion was initiated through the right carotid artery at 19:32 hrs. Securing the right cannula had become problematic due to it not advancing far enough into the artery, so the decision was made to cannulate past the bifurcation into the internal carotid artery to improve flow. Perfusion of the right carotid artery was stopped, and a 14 Fr catheter was used to replace the 18 Fr catheter at 19:37 hrs and perfusion was reinitiated. The temperature probe was placed in the burr hole at 19:50 hrs.

The vertebral arteries were draining, which confirmed that the Circle of Willis was intact and there would be reasonable perfusion pressure at the back of the brain. The vertebral arteries were clamped off at 19:54 hrs.

At 19:57 hrs the measured arterial pressure was 70 mmHg. The gravity-induced perfusion flow was initiated at 19:54 hrs with the first bladder containing nM22 cryoprotectant with a concentration of 0.05 CNV). See the below Table of Concentrations (Brix) of nM22 Solution for the precalculated refractive index of the individual bladders, times when the bladders were started, and the refractive index of the effluent samples. Bladder #4 was not infused because it had been damaged during the flight to Washington.

Cryoprotectant perfusion was terminated when bladder #12 was 75 percent expended at 22:51 hrs (see the Discussion section). The final refractive index readings were 50.1 Brix right venous (100% of perfusate concentration needed to vitrify (CNV)) and 49.7 Brix left venous (99% of CNV).

Patient Transport

T-0 days

The NPT was -5.1°C at 22:54 hrs. The cephalon was placed in the dry ice shipper and covered with dry ice. A power supply error caused the temperature logger to fail (see the Discussion section).

T+1 days

The NPT was -70°C at 07:10 hrs. The patient was taken to a local airport and departed for Alcor at approximately 11:10 hrs, and arrived at Alcor at 14:44 hrs.

Cooling to Liquid Nitrogen Temperature

A computer program was used to initiate cryogenic cooldown at 15:05 hrs on T+1 days, starting at -79°C and plunging to -110°C and descending thereafter at -1°C/hour to LN_2 temperature. At 18:33 hrs on T+5 days, an uneventful cooldown was terminated at LN_2 temperature (-196°C). At 11:00 hrs on T+8 days CT scans of the cephalon at LN_2 temperature were obtained. The patient was transferred to long-term maintenance at LN_2 temperature at 15:38 hrs on T+56 days.

Timeline and Time Summaries

Timeline

T-0	17:15	Estimated time of cardiac arrest
T-0	17:17	Pronouncement of legal death
T-0	17:19	Placed water ice on patient
T-0	17:19	Placement of airway
T-0	17:20	Placement of first intraosseous (IO) device
T-0	17:20	Placement of first ETCO2 device (first reading = 26)
T-0	17:21	Start of mechanical chest compressions
T-0	17:21	Start ventilator and capnograph
T-0	17:23	Administration of first medication (propofol)
T-0	17:28	Placement of second intraosseous (IO) device
T-0	17:46	Final reading from ETCO2 device (reading = 10)
T-0	17:47	Administration of final medication (decaglycerol/THAM)
T-0	17:58	Start transport of patient to funeral home
T-0	18:18	Arrival of patient at funeral home (NPT 28°C)
T-0	18:40	Start field surgery (cannulation)
T-0	18:57	Termination of cardiopulmonary support (NPT 26°C)
T-0	19:15	Start of cephalic isolation
T-0	19:21	Completed cephalic isolation (cephalon not weighed)
T-0	19:32	Start of open circuit cryoprotection (left carotid artery)
T-0	22:51	End open circuit (Brix = 50.1 rt venous, 49.7 left venous)
T-0	22:54	Start of dry ice cooling (NPT = -5.1°C)
T+1	07:10	Near dry ice temperature reached (-70°C)
T+1	11:06	Departure of patient for local airport
T+1	14:18	Departure from the airport for Arizona
T+1	14:44	Arrival of patient at Alcor (temperature not recorded)
T+1	15:05	Start of patient cryogenic cooldown
T+5	18:33	End of cooldown at LN2 temperature
T+8	11:00	CT scans of cephalon at LN2 temperature
T+56	15:38	Transfer of patient to long-term maintenance at LN2 temperature

Time Summaries

Event Duration hr:min		days	time	
Stabilization				
00:02	From:	T-0	17:15	Estimated time of cardiac arrest
	Till:	T-0	17:17	Pronouncement of legal death
00:04	From:	T-0	17:15	Estimated time of cardiac arrest
	Till:	T-0	17:19	Placed water ice on patient

00:06	From:	T-0	17:15	Estimated time of cardiac arrest
	Till:	T-0	17:21	Start of mechanical chest compressions
00:08	From:	T-0	17:15	Estimated time of cardiac arrest
	Till:	T-0	17:23	Administration of first medication (propofol)
00:24	From:	T-0	17:23	Administration of first medication (propofol)
	Till:	T-0	17:47	Administration of final medication (decaglycerol/ THAM)
Field Surgery and Field Cryoprotectant Perfusion				
00:22	From:	T-0	18:18	Arrival of patient at funeral home (NPT 28°C)
	Till:	T-0	18:40	Start field surgery (cannulation)
01:25	From:	T-0	17:15	Estimated time of cardiac arrest
	Till:	T-0	18:40	Start field surgery (cannulation)
00:41	From:	T-0	18:40	Start field surgery (cannulation)
	Till:	T-0	19:21	Completed cephalic isolation (cephalon not weighed)
02:17	From:	T-0	17:15	Estimated time of cardiac arrest
	Till:	T-0	19:32	Start of open circuit cryoprotection (left carotid artery)
03:19	From:	T-0	19:32	Start of open circuit cryoprotection (left carotid artery)
	Till:	T-0	22:51	End open circuit (Brix = 50.1 rt venous, 49.7 left venous)
05:36	From:	T-0	17:15	Estimated time of cardiac arrest
	Till:	T-0	22:51	End open circuit (Brix = 50.1 rt venous, 49.7 left venous)
00:41	From:	T-0	18:40	Start field surgery (cannulation)
	Till:	T-0	19:21	Completed cephalic isolation (cephalon not weighed)
00:52	From:	T-0	18:40	Start field surgery (cannulation)
	Till:	T-0	19:32	Start of open circuit cryoprotection (left carotid artery)
04:11	From:	T-0	18:40	Start field surgery (cannulation)
	Till:	T-0	22:51	End open circuit (Brix = 50.1 rt venous, 49.7 left venous)
03:19	From:	T-0	19:32	Start of open circuit cryoprotection (left carotid artery)
	Till:	T-0	22:51	End open circuit (Brix = 50.1 rt venous, 49.7 left venous)
Dry Ice Cooling and Cryogenic Cooldown				
00:03	From:	T-0	22:51	End open circuit (Brix = 50.1 rt venous, 49.7 left venous)
	Till:	T-0	22:54	Start of dry ice cooling (NPT = -5.1°C)
05:34	From:	T-0	17:20	Placement of first ETCO2 device (first reading = 26)
	Till:	T-0	22:54	Start of dry ice cooling (NPT = -5.1°C)
04:36	From:	T-0	18:18	Arrival of patient at funeral home (NPT 28°C)
	Till:	T-0	22:54	Start of dry ice cooling (NPT = -5.1°C)
00:21	From:	T+1	14:44	Arrival of patient at Alcor (temperature not recorded)
	Till:	T+1	15:05	Start of patient cryogenic cooldown

Table of Medications Administered

Time	Medication	Dose	Purpose
17:23 hrs	Propofol (Diprivan)	200 mg	Anesthetic; reduces cerebral metabolic demand; reduces the theoretic possibility of increased awareness during aggressive CPS.

17:23 hrs	Sodium citrate	**(1st dose 60 cc)** Note 2	Anticoagulant; prevents blood clot formation.
17:25 hrs	Sodium citrate	**(2nd dose 40 cc)** Note 2	Anticoagulant; prevents blood clot formation.
17:27	Antacid	240 cc Note 3	A buffer used to protect the stomach from acid erosion.
17:28 hrs	Heparin	50,000 IU	Anticoagulant; prevents blood clot formation.
17:29 hrs	Minocycline	200 mg	Antibiotic and neuroprotectant
17:29 hrs	Decaglycerol/ THAM [tris (hydroxymethyl) aminomethane]	200 cc total **(1st dose 50 cc)** Note 4	Decaglycerol inhibits cerebral edema. THAM is a buffer to mitigate acidosis.
17:29 hrs	Vasopressin	80 IU total **(1st dose 40 IU)** Note 5	Vasopressor; increases blood pressure during CPS.
17:30 hrs	SMT (S-methyl-isothiourea)	400 mg Note 6	Neuroprotectant (iNOS inhibitor); protects the brain from ischemic injury; raises blood pressure.
17:31 hrs	Vital Oxy	200 cc total **(1st dose 60 cc)** Note 7	Antioxidants: melatonin, vitamin E (D-alpha tocopherol), PBN (alpha Phenyl t-Butyl Nitrone) and anti-inflammatory carprofen.
17:37 hrs	Vital Oxy	200 cc total **(2nd dose 60 cc)** Note 7	Antioxidants: melatonin, vitamin E (D-alpha tocopherol), PBN (alpha Phenyl t-Butyl Nitrone) and anti-inflammatory carprofen.
17:38 hrs	Vasopressin	80 IU total **(2nd dose 40 IU)** Note 5	Vasopressor; increases blood pressure during CPS.
17:39 hrs	Vital Oxy	200 cc total **(3rd dose 60 cc)** Note 7	Antioxidants: melatonin, vitamin E (D-alpha tocopherol), PBN (alpha Phenyl t-Butyl Nitrone) and anti-inflammatory carprofen.
17:41 hrs	Decaglycerol/ THAM [tris (hydroxymethyl) aminomethane]	200 cc total **(2nd dose 50 cc)** Note 4	Decaglycerol inhibits cerebral edema. THAM is a buffer to mitigate acidosis.
17:41 hrs	Vital Oxy	420 cc total **(4th dose 20 cc)** Note 7	Antioxidants: melatonin, vitamin E (D-alpha tocopherol), PBN (alpha Phenyl t-Butyl Nitrone) and anti-inflammatory carprofen.
17:44 hrs	Decaglycerol/ THAM [tris (hydroxymethyl) aminomethane]	200 cc total **(3rd dose 50 cc)** Note 4	Decaglycerol inhibits cerebral edema. THAM is a buffer to mitigate acidosis.

17:48 hrs	Decaglycerol/ THAM [tris (hydroxymethyl) aminomethane]	200 cc total **(4th dose 50 cc)** Note 4	Decaglycerol inhibits cerebral edema. THAM is a buffer to mitigate acidosis.
18:30hrs	Streptokinase	250,000 IU Note 8	A thrombolytic used to break up existing blood clots.

Notes:

1. All the medications that were in the field kit were administered.

2. The standard formulation for sodium citrate is 50 cc vials of 20% w/v = 10 grams sodium citrate, with a maximum of two vials being administered depending on patient weight. This patient received 20 grams of sodium citrate as per protocol, administered in two doses because his weight was over 40 kg.

3. Antacid was given in a single dose and was inserted through the nasogastric tube.

4. 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).

5. Vasopressin is a fixed dosage of 40 IU, per dose for two doses. The second 40 IU dose 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.

6. 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.

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. The standard administration of streptokinase is 250,000 IU dissolved in 5 mL of 9% sodium chloride.

Table of Concentrations (Brix) of nM22 Solution

Step-ramp calculator, nM22			
sixth root of [% CNV]/5 =		1.6636	step ratio
bag #	contents	[nM22], CNV	Brix (calc)
1	washout	0.00	9.80
2	0.05	0.05	11.81
3	0.08	0.08	13.14
4			
5	0.23	0.23	19.03
6	0.38	0.50	29.85
7	0.64	0.50	29.85
8	1.06	1.06	52.31
9	1.06	1.06	52.31
10	1.06	1.06	52.31
11	1.06	1.06	52.31
12	1.06	1.06	52.31

Note: Bladder #4 was not infused because it had been damaged during the flight to Washington. This case took place in 2020 which was before the more detailed reporting on field cryoprotection cases. This case was one of the first FCP cases during the Covid-19 pandemic and recording the times that individual bladders were hung had not yet become protocol.

Discussion

Standby, Stabilization and Transport

Alcor's standby, stabilization and transport (SST) personnel held phone and email conversations regarding when the member would take his end-of-life medications in accordance with the death with dignity laws in the state of Washington. The member was advised by Alcor that there could be increased challenges (such as obtaining the death certificate and transit permit) if he were to take the medications late in the day or near or on the weekend. Alcor wanted to be sensitive to the member's autonomy. That being said, since field cryoprotection (FCP) was being utilized, the cephalon needed to be stored on dry ice for an extended period of time, a temperature which favors ice formation.

There might have been an issue with the RespUSense $ETCO_2$ monitor. The monitor appeared to work at Alcor and was tested both before and after the case. There are different reasons that an $ETCO_2$ reading could be low. Alcor is continuing to seek more data before suggesting that there were technical issues the first time the monitor was used.

The first $ETCO_2$ reading taken at 17:20 hrs was 26, which would have been a good reading for a typical out-of-hospital

resuscitation case. Readings above 20 persisted at least during the first 15 minutes of the case, followed by a decline. An effort was made to reposition the airway and the monitor, but a more appropriate reading could not be obtained, and the reading continued to fall until the last reading of 10 at 17:46 hrs and the use of the monitor was discontinued. The SST kit did have a colorimetric device that could have been used to confirm placement but would not have provided specific $ETCO_2$ data. For future cases, an adapter will be used that will record data and provide more meaningful readings.

During patient transport to the funeral home, there was a rise in temperature. As the cause was not observed at the time, there are a couple of possible explanations. First, the nasopharyngeal probe could have shifted and become exposed to air. Another possibility is that the patient's mouth was not totally occluded and water from the recirculation mask leaked into the nasopharynx, and this would cool the probe, and then acclimate with the surrounding tissue. And another possibility could have been that additional air was introduced into the King airway due to a potential leak. If the air was escaping up into the nasopharynx there would be a cooling effect as well.

One of the results of this probe failure is that individual temperature data during CPS are unreliable. As a consequence, the initial cooling rates and S-MIX were based on linear estimates instead of individual readings. It is important to emphasize that the Alcor protocol stipulates the use of at least two separate temperature probes. In this case a tympanic probe and rectal probe (attached to the rectal plug) might have yielded more reliable readings.

Field Surgery and Cryoprotection

The gravity feed system uses a tripod that can be adjusted for height to control the arterial pressure. The pre-mixed cryoprotectant is in a series of bladders with graduated concentrations (measured by the refractive index (RI) in Brix units). By hanging two bladders with different RI concentration on a teeter-totter atop the tripod, as the bladder with the lower RI runs out and becomes lighter, at the mid-way point the teeter-totter will allow both bladders to flow, essentially mixing the two concentrations and creating a smoother transition from one concentration to the next. When the bladder with the lower RI runs out, the full concentration of the bladder with higher RI is then flowing exclusively. This process allows for a smoother curve in the increasing concentrations of cryoprotectant.

Transport temperature logging began immediately upon placement of the nasopharyngeal probe and continued through cephalic isolation until the pressure sensor was connected. When the arterial pressure sensor was connected, a power supply error caused the temperature logger to fail. The team immediately replaced the logger with a second unit without interrupting the procedure. However, due to an unknown cause, the second logger did not begin recording the temperature data. The team continued to take visual temperature measurements from the second logger.

The 12-bladder system (and here bladder #4 was missing so in reality this case was an 11-bladder system) is probably not perfusate enough to realistically achieve 100% concentration needed to vitrify (CNV) on the single-pass step ramp. Due to the results of this case, 105% CNV 2-liter bladders (12 liters) have been added to the FCP system to prolong the endpoint and get more cryoprotectant uptake into the brain.

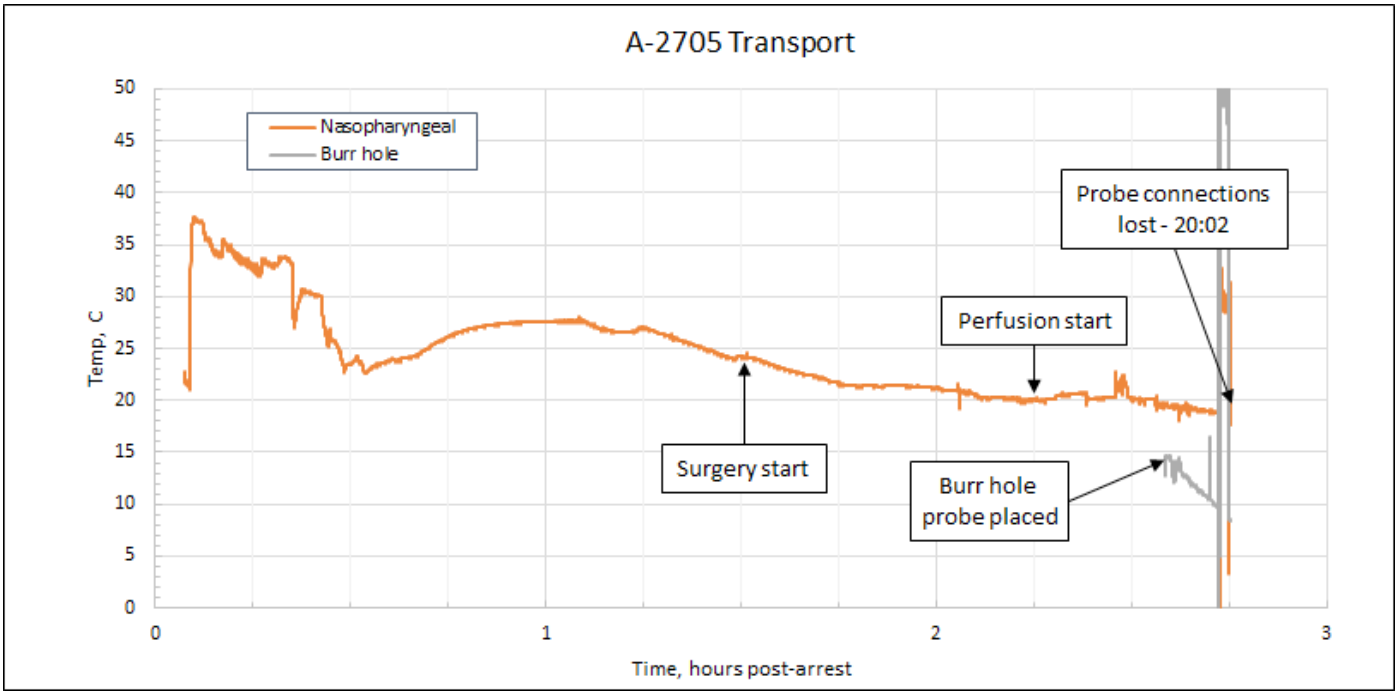
Closed circuit perfusion was terminated when there was still 25 percent of the perfusate in bladder #12. This information came from bodycam footage, but unfortunately there was no audible explanation for termination prior to the bladder being expended. As this was a 2020 case, those individuals who participated in the case do not remember with certainty, however, since the final refractive index readings were 50.1 Brix right venous (100% of perfusate concentration needed to vitrify (CNV) and 49.7 Brix left venous (99% of CNV), it is assumed that perfusion was terminated because the proper concentration for termination of perfusion had been reached before the last 25 percent of the perfusate was expended.

This is not optimal because standard protocol is to maintain perfusion after reaching 100% CNV venous concentration for at least 30 minutes (per protocol), especially in cases where not all perfusate is available (bladder 4). For this case, it is not evident whether there was enough perfusate to conform to this protocol. As can be expected in right-to-die cases with rapid CPS and cooling, flow rates were relatively high for a given pressure, requiring more perfusate.

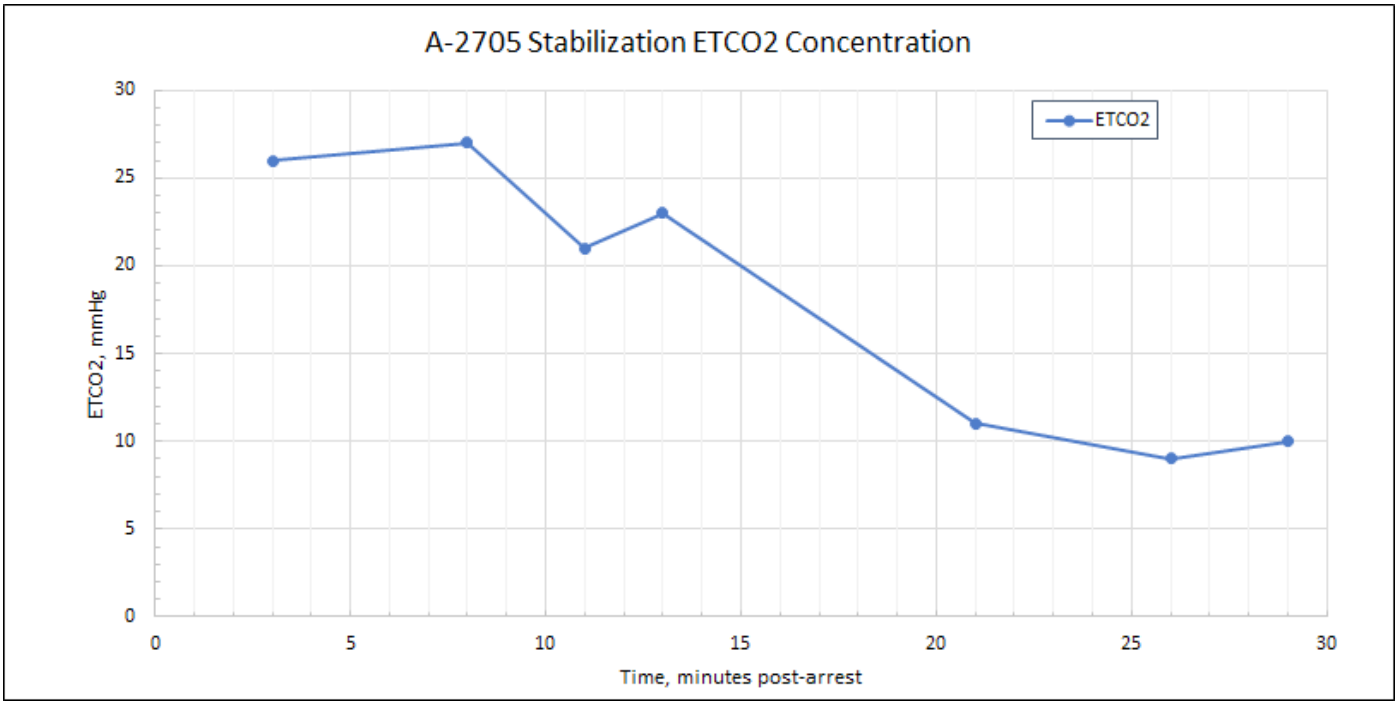
CT scans of this patient reveal reasonably good cryoprotection results, with many areas in the 80% CNV range and some areas indicating complete equilibration. The outcome of this case was greatly favored by the conditions under which the patient died, both in terms of logistics and time between circulatory arrest and start of procedures.

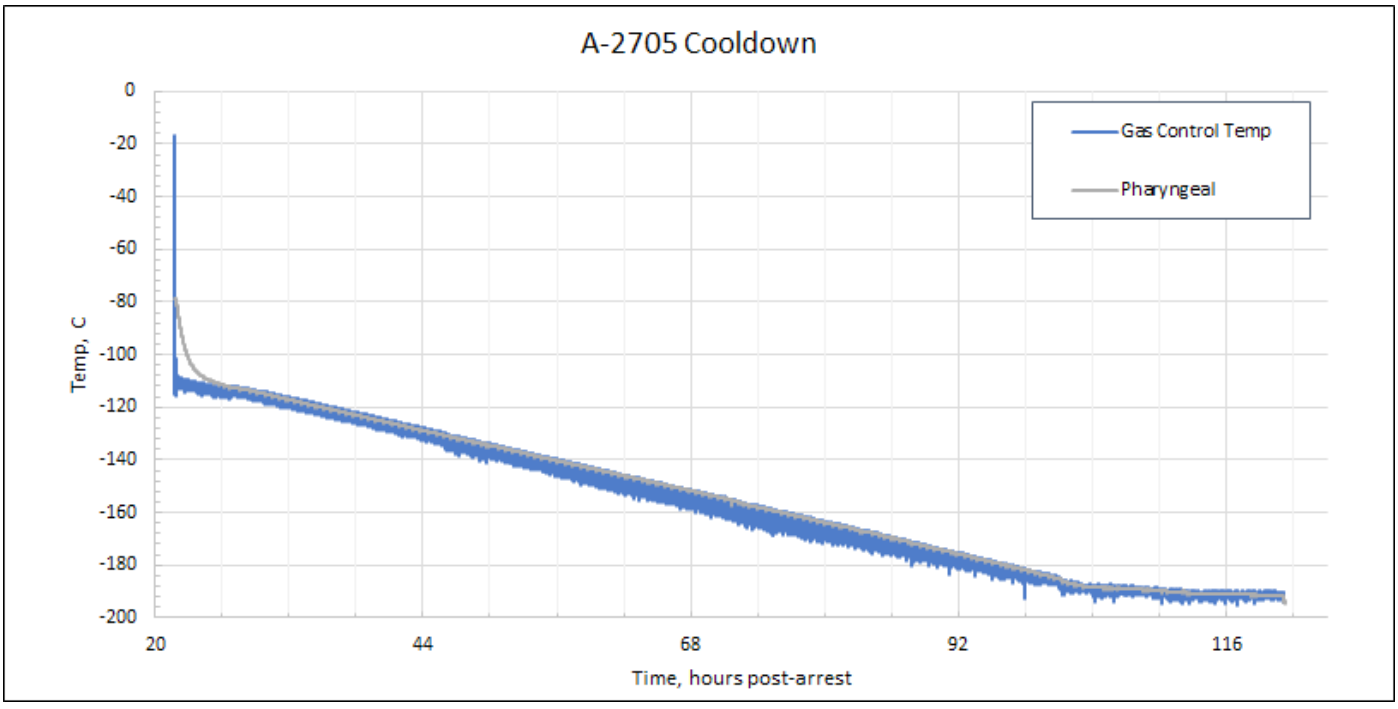
Further improvements in outcome could have included the use of a true portable ice bath to further accelerate external cooling, aggressive pressor support based on declining ETCO2 readings, complete 4-vessel cannulation, and extended perfusion times at the highest concentration to bring more areas of the brain towards 100% CNV.

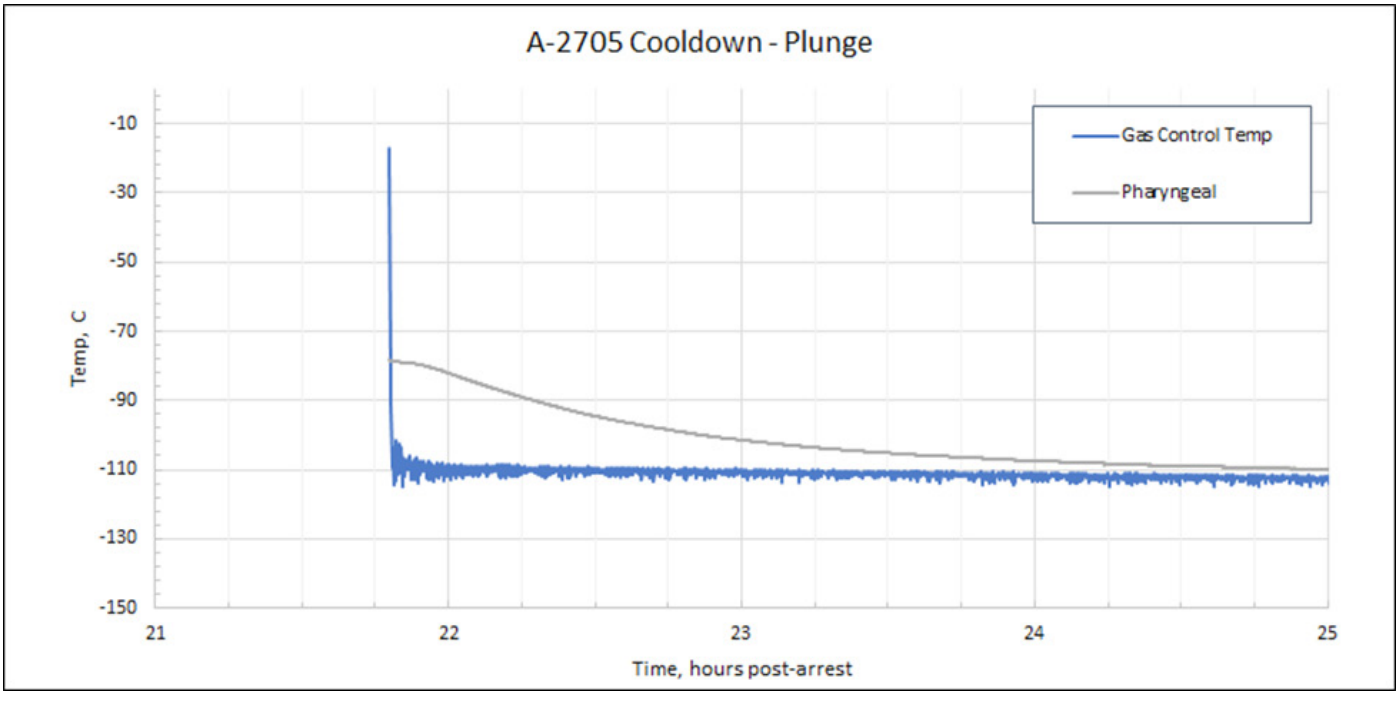
Cryoprotection and Temperature Graphs



Note: Two critical measurements that were taken during the period where no other temperature data exists are -5.1°C at 22:49 hrs on T-0, and -70.0°C at 7:10 hrs on T+1. No further visual measurements were made until the patient arrived at Alcor, at which point the data trail picks back up with the patient connected to the cooldown cart.




A-2705 Cooldown
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
Temp, C
20
44
68
92
116
Time, hours post-arrest
Gas Control Temp
Pharyngeal


A-2705 Cooldown - Plunge
-10
-30
-50
-70
-90
-110
-130
-150
Temp, C
21
22
23
24
25
Time, hours post-arrest
Gas Control Temp
Pharyngeal

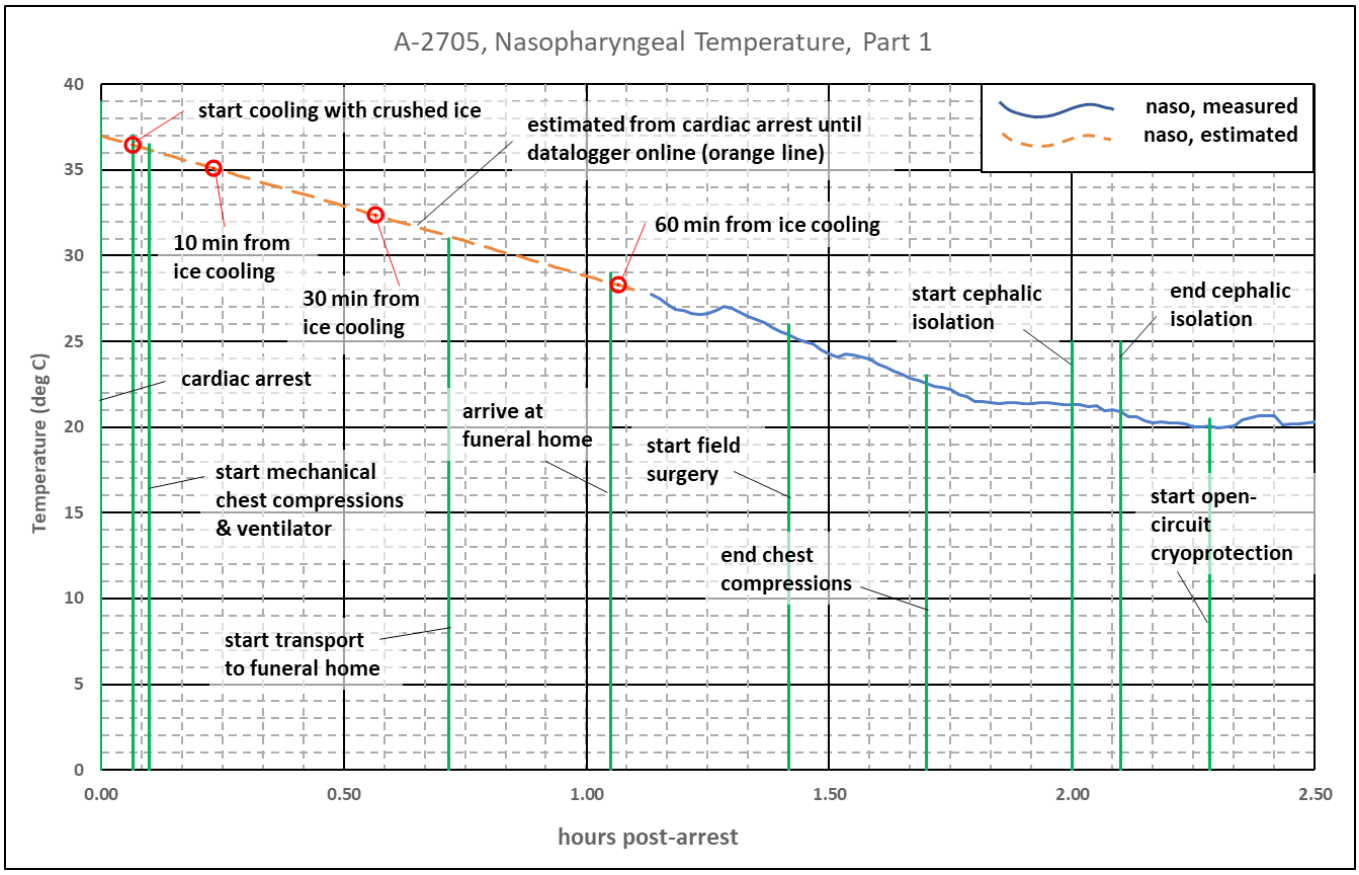
S-MIX

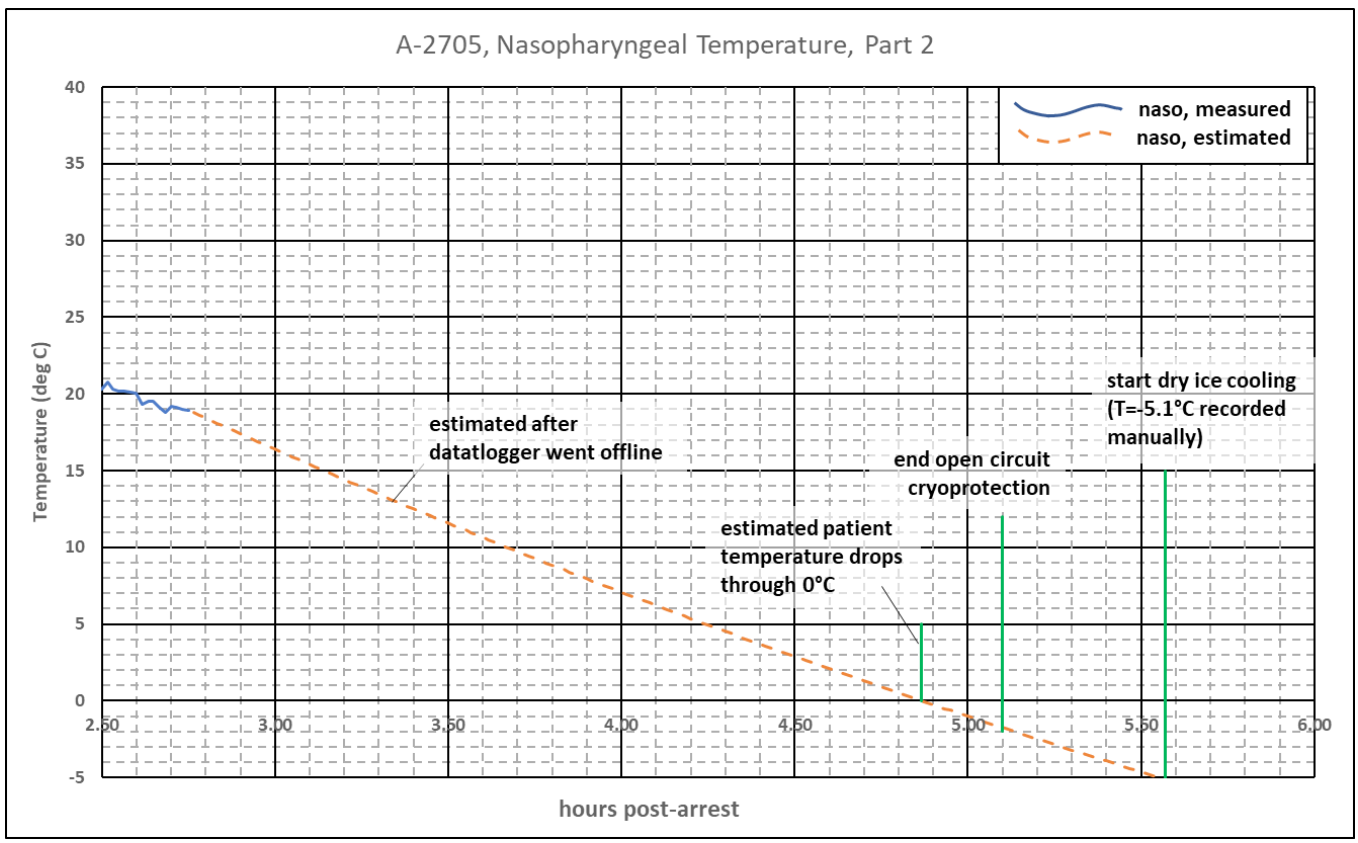
The Standardized Measure of Ischemic Exposure (S-MIX) expresses the total ischemic exposure prior to the start of cryogenic cooling as the equivalent duration of normothermic ischemia. An S-MIX of 00:00 (hh:mm) is the ideal case of no ischemic damage. The higher the S-MIX time, the more damage. Factors 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 4:52. As shown below, and due to lowering of the body temperature, S-MIX duration is shorter, at 01:15.

event	segment #	days (T+X)	time (MST) duration	post-arrest	Tnaso (deg C)	CPS w/ ventil.	washout oxygen.	S-MIX (hh:mm)
Estimated time of cardiac arrest		T-0	17:15	00:00	37.0			
	seg 1		00:04	00:04	-0.5	no	no	00:04
Start cooling with crushed ice & place airway		T-0	17:19	00:04	36.5			
	seg 2		00:02	00:02	-0.3	no	no	00:02
Start mechanical chest compressions & ventilator		T-0	17:21	00:06	36.2			
	seg 3		00:37	00:37	-5.0	yes	no	00:15
Start transport of patient to funeral home		T-0	17:58	00:43	31.1			
	seg 4		00:20	00:20	-2.7	yes	no	00:06
Arrival of patient at funeral home		T-0	18:18	01:03	28.4			
	seg 5		00:22	00:22	-3.0	yes	no	00:05
Start field surgery (cannulation)		T-0	18:40	01:25	25.4			
	seg 6		00:17	00:17	-2.9	yes	no	00:03
Termination of cardiopulmonary support		T-0	18:57	01:42	22.5			
	seg 7		00:18	00:18	-1.3	no	no	00:06
Start of cephalic isolation		T-0	19:15	02:00	21.3			
	seg 8		00:06	00:06	-0.4	no	no	00:02
Completed cephalic isolation		T-0	19:21	02:06	20.9			
	seg 9		00:11	00:11	-0.8	no	no	00:03
Start of open circuit cryoprotection		T-0	19:32	02:17	20.1			
	seg 10		02:35	02:35	-20.0	no	no	00:28
Estimated temperature thru 0C		T-0	22:07	04:52	0.0			
totals:			04:52	04:52	-37.0			01:15

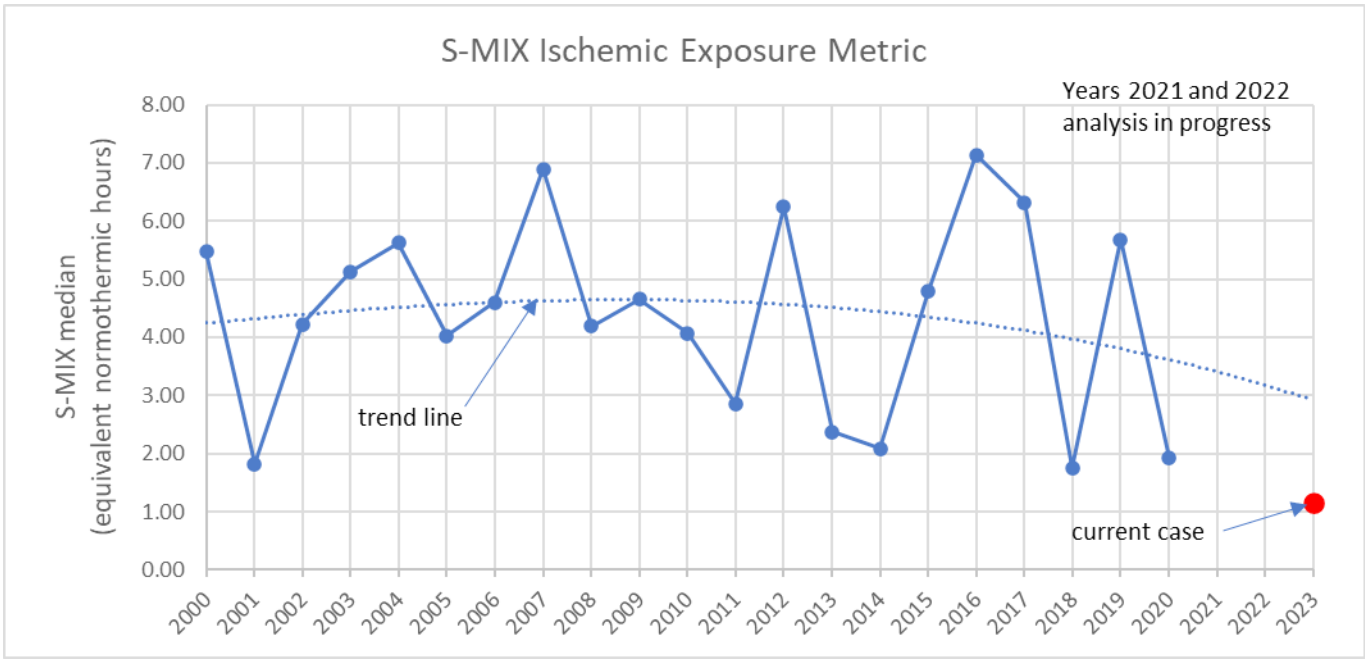
The below plots show events related to the S-MIX calculation. The red dots provide a metric for how fast the patient is cooled. This is a critical period since body temperature is highest and ischemic damage most rapid. The below table provides cooling data for 0, 10, 30, and 60 minutes after the team first applies water ice.

Patient Cooling Rate				
Note: time = 0 at start of ice cooling	0 min elapsed	10 min elapsed	30 min elapsed	60 min elapsed
Naso temperature (°C)	36.5	35.1	32.4	28.3
Temperature drop (°C) from t = 0	0.0	-1.4	-4.1	-8.2
Cooling rate (°C/min) from t = 0	N/A	-0.14	-0.14	-0.14


A-2705, Nasopharyngeal Temperature, Part 1
Temperature (deg C)
hours post-arrest
naso, measured
naso, estimated
start cooling with crushed ice
estimated from cardiac arrest until datalogger online (orange line)
10 min from ice cooling
30 min from ice cooling
60 min from ice cooling
cardiac arrest
start mechanical chest compressions & ventilator
start transport to funeral home
arrive at funeral home
start field surgery
end chest compressions
start cephalic isolation
end cephalic isolation
start open-circuit cryoprotection

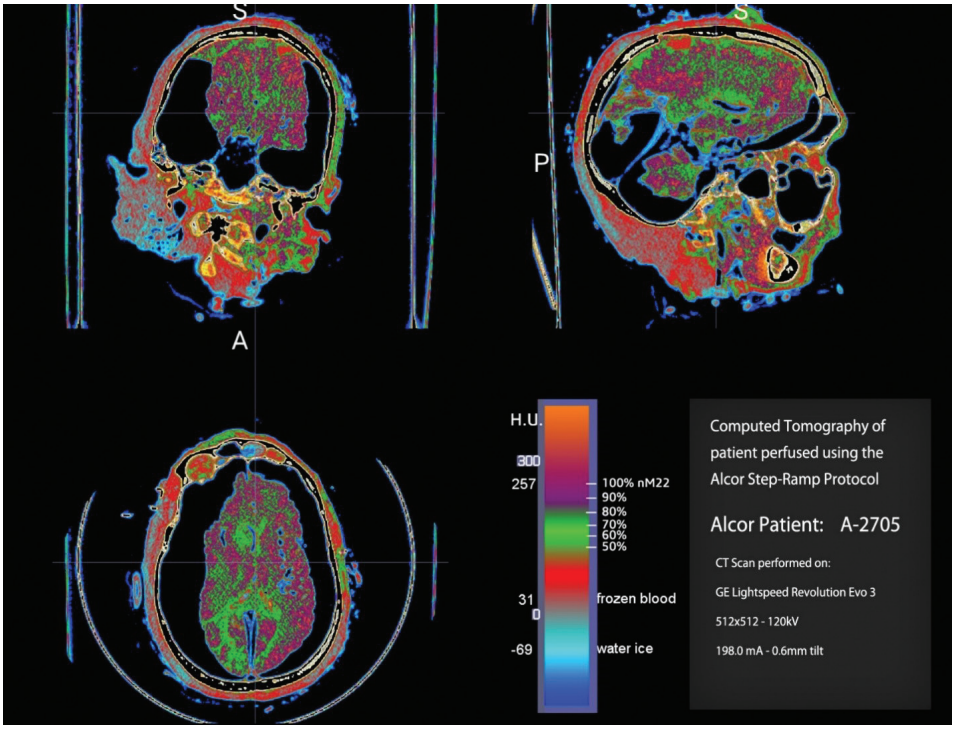

A-2705, Nasopharyngeal Temperature, Part 2
Temperature (deg C)
hours post-arrest
naso, measured
naso, estimated
estimated after datatlogger went offline
estimated patient temperature drops through 0°C
end open circuit cryoprotection
start dry ice cooling (T=-5.1°C recorded manually)

The following plot shows how the current case compares to prior years.



CT Scans

Cryoprotectant Distribution (Post-cryopreservation CT scan)



The post-cryogenic cooldown CT scans were obtained at 11:00 hrs on T+8 days; the patient was at liquid nitrogen temperature (-196°C).

CT visual analysis indicates that this patient's brain achieved between approximately 80% to 100% concentration needed to vitrify (CNV) and this can be seen on the single-slice 3-view CT image provided. The CT scans also indicate significant CPA-induced brain shrinking, an observation that is typically only seen in local or rapid field cryoprotection cases with rapid cardiopulmonary support (CPS). ■

Tissue Fixation: Preliminary Thoughts for a Proposed Experimental Study

By R. Michael Perry and Aschwin de Wolf

Here we report briefly on some plans to study immersion fixation of whole human brains. This is not a detailed "proposal" nor is it a technical article but some preliminary thoughts, as the title suggests. But we think it is a desirable step, as a followup of a recent theoretical study, in which a spherical model was used to approximate the human brain.[1] We need to proceed toward studies that use real fixatives and actual tissues, to assess the possible advantages of inducing biostasis through chemical means, either alone or in connection with some form of low-temperature storage.

In cryonics, currently and historically, the main step is to cool a legally deceased patient to cryogenic temperature, to achieve biostasis, the total arrest of biological activity. Once in biostasis the patient can wait indefinitely so that, hopefully, future technology can eventually be applied to revive them in a healthy, functioning state. Cryogenic cooling is assisted, when possible, by cryoprotective perfusion, to minimize damage to the tissues from the cooling process. Today cryopreservation is regarded as the best means of achieving biostasis for possible later revival, but it is costly and has other disadvantages that could be important, such as vulnerability to episodes of thawing/refreezing which could occur during future periods of economic hardship or social unrest. An alternative is chemical fixation of the tissues. Despite some difficulties, it offers a lower-cost pathway to biostasis which might also be used in combination with cryopreservation itself to improve the preservation process or make the patient less vulnerable to temporary warming.

Studies of fixation are warranted to assess its efficacy under varied conditions. We must consider which fixatives to use and their properties such as penetration rates into the specimen being fixed and details of the fixation process, including time requirements, after penetration has occurred. The brain, as the seat of personality, is the most important organ to preserve and accordingly deserves major emphasis. If at all possible, the vasculature of the brain should be used as the pathway of entry; the fixative then will much more rapidly reach its target volume. An issue then is how much time will be needed for fixation to occur given the tissue is well-exposed to fixative. Although we would like to think the fixation, once full exposure to fixative is achieved, will be near-instantaneous, such is not in general the case and may take some appreciable time, as with the commonly used fixative, formaldehyde (see discussion below). If the vasculature cannot be used, as is often the case, then fixation must occur through diffusion of the fixative into the specimen, a slower process in which the size, shape, and internal configuration of the target object are important.

A previous study of ours [1] considered diffusion fixation, which is also to be the focus for the proposed experimental work. A theoretical model was developed in which the human brain was approximated by a sphere and penetration rates comparable to experimental findings for formaldehyde and glutaraldehyde were assumed. The penetration itself was based on the mathematically equivalent theory of heat flow adapted and developed by Art Quaife as an approximation to conditions occurring in cryopreservation. [2] We considered (as did Quaife), in addition to the spherical model, a semi-infinite solid which has a planar face but extends behind it without limit. This model is useful and (despite possible appearances) not hard to duplicate to reasonable accuracy in a laboratory setting. (For instance, one can use glass pipettes sealed at one end, filled with gelatin, and immersed in a fixative bath. Fixation occurs through the open end only and forms a plug whose measured thickness indicates the depth of fixation, with modest allowance for shrinkage or swelling. The thin pipette of gelatin behaves in its penetration and fixation details as would an infinite, laterally uniform slab of material.[3].)

With a semi-infinite solid the penetration depth of diffusate (heat or other fluid, including, to a first approximation, fixative) is proportional to the square of time. This would result in times of hundreds of hours to penetrate to the center of the brain, approximately 7.2 cm, with penetration into a sphere of this radius assumed to be 0.36 cm in the first hour. (Penetration is said to occur when the concentration of fluid reaches 1 -1/*e* or about 63% of its value at the surface, where it is normalized to 1 and assumed to be maintained at this constant level throughout an experiment.) The spherical geometry, however, reduces this penetration time from hundreds to dozens of hours, on the order of a factor of ten. (It is noted too that the brain itself has a highly convoluted shape that should allow for more rapid penetration than a smooth, spherical surface. In addition, a very important component, the cerebral cortex, occupies a thin layer along the surface, which should further greatly reduce the time for significant penetration and fixation.)

As noted in the study, major simplifying assumptions, compared to actual or expected cases, were necessary to make the calculations reported, including the results shown in fig. 1. Much research is needed to clarify, correct, and extend such results. Two important

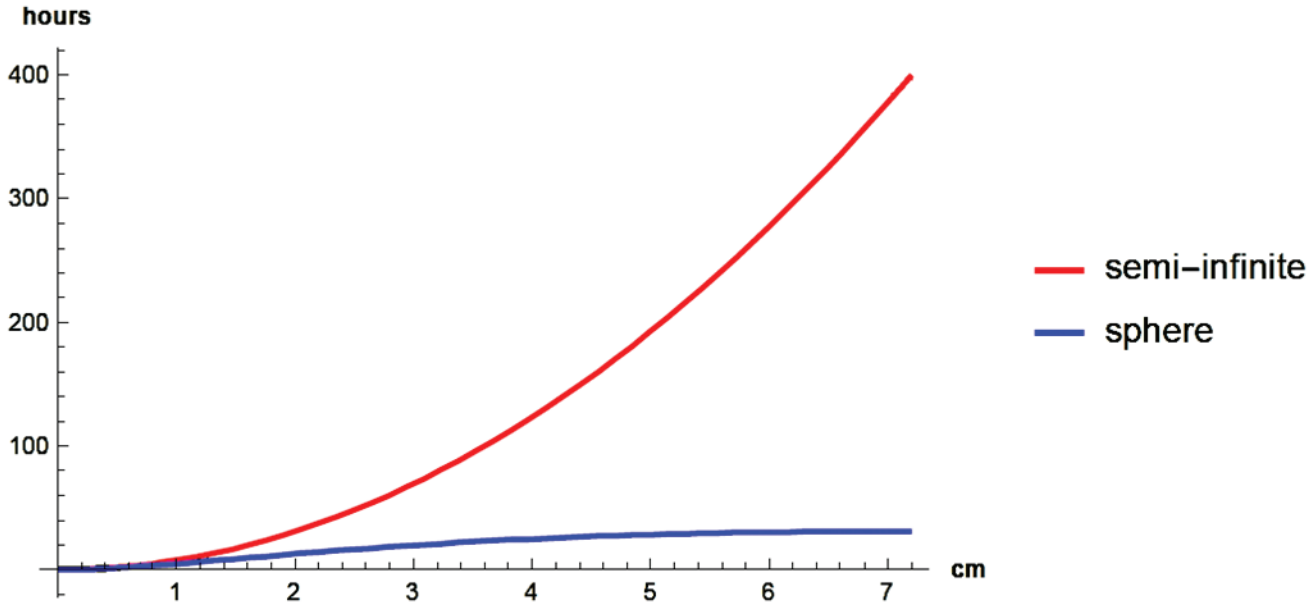


Fig. 1 (from [1]). Penetration time of hypothetical fixative under diffusion, chosen to approximate reported performance of formaldehyde and glutaraldehyde, as a function of distance, for the two cases of (1) sphere of radius 7.2 cm, approximating the human brain, and (2) the semi-infinite solid. (Temperature is 20°C.) Except near the beginning, penetration is much faster for the sphere (penetration times are less).

fixatives are formaldehyde and glutaraldehyde, and these would make good starting points for a fixative study. We are interested, specifically, in preserving entire human brains and in comparing the two fixatives, both acting independently and together, where each should offer advantages so that a mixture of the two could be better than each fixative alone.

Starting, then, with these two fixatives, there are two important issues that deserve clarification. First would be comparing the two side-by-side to assess their penetration and fixation properties as a function of time. This might be done in an "idealized" setting using gelatin in pipettes as in the experiment reported above. We then would want to determine how each fixative performs "in situ" in an actual human (cadaver) brain. Combinations of the two fixatives should also be assessed as to their performance, both idealized and in situ.

Fixation of tissues has a long history in laboratory use, and much literature is devoted to the performance of different fixatives in various settings. However, these settings by and large are very different from the possible use in cryonics of, mainly, preserving a whole human brain so that every significant portion will be decipherable by future technology. Thus we must be concerned with issues beyond the usual histological concern that *some* significant fraction of the cells will be well-preserved. The preservation should be as uniform as possible and it must be stable for at least a period of decades, maybe even centuries or more. (The stability issue might be mitigated somewhat by the prospect that the fixed brain might eventually be stored at cryogenic temperatures, but in any case stability beyond that needed for the usual histological studies is a requirement.)

In the proposed study we will also have to confront special properties of the fixatives tested. Formaldehyde in this respect is peculiar. It penetrates relatively rapidly but then takes time to fix the tissue. The fixation in fact occurs in two stages. The initial fixation is said to take 24-48 hours and to be reversible. The "harder," more complete fixation takes order of 30 days (768 hours)![4] The graph of fig. 1 will be relevant only if the first, lesser fixation is at least adequate to "hold the line" until the second, stronger variety can take over (or until some other fixative that is also part of the mixture, e.g. glutaraldehyde, can perform this function).

Another important consideration is temperature. In our initial article (referenced above), we calculated that perfusion at low temperatures (between 0 degrees and 4 degrees Celsius) gives the best trade-off between diffusion time and minimizing ischemia. This result reinforces the premise from hypothermic organ preservation that metabolic activity is reduced by a greater rate than diffusion time at low temperature.

One of the biggest challenges for immersion fixation of human brains is how to determine whether a fixative has penetrated the core of the brain. Imaging and ultrasound techniques have

been used by researchers to understand this, but not all of these techniques will be practical or cost-effective for our purposes. If we determine the brain penetration rate of a given fixative at a given temperature, can we assume that this finding can be used for all future cases? If we follow fixation by immersion cryoprotection, when do we move from one concentration to another? Can we use a very high concentration of CPA (cryoprotectant agent) and store at subzero but not cryogenic temperatures?

These, then, are some thoughts as we contemplate a series of tissue and cadaver experiments to further assess the suitability of using fixatives to achieve biostasis for cryonics purposes. Criticism, comments, and suggestions are welcome. ■

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Evan Cooper: An Untold Story

By R. Michael Perry, Ph.D.

Robert Ettinger is usually considered to be the "founder of cryonics." His book exploring the idea (before the term "cryonics" had been invented for it) under the title, *The Prospect of Immortality*, was published commercially by Doubleday in June 1964. (Before this, he had written a science fiction story exploring the cryonics idea, "The Penultimate Trump," published in the March 1948 issue of *Startling Stories*.) And Ettinger's later involvement was long and productive and culminated in his own cryopreservation, in 2011 at age 92, at the organization he largely founded, the Cryonics Institute.[1]

Even before the Doubleday sendoff, though, there was a well-organized movement devoted to the cryonics idea, with a newsletter and a band of loyal devotees who sometimes contributed their thoughts in a reader's column. The organizer of this group was not Ettinger, who lived in Michigan, but a shy, secretive thinker and boating enthusiast who hailed from Washington, D.C. Evan "Ev" Cooper, as he called himself, also independently wrote a book of his own, under the pen name Nathan Duhring, at nearly the same time as Ettinger, which he titled *Immortality: Physically, Scientifically, Now*. Essentially it offered the same argument as Ettinger's, namely, that by freezing and storing the newly deceased, you provided a chance of reviving them someday, when ever-advancing technology might make it possible. Cooper also started an organization, the Life Extension Society, which was the first ever to promote the cryonics idea and was the first to stage conferences and as noted, publish a newsletter.[2]

So, just who was Evan Cooper? Where was he born and when, who were his parents, where did he grow up, what education did he have, what led him to help start the field of cryonics? Cooper had been married, that much had long been known. He and his wife Mildred separated in 1970; the marriage had been childless, and it appeared that Cooper had never had any other offspring. Some tantalizing further clues seemed to be obtainable from Alcor's archives. There is a taped interview with Mike Darwin from January 1983, about three months after Cooper was lost at sea. Mildred gave Mike a few details about her former husband. He was born in Seattle, Washington, she said, in April 1926. She wasn't sure of the exact day but thought it might be the 7th.

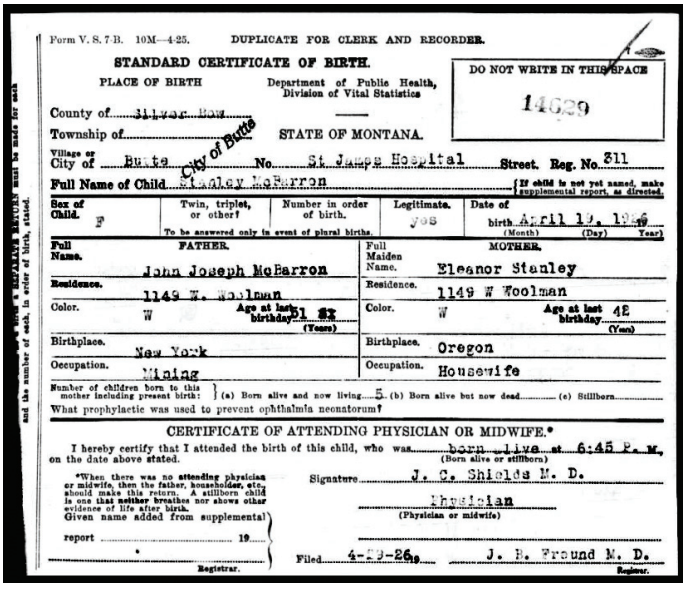
Not sure of the day of the month your husband was born on? Well, that seemed a little odd, but Cooper was secretive, wasn't he? Mildred explained in the interview that he didn't like to be reminded of his birthday. I tried to follow up on the few other details she had provided. I looked in the 1930 census (many of these records are online now) for a boy of about 4 years' age named Evan Cooper living in or around Seattle but came up with no good candidates. Did Cooper have another name then, or was he already living somewhere else? I did find in this census an adult gentleman in Seattle named Evan G. Cooper who possibly, I thought, could have been the boy's father, and I did more research.[3]

Evan Goodwin Cooper was born in Tustin, California in 1885 and died in Reno, Nevada in 1951. Between these limits he lived at various residences in California, Wyoming, Washington State, and finally, Nevada. In the records where "Evan G. Cooper" occurs it seems to refer to this one person and I have made that assumption. In the 1910 Census in San Francisco he is a soldier in the U.S. Army. After this his occupation in the different censuses (1920, '30, '40) is "carpenter," until. in 1950, he is "retired." He is buried in the Golden Gate National Cemetery in San Bruno, California. The tombstone says he was "Musician 1st Class" in the Army. In no place is he ever said to have a wife or family, including the 1930 census in Seattle. If the younger Evan was his son, the records were certainly being reticent. And I find no clear candidates in the records for a Mr. Cooper who could have been this younger man, at least into the 1960s. (After this you start to find him in records relating to cryonics and life extension, that's about all, given that "Evan Cooper" is a fairly common name so that disambiguating scant mentions can be difficult.)

There the matter might have rested, but for a fortunate break. Some years ago I was contacted by someone whom I'll anonymize to a gentleman named "Morgan." Morgan was born out of wedlock in the mid-1970s. His father was known to his mother and others as Evan Cooper, the same man who started a "Life Extension Society" to promote the idea of extending the human life-span through science. Later, they knew, Cooper abandoned the life extension movement and spent his time sailing up and down the Atlantic coast in a privately owned boat, stopping off at different ports. After his loss at sea in October 1982, a friend commented:

> "Ev Cooper may not have kept interest in expanding the length of human life, but in the last fifteen years he certainly added to the quality of it, both his own and those lucky enough to have known him. He was absolutely free – he made a science of self-sufficiency – and was well liked wherever he went. I know this sounds suspiciously like I'm eulogizing, but it's true: Ev was quite happy and thoroughly good humored about everything, even negative things. Ev was never bitter, never spoke of the past, and remained 100% cheerful and optimistic, completely content with his existence, never frustrated."[4]

Though very young at the start of our correspondence, Morgan was interested in Mr. Cooper both as his father and in terms of the life-extension movement Cooper had helped get started. Later Morgan started researching his family history with the help of online tools. Meanwhile a new technique, DNA matching, became available to help establish family ties when records were scarce or unavailable. Finally, some results of interest came in: there was a close match with a family having the surname McBarron. A search of records showed one Stanley Edward McBarron, who it seems is the father of Morgan and thus one and the same as the life extensionist Evan Cooper.[5][6]



Form V. S. 7-B. 10M—4-25. DUPLICATE FOR CLERK AND RECORDER.

STANDARD CERTIFICATE OF BIRTH.

PLACE OF BIRTH — Department of Public Health, Division of Vital Statistics

DO NOT WRITE IN THIS SPACE 14629

County of Silver Bow

Township of — STATE OF MONTANA.

Village or City of Butte (City of Butte) No. St James Hospital Street. Reg. No. 811

Full Name of Child Stanley McBarron (If child is not yet named, make supplemental report, as directed.)

Sex of Child.	Twin, triplet, or other?	Number in order of birth.	Legitimate.	Date of birth
F	To be answered only in event of plural births.		yes	April 19, 1926 (Month) (Day) Year

FATHER.		MOTHER.	
Full Name.	John Joseph McBarron	Full Maiden Name.	Eleanor Stanley
Residence.	1149 W. Woolman	Residence.	1149 W Woolman
Color.	W	Color.	W
Age at last birthday.	51 yr (Years)	Age at last birthday.	48 (Years)
Birthplace.	New York	Birthplace.	Oregon
Occupation.	Mining	Occupation.	Housewife

Number of children born to this mother including present birth: (a) Born alive and now living 5 (b) Born alive but now dead (c) Stillborn

What prophylactic was used to prevent ophthalmia neonatorum?

CERTIFICATE OF ATTENDING PHYSICIAN OR MIDWIFE.*

I hereby certify that I attended the birth of this child, who was born alive at 6:45 P. M. on the date above stated. (Born alive or stillborn)

*When there was no attending physician or midwife, then the father, householder, etc., should make this return. A stillborn child is one that neither breathes nor shows other evidence of life after birth.

Given name added from supplemental report ..... 19.....

Signature J. C. Shields M. D. Physician (Physician or midwife)

Filed 4-29-26 J. B. Freund M. D. Registrar. Registrar.

Stanley was born April 19, 1926, not in Seattle but in Butte, Montana, the son of John Joseph McBarron and Eleanor "Elsie" Stanley.[7] The McBarrons appear to have lived in Butte for many years but had relocated to Seattle by May 1929 when Stanley would have been 3 years old, and possibly some time before this, so he may have felt or just assumed he was born there. His father was a wealthy mining entrepreneur, whose activities were occasionally reported in local news publications, like this short article from 1904:

> "John McBarron, who is connected with the Green Campbell Mining Company, exhibited in the city Iast week several specimens of rich gold quartz that were taken from the Green Campbell mine near Silver Star in 1867. They were literally full of gold. The specimens were in the possession of Mrs. Everett, whose husband owned the property, from the time of their extraction from the vein until a few weeks ago, at which time they were turned over to Mr. McBarron by her in Cleveland, Ohio. Mrs. Everett is now a resident of that city, but was in Madison county [Montana] with her husband In the '60s. The transfer of the specimens was made at the time Mr. McBarron bought the interest in the property held by the Everett estate."

In another note, this on "new corporations," the Great Falls, MT *Daily Tribune*, Oct. 29, 1920, refers to the "Obelisk Mining company of Butte, formed by John McBarron, Frank V. Whitman, William Hogan, F. A. Gilbert, Richard McCarthy, with a capitalization of $1,000,000 …"

And in May 1929, young Stanley is mentioned by name:

> "Mr. and Mrs. John McBarron and small son Stanley Edward, of Seattle are house guests of Mr. and Mrs. C. F. McBarron at a Mother's Day family re-union. C. F. McBarron presented Mrs. McBarron with a new automobile."

Stanley's mother died in 1943 before his father, who passed in 1947. During the closing years of WWII young Stanley served in the Navy, already showing an interest in seafaring. In 1947 Stanley attended the University of Washington and also, in December, got married, to Miss Frances O'Brien, and would have been in line to inherit a share of the family estate, divided presumably among several siblings.

I have so far been unable to find any record of the settlement of the McBarron estate. Did the family contrive to keep it secret, or are the relevant records still not public or not easily available? In any case Cooper was said on good authority to be a "remittance man" who, though not wealthy, evidently lived on a small inheritance.[8]

Three views, one person. (1) Stan McBarron, 1947 U. of Wash. yearbook; (2) Ev Cooper the life extensionist, Maclean's, Apr. 2, 1966; (3) Cooper the "bearded boatman" of later years.

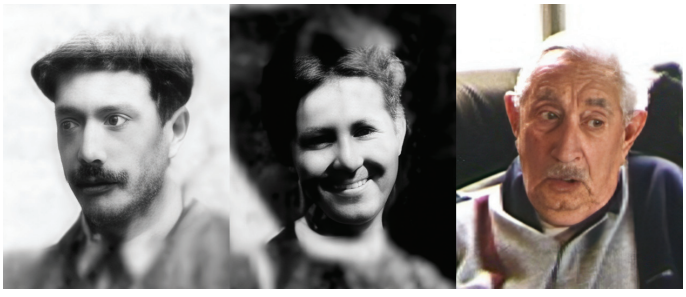
Going on, however, the 1950 Census shows, in Skagit, Washington, Stanley E and Frances T McBarron with two young children; a third was born the following year, when the family was living in Portland, Oregon.[9] After this it appears the family moved to Florida. The Miami News, Sunday, 28 Nov. 1954, p. 22 has an article with the title "Miami Star Class Eliminations Start." The opening paragraph gives details:

> "Miami's Star class racing fleet, which includes one former title-holder and an Olympic representative, begin eliminations Saturday to select a team for three

important events in neighboring waters next year – the Mid-Winter, Spring, and World sailing championships."

Continuing on, the article notes that there are seven craft in the Miami organization and eight skippers, two of them sharing one boat – it doesn't say which. One of the skippers, however, is Stan McBarron – so it appears that by then he was involved in sailboat racing – though it doesn't appear that he won any major races.

The next event I find in the records, from September 1956, Dade, Florida, may be the trigger that led to a major life change. Stanley and Frances McBarron divorced. Within a few years Stanley, under the new name Evan Cooper, would be living in Washington, DC and starting his life extension organization. It appeared he decided to make a complete break with the past, or did he continue to support his young family? These and many other questions must remain mysteries for now. We can still speculate, however, that the name Evan Cooper was not just random but derived from the man who had lived close to the McBarrons around 1930. (With online help I was able to determine that they were only about 6 miles apart. The McBarrons lived at 7510 14th Ave. NE and Mr. Cooper at 3409 15th Ave. W.) Perhaps Mr. Cooper was acquainted with the McBarrons and did carpentry work for them, or even worked on their boat, if they had one. In any case, the man we know as Evan Cooper would make an interesting subject for a full-length biography. ■



Family. From left: father John Joseph McBarron; (probably) mother Elsie (Stanley) McBarron, both from a family outing photo around 1900-1910; a later photo of their oldest son, John Stanley McBarron (1908-2004) [10], who shows a striking resemblance to his younger brother.

Significant others, from left: Francis (O'Brien) McBarron; Mildred Cooper; Morgan's mother.

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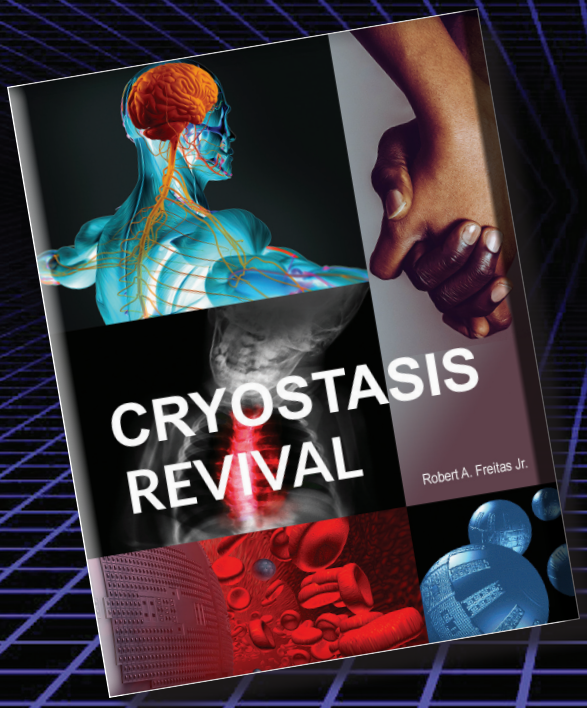
Sharpening, colorization, and/or other possible rendering were used in some of the images in this article.

New Book by Robert A. Freitas Jr.

Cryostasis Revival: The Recovery of Cryonics Patients through Nanomedicine



Cryostasis is an emergency medical procedure in which a human patient is placed in biological stasis at cryogenic temperatures. A cryopreserved patient can be maintained in this condition indefinitely without suffering additional degradation, but cannot yet be revived using currently available technology. This book presents the first comprehensive conceptual protocol for revival from human cryopreservation, using medical nanorobots. The revival methods presented in this book involve three stages: (1) collecting information from preserved structure, (2) computing how to fix damaged structure, and (3) implementing the repair procedure using nanorobots manufactured in a nanofactory – a system for atomically precise manufacturing that is now visible on the technological horizon.

"Robert Freitas is an extraordinary thinker and author whose previous works have been transformational for our ability to visualize the extraordinary capabilities of future medical technology. In Cryostasis Revival, he now puts his prodigious previous knowledge of nanomedicine to the task of envisioning methods for healing those whose injuries challenge even the ultimate limits of future medicine. His illuminating results and new insights will greatly inform debate over, and may even help to resolve, controversies that have persisted for decades." — ***Gregory M. Fahy, Ph.D., Fellow, Society for Cryobiology & Executive Director, 21st Century Medicine, Inc.***

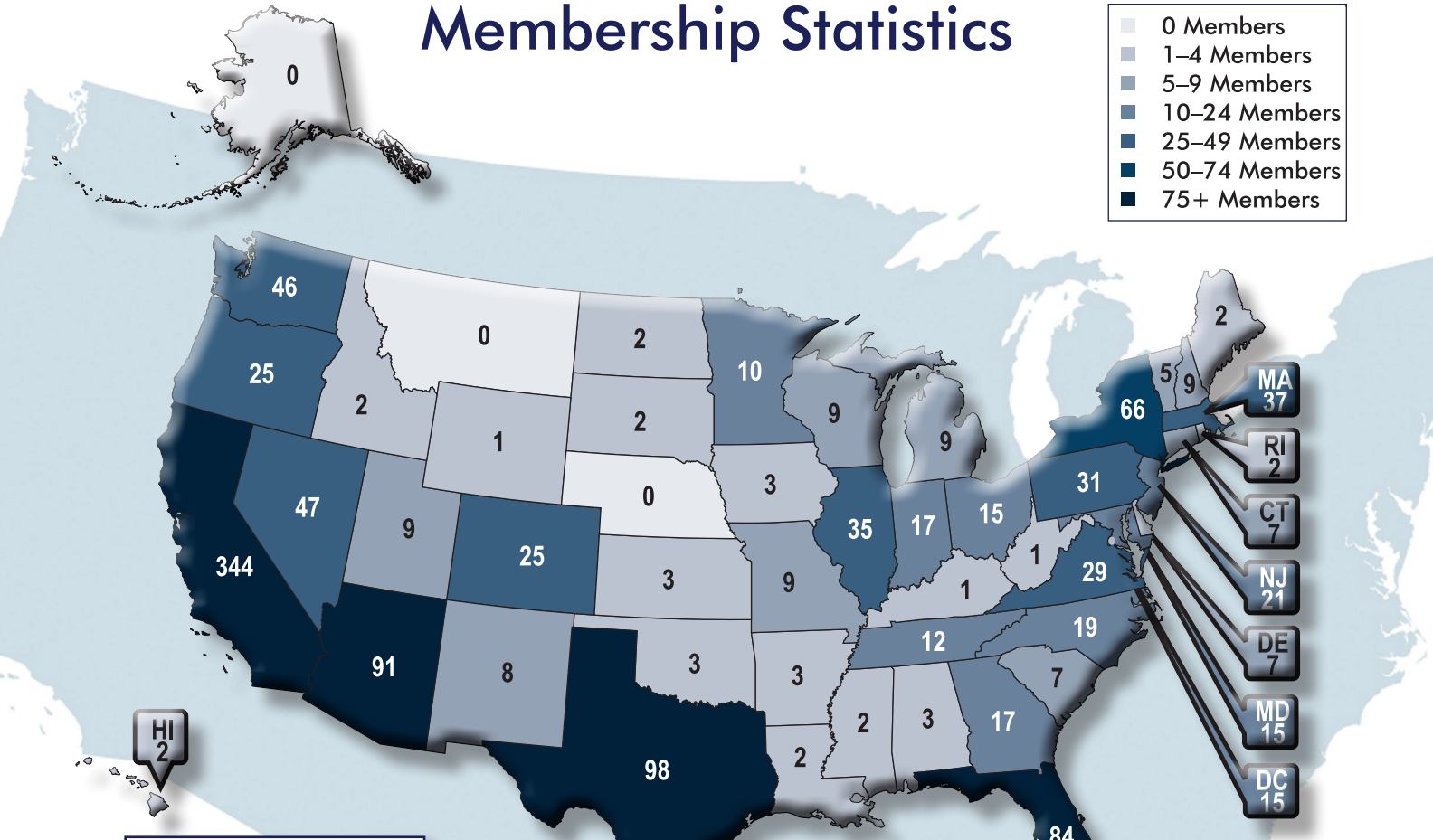
"Future repair and revival of damaged cryopreserved tissue has been the subject of speculation for decades. This book by a nanomedicine expert examines the problem in detail far beyond anything ever written before. With more than 3000 references, it's both wide-ranging and intensely specific about diverse technical aspects of the problem. It will surely stimulate much discussion, and be an invaluable resource for thinkers about nanomedical cell repair for years to come." — ***Brian Wowk, Ph.D., complex systems cryobiologist, Chief Technology Officer, 21st Century Medicine, Inc.***

"We now have considerable evidence that cryopreserved patients retain the physical structures encoding memory and personality. For most people, the difficulty lies in understanding how it could ever be possible to repair and revive patients. Leading nanomedicine expert Robert Freitas fills in that gap with admirable and remarkable depth. Cryostasis Revival provides an unparalleled clarification of pathways for researchers to explore in the quest to make human cryopreservation reversible." — ***Max More, Ph.D., Ambassador, Alcor Life Extension Foundation***

"Cryostasis Revival is the most magnificent tour de force on cryonics ever done with the signature flair, comprehensive coverage and authoritative style of Robert A. Freitas Jr. It describes all the issues involved in reviving cryopreserved patients: from the philosophical (what is "information theoretic death") to the practical (what damage actually takes place during a cryopreservation) to the technological (how to apply nanotechnology to restore a cryopreserved patient) and more. Nothing else even approaches such a complete and incisive treatment of this life-saving subject. Cryostasis Revival is the book to give anyone who's thinking about cryonics but "isn't sure about the science." — ***Ralph C. Merkle, Ph.D., Senior Research Fellow, Institute for Molecular Manufacturing***

Free electronic book and hardback copies for sale at:
https://www.alcor.org/cryostasis-revival or Amazon.com

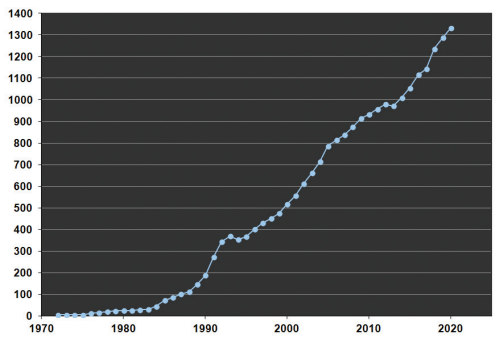
Membership Statistics



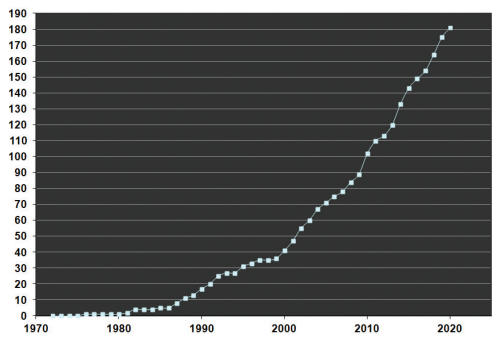
International Members & Patients

Country	Members	Patients
Australia	15	3
Austria	1	0
Belgium	1	0
Brazil	1	0
Bulgaria	1	0
Canada	68	5
China	0	1
Croatia	1	0
Finland	1	0
France	2	1
Germany	19	0
Hong Kong	2	0
Hungary	1	0
Israel	1	1
Italy	1	0
Japan	6	0
Luxembourg	1	0
Mexico	5	0
Monaco	1	0
Netherlands	1	0
New Zealand	2	0
Norway	2	0
Portugal	5	1
Puerto Rico	3	0
Slovenia	1	0
Spain	4	1
Sweden	1	0
Switzerland	7	0
Taiwan	1	0
Thailand	3	1
United Kingdom	47	4
Virgin Islands	1	0
TOTAL	206	18

2023	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Cryo Members	1411	1412	1417									
Basic Members	32	33	36									
Patients	203	204	205									
Assoc./Apps	218	221	218									
TOTAL	**1864**	**1870**	**1876**									



Number of Alcor members



Number of Alcor patients

Alcor Longevity Circle of Distinguished Donors

The Alcor Board of Directors is pleased to announce the formation of the **Alcor Longevity Circle of Distinguished Donors**. This new organization will honor those members and their foundations that have donated in excess of $100,000 over the past few years to support Alcor and its affiliated organizations. In addition to being recognized in Alcor publications and at conferences and other events, members will also be entitled to:

- Exclusive access and a quarterly conference call with Alcor Directors, officers, and officials to get in-depth briefings and ask questions and make suggestions.
- Special recognition, seating, and access to officials at Alcor conferences.
- An exclusive yearly, hosted in-person event honoring members with face-to-face interaction with Alcor Directors, officers, and officials.
- A unique, professionally designed and engraved memento of their membership.

These benefits are, of course, overshadowed by the immense gratitude members' and patients' families will always have for these especially generous individuals. New levels of membership (higher and lower levels of participation) may also be announced in the future. ■

Support Alcor's **RAPID** Research

Readiness **A**nd **P**rocedure **I**nnovation/**D**eployment **(RAPID)**

In order to advance the science and reputation of cryonics, Alcor plans to conduct ongoing research to develop novel and near-future products related to cryopreservation procedures and protocols. The RAPID team is developing relationships and contracts to procure recently deceased human cadavers, which are not Alcor members or patients, but are already earmarked for medical research. The idea is to procure one to two cadavers per month to conduct research. We would go on a "light standby" to enable fast access to cadavers.

The RAPID initiative will support cryonics research in multiple ways. Most immediately, it will help advance research into liquid ventilation – using a patient's lungs as a heat exchanger to induce very rapid hypothermia. Animal studies alone cannot take LV development to the next level due to different chest anatomy. LV research will include cooling rate control; chest compression studies; and timing and sensor feedback.

RAPID will also enable research comparing chemical fixation to cryoprotection and will support rewarming studies. Another benefit will be a great improvement in cryonics-specific surgical training. That includes raising and cannulating the carotids; cephalic isolation; raising and cannulating the femoral arteries; field neuro procedure training; median sternotomy training; and alternate surgical approaches.

Alcor is requesting donations through GoFundMe. All donors will receive quarterly reports from Alcor regarding the progress with fundraising and milestone achievements rising from the RAPID program! Please donate today to support Alcor's RAPID initiative. Alcor is a non-profit, federally tax-exempt, 501(c)(3) corporation and your donation may be tax deductible. ■

Donate here: https://charity.gofundme.com/o/en/campaign/rapid-research/alcorlifeextensionfo

For more information, see the presentation here: https://www.youtube.com/watch?v=BUaVcVMuFWQ&feature=youtu.be

Fight Aging!

Reports From the Front Line in the Fight Against Aging

Reported by Reason

Fight Aging! exists to help ensure that initiatives with a good shot at greatly extending healthy human longevity become well known, supported, and accepted throughout the world. To this end, Fight Aging! publishes material intended to publicize, educate, and raise awareness of progress in longevity science, as well as the potential offered by future research. These are activities that form a vital step on the road towards far healthier, far longer lives for all.

A Small Lifespan Study of Combined Interventions

September, 2022

My attention was drawn recently to a small mouse life span study run by one of the groups that has been in the longevity community for a while now. It is interesting for testing combinations of interventions that have in the past been demonstrated to modestly slow aging in mice (such as rapamycin), or modestly improve aspects of cell function in old tissues (such as nicotinamide mononucleotide). Combinatorial studies are rare in academia and industry, for reasons that have a lot to do with (a) the perverse incentives produced by the existence of intellectual property, in that the rights to use specific interventions can be owned, granted, refused and (b) the way in which the huge cost of regulatory approval determines which projects that can be successfully funded, typically only those in which patents grant a monopoly on use.

The results are much as one might expect, given the interventions chosen, in that most of the combinations did little to nothing to mouse survival and life span. The only one that appears to have an effect is the use of C60 - an intervention that, you might recall, has a checkered history in animal studies. The most recent data, from Ichor Therapeutics and others, who spent some years working with C60, is that it is not a useful intervention in the matter of modestly slowing aging.

Unfortunately, this study did not control for inadvertent calorie restriction. When an intervention makes mice feel ill, they will eat less. Mou**se weight is a sensitive barometer of mouse well-being. Even minor degrees of calorie restriction can extend mouse life span, distorting the effects of interventions.** This is one of the reasons why rigorous studies, such as those conducted by the Interventions Testing Program, tend to find no effect when repeating earlier studies in which an intervention was claimed to modestly slow aging. Sadly, this means that positive outcomes here don't have all that much weight, and it is possible that some of the neutral outcomes are actually poor outcomes.

Bucky Labs Longevity Study

Our mouse longevity study completed with interesting results. Frankly, we did not know what to expect. We tested our products and other promising substances on 245 interbred male C57BL/6 mice. We started the interventions when mice were 300 days old (about 50 in human yrs). Caveats: the sample sizes were very small, optimal dosages were guesses, and we did not weigh the mice - so some effects may be from dietary restriction, etc.

1 C60 99.95 Olive Oil 10%

2 C60 in MCT oil 10%

4 cycloastragenol, NMN, fisetin, icariin, berberine, cistanche, AFA algae

5 exosomes, klotho, FOXO4-DRI, gdf11, epitalon

6 rapamycin, Azithromycin, metformin, NMN, spermidine, echinacea

7 NMN, fisetin, C60

8 RG7834, DHEA, berberine, fisetin, NMN

9 berberine, BHB, NMN, ALA, cycloastragenol, spermidine, DHEA, rhodiola, fisetin, icariin, echinacea, cistanche

10 rapamycin, metformin, aspirin, niacin, RG7834, spermidine, FOXO4-DRI, gdf11

11 centrophenoxine, exosomes, fisetin, metformin

12 double dose fisetin, double NMN, double cycloastragenol

13 klotho, RG7834, spermidine

15 MOTS-C

16 gdf11

17 spermidine

18 double NMN, double berberine, double centrophenoxine, double cycloastragenol, double fisetin

20 NMN, ALA, pterostilbene, cycloastragenol, centrophenoxine, spermidine, DHEA, melatonin, rhodiola, luteolin, fisetin, icariin, echinacea, cistanche, carnitine

21 double fisetin, double NMN, double berberine, NAC, DHEA, echinacea, cistanche

The best intervention was Intervention 1 (red line), C60 Olive Oil (the mouse feed was supplemented with about 10% C60 in organic olive oil). This group also had the largest number of mice (16), so the confidence that something real is happening is greatest with this intervention. The next best group was Intervention 9 (NMN, spermidine, berberine, BHB, ALA, cycloastragenol, dhea, rhodiola, fisetin, icariin, echinacea, cistanche). The following next best interventions are clustered closely around the control, so no conclusions should be made. Surprising that the poorest performer was Intervention #20 (NMN, ALA, pterostilbene, cycloastragenol, centrophenoxine, spermidine, DHEA, melatonin, rhodiola, luteolin, fisetin, icariin, echinacea, cistanche, carnitine) which is similar to the 2nd best performer. Also, Intervention #8 (RG7834, DHEA, berberine, fisetin, NMN) did not do well.

The results with our peptides/proteins did not appear to result in any significant longevity increases. Also, surprising was that the interventions with rapamycin did not appear to produce significant improvements. Lastly, ours is the first lifespan study to investigate C60 with an alternative lipid, we tried MCT oil (basically coconut), and there was no lifespan improvement.

Link: https://www.buckylabs.com/longevity-study/

Testing Narrow Epigenetic Clocks in Centenarians

October, 2022

Work on immunotherapies that can clear amyloid-β from the brain, an approach to treating

Many different epigenetic clocks have been proposed and tested in recent years, all using different weighted combinations of DNA methylation status at various CpG sites on the genome, some using fewer than ten sites, others using hundreds of sites. DNA methylation is in constant flux, regulating gene expression in cells, but some changes are characteristic of age, and machine learning approaches have produced clocks with strong correlations to chronological age. Where clock age is higher than chronological age, individuals have been shown to have greater incidence and risk of age-related disease and mortality.

Researchers still, however, do not have more than the rudimentary beginnings of a map to link methylation at specific CpG sites to the underlying damage and dysfunction of aging. Thus it is hard to treat epigenetic clock data as actionable for any given individual and their treatments. The clocks are quite good for unmodified aging, but what we really want is a way to cost-effectively, rapidly assess the outcome of potential rejuvenation therapies, each of which will tend to only directly affect one of the many mechanisms of aging, without undertaking the time and expense of life span studies.

Given this, it is hard to trust narrow epigenetic clocks that use few CpG sites. They seem very unlikely to accurately reflect all of the processes of aging, and thus even trying to calibrate them against specific therapies seems likely to produce poor results. Nonetheless, since such narrow clocks are cheaper than broad clocks using hundreds of CpG sites, many research groups are working in this direction.

Centenarians consistently present a younger epigenetic age than their chronological age with four epigenetic clocks based on a small number of CpG sites

The study of DNA methylation in human aging has revealed the occurrence of two types of age-related DNA methylation changes. The first, known as epigenetic drift, is characterized by the progressive divergence of the methylome of individuals acquired environmentally and stochastically across their lifespan, which even affects monozygotic twins. The second type of DNA methylation changes is called the epigenetic clock and refers to all age-related DNA methylation variations that consistently increase or decrease in every individual, thereby correlating to their chronological age.

The latter type of epigenetic modifications has been widely used as biomarkers of aging in several age-prediction models to estimate the chronological and biological age of individuals, mainly from blood DNA samples. These models are based on multiple regression, machine learning, and deep learning approaches using either a large number of CpGs requiring high-throughput technologies such as genome-wide epigenotyping array or a smaller number of CpGs requiring high resolution locus-specific methods such as pyrosequencing. DNA methylation-based age (DNAmage) prediction has proven to be of great interest in several bio-medical applications. It could notably give a better estimation of the biological age than chronological age and could also be a good indicator or predicator of different risks, health conditions and age-related diseases when compared to the chronological age.

In the present study, we investigated the DNAmage of French long-lived individuals (LLI) including centenarians and semi-supercentenarians (n = 214), as well as nonagenarian's and centenarian's offspring (n = 143) of the CEPH aging cohort using blood extracted DNA and four epigenetic clocks based on a small number of CpGs and locus-specific pyrosequencing. These clocks, known as Bekaert, Thong, Garali MQR and Garali GBR clocks, were developed from 2 to 4 CpGs located in the promoters of 1 to 4 genes (ASPA, EDARADD, ELOVL2, KLF14, PDE4C, and TRIM59).

Compared to their chronological age, DNAmage of centenarians and semi-supercentenarians was strongly underestimated (15 to 28.5 years in average), which was still strongly significantly underestimated when compared to control group DNAmage (10.8 to 21 years in average). This might indicate that the epigenetic clock and potentially aging were decelerated in exceptionally long-lived individuals, who presented younger DNAmage and potentially also younger biological age.

Link: https://www.aging-us.com/article/204316/text

Notes from the Rejuvenation Startup Summit, Held in Berlin in October 2022

October, 2022

A fair number of longevity industry and related companies presented this past weekend in Berlin, at the Rejuvenation Startup Summit hosted by the Forever Healthy Foundation. Unlike the past Undoing Aging events, this is much more focused on the industry rather than on scientific programs, but there was nonetheless a great deal of science on display. I took a few notes in between other activities, for posterity. As Michael Greve noted in his introduction to the participants, these are the early years of what will become the largest industry on the planet. Everyone ages, and everyone is a customer for the rejuvenation therapies and related technologies that lie just around the corner.

Eric Verdin of the Buck Institute gave the opening keynote, discussing recent work on biomarkers of aging, and specifically the most promising line of epigenetic clocks based on assessment of DNA methylation status. Given ways to reliably extend life in mice (e.g. rapamycin and senolytics), we now need a way to measure that outcome that doesn't involve waiting around for the results of a life span study. The point made in this presentation was that different immune cell populations exhibit sizable differences in assessed epigenetic age, which probably means that all clock data based on blood samples is suspect. The Buck Institute researchers have found differences of as much as ~20 years in epigenetic age between immune cell types, as well as differences based on infection and inflammation status, so clearly more care needs to be taken here. In an attempt to address this issue, the team built a new clock that is invariant across immune cell subpopulations. We will no doubt be hearing more about this as it progresses, given the prevalence of work that uses epigenetic age derived from blood samples.

Lou Hawthorne of Nanotics gave an outline of their technology platform, a way to produce particles that bind specific molecules in the blood stream in a controllable way, depleting them for minutes to hours. Heterochronic parabiosis research has led to evidence for harmful factors to circulate in the aged bloodstream, maintaining inflammation and dysfunction, which naturally leads to the desire to remove these factors in a targeted way. Nanotics is particularly focused on pro-inflammatory factors, and their view of aging is inflammation-centric. Thus the cytokine storm of sepsis is not a bad starting place to test this sort of therapy in the clinic. The Nanotics platform allows the targeting of signal processes that are inaccessible to small molecule therapeutics, so offers ways to potentially dial down inflammatory signaling without also blocking necessary immune functions.

Alexander Schueller of cellvie discussed their view of the mitochondrial dysfunction observed in aging, and the relevance of mitochondrial damage caused by ischemia to the cell death and dysfunction following ischemic injury. The cellvie approach is to deliver replacement mitochondria to be taken up by cells in need. Like the other companies working on this approach, they are near entirely focused on the logistics and process development needed for this goal. Their intent to is to generate allogeneic mitochondria, harvested from standard cell lines. Once ready, cellvie is looking at sarcopenia as a first indication for clinical development, based on promising animal data.

Vlad Vitoc of Maia Biotechnology gave an impressive overview of their progress towards a near-universal cancer therapy. They develop therapies based delivery of THIO, a compound that is metabolised and utilized by telomerase, and then incorporated into telomeres to produce cell death. Since nearly all cancer cells aggressively utilize telomerase, these are the cells that die when THIO is introduced. The company has orphan drug designations for a variety of cancers, and are well advanced in the path to clinical trials. A first phase 1 is running now, with further trials coming up in next few years, including one phase 2 just starting in Australia. These trials are conducted in partnership with Regeneron, and they put THIO into patients in conjunction with a Regeneron-developed checkpoint inhibitor therapy. The company went public recently, and they are using the sizable funding they have raised to date in order to build new and more efficient versions of THIO. We should expect the important questions regarding telomerase as a target to be answered in the years ahead, now that it is an ongoing project, such as how to manage the effects of telomerase-targeted therapeutics on stem cell function, and what those effects are in practice.

Chris Rinsch of Amazentis talked about the use of urolithin

A as supplement-based approach to improving mitochondrial function in aged individuals. Their initial aim is to look at muscle function in aging, attempting to produce modest improvements via this approach. They hold the consensus view that urolithin A works by improving both mitophagy and mitochondrial biosynthesis, though as for many such compounds exactly how it achieves this outcome is far from settled.

Unfortunately, I had to miss the presentation by Alex Blyth of LifT Biosciences. This company pursues an interesting approach to cancer via transplantation of donor leukocytes; you might recall the original work on granulocyte transfer presented at SENS meetings back in the day. The original research showed great promise, and the company has been doing well these past few years, judging from the public updates.

Dobri Kiprov of Lyfspn presented on the merits of therapeutic plasma exchange. He presented a range of human data from patients in past years, including a reduction of epigenetic age via this approach, as well as immune improvements, improvement in joint issues, and improved liver and kidney function. Their view is that the most important aspect of this removal of bad factors is that it modulates the immune system, reducing the state of inflammaging and consequent harm and dysfunction. But the data they have is not rigorous, it results from clinical practice, and thus they founded this company to generate high quality data via clinical trials. They acknowledge that these are still the early days for therapeutic plasma exchange, and they still lack firm, defensible answers to even simple questions such as how long the benefits last from one treatment.

Pankaj Kapahi of Juvify discussed the science supporting this supplement company spinout from the Buck Institute. Their product is a modulator of glycation, acting to reducing the impact of sugar consumption and obesity on long-term health. Their hypothesis is that the generation of advanced glycation endproducts (AGEs) is the major problem that is produced by sugar metabolism. They work with compounds that target one type of shorter-lived AGE, methylglycoxal AGEs. Thus benefits may be a matter of reducing inflammatory signaling caused by AGEs via the RAGE pathway, but they think that RAGE is not the only mechanism of interest here. Interestingly, these compounds suppress appetite, so somehow short-lived AGEs are acting as appetite enhancers. Ongoing studies in mice also indicate that this interference in short-lived AGEs, conducted over the long term, decreases growth hormone signaling and reduces the burden of cellular senescence, among other benefits. Since appetite is reduced, is it possible that the benefits are all simply benefits of calorie restriction? They think that this is a factor, but only part of story.

Yuri Deigin of Youth Bio presented on partial reprogramming, a huge potential market, based on evidence from animal studies showing rejuvenation in many different tissues. Certainly, investors believe it will be huge, judging by the vast financial support for this part of the industry, dwarfing investment elsewhere. Youth Bio are an early stage preclinical company, at the point of having completed mouse studies showing reversal of measures of aging. They are working on a few different projects in parallel. Firstly they are attempting to produce new reprogramming approaches with novel factors and tissue-specificity. They avoid the liver and intestine for safety reasons, as mice tend to die when these are repeatedly reprogrammed. Secondly, they are working towards viable therapies based on use of the existing OSKM factors. Alzheimer's disease is their first indication on this side of the house, and they propose the use of a one-time gene therapy that introduces inducible genes, followed by delivery of small molecules for periodic activation of those genes.

Silke Hüttner of Rejuvenate Biomed outlined their approach to combinatorial therapies using small molecules identified through screening and later optimization. They are, unfortunately, cagey about the details of their compounds, but their present lead therapeutic candidate is a combination of two compounds that they have developed, which positively affects inflammation and other properties relevant to aging. The company is initially focused on sarcopenia, but they want to move on from there to other age-related conditions and then aging itself as a target. The company has produced successful studies in both progeroid mice and naturally aged mice, with early human trials ongoing.

Mourad Topors presented as the CSO of Repair Biotechnologies, the company that I co-founded with Bill Cherman. We develop a means of safely breaking down excess intracellular free cholesterol, delivered as a gene therapy to arbitrary cells in the body, or as a cell therapy of engineered cells equipped with this capability. We work towards reversal of atherosclerosis, the primary cause of human mortality, resulting at root from the presence of excessive cholesterol deposits in arterial walls. We are finding a faster path to the clinic in treatment of nonalcoholic steatohepatitis (NASH), however, largely because the delivery systems for liver-targeted gene therapies are far more developed. We presented recent results showing reversal of liver inflammation and fibrosis in NASH model mice, and noted that we're raising funds to start our clinical development program leading to human trials. Therapies to reverse atherosclerosis progression will follow shortly on the heels of this work on NASH.

Robin Mansukhani of Deciduous Therapeutics discussed their approach to immune system modulation via small molecules, training invariant natural killer cells to attack senescent cells. The point was made that engaging the immune system may be a way to work around many of the present unknowns regarding senescent cell status, biomarkers, and subtypes. Interestingly, a one-time treatment via their approach rouses immune cells for at least months thereafter, consistently clearing senescent cells over that time.

Mike Kope of Cyclarity presented on their approach. Cyclarity is the renamed Underdog Pharmaceuticals, a spinout from the SENS Research Foundation that employs engineered cyclodextrins to bind 7-ketocholesterol. This is essentially a test of the degree to which 7-ketocholesterol is a meaningful cause of pathology in human atherosclerosis and other conditions. They have great cell data, showing that they can reverse the foam cell state that arises from 7-ketocholesterol exposure, and they also test in human plaques obtained from cadavers and surgical procedures. Despite a lack of animal models for 7-ketocholesterol presence in atherosclerotic disease, Clarity has engineered a fast path to the clinic, based on the safety profile of cyclodextrins as a class. They will begin their first clinical trials next year.

Cristiana Banila of Mitra Bio discussed the need for better ways to measure skin aging. They have developed a way to measure epigenetic age in skin non-invasively, with no biopsy. They obtain cells from the skin surface via adhesive tape and have shown that this produces the same results as are obtained using biopsies. The company uses this approach to assess methods that are alleged to reverse skin aging, and presented data for an example treatment that can in fact reverse epigenetic age in UV-damaged skin. They plan to test many more of the established and potential skin-focused interventions that exist, to generate personalized recommendations for patients.

Brian Kennedy talked about his scientific work at the National University of Singapore. This spans a range of preclinical studies, including efforts to produce treatments based on the hallmarks of aging and work on biomarkers and epigenetic clocks. They tend to run 6-9 month interventions in mice, starting at 18 months of age, and assessing frailty and biomarkers of aging rather than using life span as a measure of success. Similarly, they run human studies, presently small ones, and again 6-9 months of intervention in healthy older people, while assessing biomarkers. The researchers are focused on the standard panoply of well-known small molecule geroprotectors, such as rapamycin, largely calorie restriction mimetics. In nematode worms, the development of automation now allows this research group to run studies of combinations of such compounds, tens of thousands of these studies every year; this capacity has led to a new company that intends to ramp up to millions of studies or more per year. One of the more interesting conclusions from the work carried out to date is that combinations produce unexpected results. The individual outcome of two small molecules is no guide as whether the combination will be better, worse, or indifferent. Any and all polypharmacy, or even combination of supplements, is a walk in the dark.

Chris Shepard of Thymofox gave an overview of the importance of thymic involution to the aging of the immune function. The insight leading to the creation of this company is that a young thymus regenerates from injury, but this capacity is much reduced in adults, and further so with aging. They are looking for the regulators of this decline, upstream of FOXN1. They aim to produce small molecules to indirectly upregulate FOXN1 expression in the thymus, searching via a high-throughput screen they they designed. They believe that along the way so far they have discovered some genetic regulators of FOXN1 level that may be useful in other ways, but details on their progress to date are light.

Mark Allen of Elevian gave his outline on their work on GDF11, one of the first candidate factors for the effects of parabiosis, back when it was thought that the effects of parabiosis might be mediated by beneficial factors in young blood, rather than a dilution of harmful factors in old blood. This line of research has been underway for a while now, and they are narrowed down to applications in stroke recovery as the first clinical indication. Their evidence in mice shows recombinant GDF11 to promote vascular regeneration, activate various stem cell and progenitor cell populations, suppress inflammation to some degree, and improve metabolism. They think that indications could be addressed via GDF11 therapies, and the first clinical trial for stroke recovery will begin in 2023. Further, they have identified a regulatory agent responsible for the age-related downregulation of GDF11 expression, and are working towards an antibody therapy as an alternative to delivery of recombinant GDF11.

Matthias Breugelmans of Elastrin Therapeutics discussed the regeneration of damaged elastin fibers in the extracellular matrix to restore elasticity in aged tissues. The company employs an albumin nanoparticle decorated with antibodies that bind to damaged elastin to deliver their therapy in a very targeted way. The nanoparticle contains EDTA and a proprietary PGG compound. In their eyes, damaged elastin in blood vessels and other tissues produces a local inflammatory response which in turn provokes calcification and other woes. They are targeting a variety of indications, including vascular calcification, aneurysm, hypertension, and a few rare orphan conditions. The company has obtained large reductions of vascular calcification in animal models, and a first phase 1 trial starts in 2023. Beyond the nanoparticle approach, they are working with delivery of mRNA encoding tropoelastin in order to stimulate the production of new elastin, but this is quite new, and earlier in development.

Matthew Rosen of CoRegen outlined a regulatory T-cell (Treg) based approach to defeating many different types of cancer. This is a spin out from Baylor College of Medicine, and uses the college infrastructure. One of the ways in which solid tumors subvert the immune system is to co-opt Treg cells, which then prevent other immune cells from attacking the cancer. Researchers have seen that gene knockdown of SRC-3 in Tregs will stop this from happening, however, and the CoRegen therapy is based on this finding. SRC-3 controls a lot of other genes, including checkpoint inhibitors, and it is thus fair to say it is a master regulator of immune capabilities against cancer. The company engineers Tregs by knocking out SRC-3, and then injects those cells, either systemically or into a tumor. This appears to provide lasting benefits in terms of resistance

to cancer, and complete remission of existing cancer in mice: a small number of engineered Tregs outweighs the effects of native Tregs. The company is aiming at a first phase 1 in 2023.

Peter Fedichev of Gero presented on the company's use of AI and animal models to characterize the split of degenerative aging into two quite different processes, which they term (a) damage (or frailty) and (b) loss of resilience. These are two quite different things, and the balance between these portions of aging is different in mice and humans. Humans are more resilient, meaning a greater resistance to perturbations to equilibrium in later life. In the Gero view, the hallmarks of aging are all linked, and a drug working on any one will have effects on all. They predict that most small molecules that slow aging in mice will have little effect on aging in healthy humans, because humans are already resilient in ways that mice are not. The company is running drug discovery programs based on this philosophy, and beginning to collaborate with big pharma entities.

Aaron Cravens of Revel Pharmaceuticals presented the company as developing a platform to produce enzyme therapies generally, at a fraction of the cost and time of past efforts. High throughput enzyme engineering, in essence. They use computational modelling of enzyme libraries to suggest new variants and desired properties, then validate in vitro. You will recall that they launched to work on enzymes to break glucosepane, CML, and other cross-links involved in aging, and that remains the initial application of their platform. This is an early stage effort, and they have not yet tested candidate molecules in animals. They intend to raise a series A next year.

Hans S. Keirstead outlined work under way at Immunis, one of the more advanced of the companies presenting at the event. They harvest the secretome of carefully tailored progenitor cell lines, and package those molecules as a therapeutic product. The result contains factors that can modulate the immune system, as well as provide other useful effects on cell behavior. This program is fairly advanced in its progression through the IND process with the FDA. They have demonstrated in IND-enabling studies that delivery of this secretome as a therapeutic helps with sarcopenia and fibrosis, reduces inflammation and arterial stiffness, and improves adaptive immunity. A phase 2 human trial for muscle atrophy is starting up now.

Robert Cargill of Glionics presented work on the use of engineered microglia to deliver therapeutic molecules throughout the brain. It is otherwise hard to get many types of compound into the brain, with good biodistribution, because of the blood-brain barrier. But microglia will naturally spread throughout the brain, provided that native microglia are cleared via some form of CSF1R inhibitor. The company is starting with klotho as the therapeutic molecule of choice. They have demonstrated repopulation following clearance in mice. Just a few thousand microglia will replicate and move throughout the brain, delivering a factor as they go, BDNF in that case. The intent is to generate therapeutic microglia from universal iPSC lines, a popular choice in research and development at the moment.

Rob Konrad Maciejewski of Biolytica outlined a vision for data-driven approach to personalized medicine and lifestyle changes. This is a software company; they build a visualization product for complex health data in order to help patients understand tests, make choices, aim towards goals, and navigate the sizable amount of data that can be obtained for these. Then on top of that add recommendations for lifestyle and supplement choices, and managing relationships with doctors and providers. The initial aim is to help people who could in principle make sizable gains in long-term health via lifestyle changes to make use of the present medical assay environment in order to achieve those gains.

Joshua McClure of Maxwell Biosciences presented on their drug discovery platform, based on producing variations on an antimicrobial peptide that is effective against pathogens of many types, including fungi, bacteria, and viruses. The story started with examination of blood plasma from young and old mice, finding a heat shock protein LL-37, a protein that is also an antimicrobial peptide that (a) seems to have broadly beneficial effects on many systems and (b) is downregulated with age. It attacks many targets from cancers to pathogens, acting via membrane disruption. Unfortunately it can't be used as a drug, as it is rapidly cleared from circulation, so instead the company makes similar peptides that have the same function while also being stable. This can be, in principle, a replacement for existing antibiotics and antivirals, with additional beneficial effects to health via heat shock protein mechanisms. The company is quite well advanced in their preclinical program, and intends to raise sizable amounts for clinical development in the coming year.

Sophie Chabloz presented on Avea, a standard issue modern dietary supplement company. They presently offer formulations incorporating nicotinamide mononucleotide and the like, aiming to produce NAD+ upregulation.

Felix Frueh of PAGE Therapeutics discussed the value of targeting metastasis in cancer therapy. Prevention of metastasis would make solid tumors far less dangerous, in any cancer. The company pursues an interesting mechanism: cancers produce circulating tumor cells, but only clusters of these circulating cells actually produce metastasis, not single cells. So why not dissolve the clusters? They found an existing drug that achieves this outcome and blocks metastasis in mice. This can then be combined with other cancer therapeutics. A trial in breast cancer patients is ongoing as a proof of concept. Despite all of this backstory from academia, they are actually in quite an early stage as a company, working to produce novel small molecule drugs targeting this cancer cell clustering mechanism via screening.

Jürgen Reeß of the very early stage company Mogling Bio introduced their work aimed at restoration of immune function

in older individuals. They wish to use derivatives of CASIN to inhibit CDC42, shown in academic work to rejuvenate the immune system with a single treatment. CASIN appears to improve function in stem cell populations generally, but in the case of hematopoietic stem cells this leads to an improved, more youthful production of immune cells. The company is just getting started, based on promising mouse data, and will target the obvious indications relating to age-related immune dysfunction.

All told, it was quite an interesting selection of ongoing work. The cancer side of the house in particular is looking very promising these days, with numerous quite general approaches under development that should be both effective and applicable to many different types of cancer.

Repeating the Point that Metformin Just Doesn't Look Good in Animal Studies

October, 2022

Based on studies conducted in mice, metformin is a terrible candidate drug for the treatment of aging. It may well benefit metabolically abnormal individuals, such as diabetics, but results for aged, metabolically normal mice are all over the map. Further, the gold standard, rigorous Interventions Testing Program found no benefit in their assessment. If the goal is to modestly slow aging, then rapamycin is way and far better: robust, replicated results on health and life span in animal studies. But the goal should not be to modestly slow aging! It should be to produce rejuvenation! The sizable fraction of academia and industry that is focused on altering metabolism to provoke greater stress responses and modestly slow aging will, unfortunately, most likely do little to reduce the suffering and death of old age.

The animal study most often cited as evidence that metformin slows aging in lab mice is no such evidence at all. The investigators tested two doses of metformin in healthy, wild-type, nonobese mice. At the lower of the two tested doses, metformin increased the animals' mean survival by a paltry 4-6%, and had no effect on maximum lifespan, meaning that the drug prevented a small number of deaths during and before middle age, but had no effect on aging. And when the mice were given the higher dose of metformin, it actually shortened the animals' lives!

The best animal study to test metformin as a potential anti-aging drug was conducted as part of the National Institute on Aging (NIA)'s Interventions Testing Program: a rigorous, systematic effort to test conventional "messing with metabolism" anti-aging agents. ITP studies are designed with several features that make them a better test than the great majority of studies of whether a potential longevity therapeutic actually works (in mice!). First, each time the ITP tests a potential longevity therapeutic, the lifespan study is done not just once, but three times independently in parallel, with three separate cohorts of mice living out their lives at three independent research sites, cared for by three different groups of scientists. Second, ITP tests all candidate longevity therapeutics in a healthy, genetically-diverse mouse population, which better resembles the normal human population than the genetically homogenous mouse strains widely used in biomedical research.

When the ITP researchers put metformin to the test, the result was unambiguous. It did not extend the lives of the mice at any site. It did not even cause the modest reduction in early deaths seen in the previous, widely-cited study. Metformin simply has no effect at all on lifespan in normal, healthy mice.

Link: https://www.sens.org/tame-attempt-slow-aging-part-1-metformin-in-mice/

Reprogramming to Improve Stem Cell Function Synergizes with Senescent Cell Clearance in Flies

November, 2022

Rejuvenation will be achieved in humans by combinations of therapies, provided periodically over time. Each individual therapy will in some way address one of the forms of cell and tissue damage that accumulate to cause the pathologies of aging. There are numerous independent sources of such damage, however. It is the case that the various types of accumulating damage, and the far greater variety of dysfunctions caused by that damage, will interact with one another to make outcomes worse than they would have been alone. Nonetheless, very different forms of rejuvenation therapy will be required to repair each of the very different forms of damage. Each individual repair therapy will produce only incremental outcomes, it will not solve all of aging.

Given this, there is, even at this comparatively early juncture in the development of rejuvenation therapies, far too little work taking place on how to best combine treatments, and on assessing the outcomes of combined treatments. Fortunately that is slowly changing, and a number of groups are at present putting earnest effort into running combinatorial studies in short-lived model organisms. Still, it is far from enough, and largely focused on metabolic adjustments that can only modestly slow aging, not repair the underlying damage.

With that in mind, today's open access paper is an interesting first step towards showing that partial reprogramming, with the effect of improving stem cell function, synergizes well with clearance of senescent cells. Both of these approaches have been shown to improve health and function in old animals, with the caveat that senolytic treatments capable of selectively destroying senescent

cells are a less recent innovation, and thus come with far more data - and more robust data - demonstrating rejuvenation. The work here uses inducible expression in genetically engineered flies rather than the delivery of therapeutics into wild-type animals in order to achieve the observed results, but that is a first step towards better studies in mice.

Combining stem cell rejuvenation and senescence targeting to synergistically extend lifespan

While the number of stem cells decreases in aging animals, senescent cells accumulate with age. Manipulating cell fates by cellular reprogramming (to rejuvenate somatic cells) and by senolytic interventions (to remove senescent cells) are two promising approaches to restore homeostasis in aged individuals and to prevent age-dependent diseases. Cellular reprogramming allows differentiated cells to regain plasticity and to take on more stem cell-like qualities. A major step towards this goal was the demonstration of cellular reprogramming of terminally differentiated cells into pluripotent embryonic-like stem cell states. Such reprogramming reverses epigenetic aging marks, demonstrating that even mature, terminally differentiated cells can be returned to a younger state. While continuous expression of the Yamanaka factors (Oct4, Klf4, Sox2, c-Myc; OKSM) in mice led to the formation of teratomas and decreased lifespan, repeated short term expression in adult mice succeeded in ameliorating cellular and physiological signs of aging. Subsequently, several studies have suggested that this approach can be applied to human aging and age-related disease, and cycling expression can rejuvenate stem cells in vitro.

Ablation of senescent cells has been shown to reverse tissue dysfunction and extend healthspan in mice. A recent study using a senolytic construct (FOXO4-DRI peptide) that induced apoptosis in senescent cells, by interfering with the binding of p53 to FOXO4 thereby freeing p53 to activate apoptosis, showed that the clearing of senescent cells both counteracted senescent cell induced chemotoxicity and restored age-dependent declines in physical performance, fur density, and renal function in aging mice. Several studies have further explored applications of different senolytic strategies to ameliorate age-related decline and disease.

Accumulation of senescent cells and loss of stem cells are not independent processes. Through the senescence-associated secretory phenotype (SASP), senescent cells release pro-inflammatory cytokines which contribute to chronic inflammation and mTOR activation, ultimately leading to stem cell exhaustion. This interaction suggests that senolytic therapies might interact with cellular reprogramming strategies in delaying age-dependent decline and disease. We have previously explored drug-drug interactions as synergistic aging interventions, and here we ask whether a combinatorial treatment of OKSM and senolytic (Sen) expression could mitigate or reverse the effects of aging more efficiently than either intervention alone.

To test this hypothesis, we induced expression of OKSM, Sen, and an OKSM-Sen combination in adult flies and compared their effects on health and lifespan. We find that each treatment alone had limited benefits, with OKSM alone benefiting maximum lifespan while Sen expression alone increased mean lifespan but had no effect on maximum lifespan. In contrast, animals subjected to the combined intervention experienced substantially longer mean and maximum lifespan. Our data is consistent with a synergistic interaction between the two interventions, simultaneously rejuvenating stem cells and removing senescent cells.

Link: https://www.aging-us.com/article/204347/text

Better Understanding the Outcome of Destroying and Rebuilding the Immune System

November, 2022

The use of chemotherapy to destroy as much of the peripheral immune system as possible, followed by some form of stem cell transplant to rebuild it, has been used for some years as a way to treat multiple sclerosis. In this autoimmune condition, the problem resides in the immune memory, and getting rid of that memory is the solution. The only approach currently demonstrated to work is this somewhat drastic treatment, and the balance of risk and cost means that it is only used for severe diseases such as multiple sclerosis. But in principle, clearance and restoration of the immune system could solve a great many of the issues present in an aged immune system, were there a way to go about it that didn't have the same level of risk and trauma.

Multiple sclerosis (MS) is an autoimmune disease in which the body's own immune system attacks the myelin sheath of the nerve cells in the brain and spinal cord. The disease leads to paralysis, pain, and permanent fatigue, among other symptoms. Fortunately, there have been great advances in therapies in recent decades. 80 percent of patients remain disease-free long-term or even forever following an autologous hematopoietic stem cell transplant. During the treatment, several chemotherapies completely destroy the patients' immune system - including the subset of T cells which mistakenly attack their own nervous system. The patients then receive a transplant of their own blood stem cells, which were harvested before the chemotherapy. The body uses these cells to build a completely new immune system without any autoreactive cells.

Previous studies have shown the basic workings of the method, but many important details and questions remained open. Some unclear aspects were what exactly happens after the immune cells are eliminated, whether any of them survive the

chemotherapy, and whether the autoreactive cells really do not return. In a recently published study, researchers systematically investigated these questions for the first time by analyzing the immune cells of 27 MS patients who received stem cell therapy. The analysis was done before, during and up to two years after treatment. This allowed the researchers to track how quickly the different types of immune cells regenerated.

Surprisingly, the cells known as memory T cells, which are responsible for ensuring the body remembers pathogens and can react quickly in case of a new infection, reappeared immediately after the transplant. Further analysis showed that these cells had not re-formed, but had survived the chemotherapy. These remnants of the original immune system nevertheless posed no risk for a return of MS, as they were pre-damaged due to the chemotherapy and therefore no longer able to trigger an autoimmune reaction.

In the months and years following the transplant, the body gradually recreates the different types of immune cells. The thymus gland plays an important role in this process. This is where the T cells learn to distinguish foreign structures, such as viruses, from the body's own. Adults have very little functioning tissue left in the thymus. But after a transplant, the organ appears to resume its function and ensures the creation of a completely new repertoire of T cells which evidently does not trigger MS or cause it to return.

Link: https://www.news.uzh.ch/en/articles/media/2022/MS-Stem-Cell-Transplantation.html

Reprogramming Alone is Not Sufficient

November, 2022

Epigenetic reprogramming is a process of exposing cells to the Yamanaka factors for a long enough period of time to shift their epigenome towards that found in youthful tissues, but not for so long as to cause any meaningful number of them to change state into pluripotent stem cells. It is an attempt to reproduce aspects of the cellular rejuvenation that occurs in the initial stages of embryogenesis, without harming the functional specialization of the cells so altered. It works surprisingly well in animal studies, considering all of the very reasonable a priori objections as to why we should believe that such an embryonic process would be harmful and cancerous (at the very least) in the very different, structured environment of adult, aging somatic tissue.

There is a school of thought-slash-marketing-to-investors regarding mechanisms of aging that suggests epigenetic reprogramming of cells in vivo will be sufficient to produce comprehensive rejuvenation, addressing near all issues. That reprogramming the epigenetic landscape to a youthful configuration will provoke tissues into repair and clearance of enough of the damage of aging that further therapies would be superfluous. This really doesn't appear to be the case, however.

Based on the animal studies to date, reprogramming will produce significant benefits, just like, say, clearance of senescent cells, but it won't be the whole of the picture. There are forms of damage that a young body cannot repair. Many forms of persistent molecular waste, such as components of lipofuscin or some advanced glycation endproducts, cannot be broken down effectively by our cells. Nuclear DNA damage won't be repaired once present. Localized excesses of cholesterol, such as that found in atherosclerotic lesions, would overwhelm the macrophages responsible for clearing this damage even in a young person. And so on and so forth.

SENSible Question: Wouldn't Cellular Reprogramming Be Enough?

Cellular reprogramming turns an old person's cells young again. So can't we fix aging by just reprogramming a person's old cells with reprogramming factors? This is a tantalizing idea that's on a lot of our supporters' minds these days. On the one hand, it's certainly true that we lose cells with aging and that other cells become dysfunctional. And on the other hand, the cellular reprogramming experiments have in some senses rejuvenated cells in a way that can and should spark excitement - first and foremost, because the technology will greatly enable cell therapy of various kinds, which will be critical to the medical defeat of aging. But the quite rational enthusiasm for a specific technology can sometimes spark a kind of irrational biomedical exuberance so great that even some very prominent geroscientists seem to have begun to fall into a kind of fallacy of composition: the body is made up of cells; therefore, if we rejuvenate all our cells, we will rejuvenate our entire bodies.

People making this intuitive leap are in for an inelegant crash. We simply are not composed entirely of cells, and replacing lost cells and restoring the original differentiation of cells with epigenetic changes won't do anything to remove or repair aging damage to the many other functional units that are lost or damaged as we age and that contribute to diseases and disabilities of aging.

For one thing, there's aging damage to the extracellular matrix (ECM). The ECM is the lattice of proteins that provide both physical structure and signaling cues for our cells and tissues, and that also have important roles of their own in the body's movement and plumbing. In addition to damage to the ECM, another critical kind of aging damage that would impair the youthful function even of pristine reprogrammed cells is the various extracellular aggregates ("amyloids") that accumulate outside cells. These are damaged proteins that either physically impede cells' ability to carry out their function, or cause cellular dysfunction in other ways.

We've been thinking about using reprogramming technology either to create replacement cells for those that have been lost to aging processes, or to reprogram cells already in the tissues in order to (as advocates would have it) rejuvenate their function. These applications could in principle deal with cells that are either missing entirely, or that are still present but behaving badly due to reversible changes in their epigenetics - but they can't do anything about cells that survive, but have suffered certain other kinds of aging damage.

For instance, cells overtaken by mitochondria with large deletion mutations (which are the most problematic kind of mitochondrial damage in aging) almost certainly can't be restored to normal functioning through reprogramming. In all probability, the presence of mitochondrial mutations and other aging damage (such as intracellular aggregates, the abnormal splice protein lamin A, and some mutations and epimutations) is one of the main reasons why only a tiny fraction of cells exposed to reprogramming factors ever actually get reprogrammed. And in addition to not repairing all aging damage, reprogramming itself causes other kinds of damage to some cells that make them useless for rejuvenation biotechnology, such as the newly-created mitochondrial DNA mutations, or abnormal numbers of chromosomes, or the paradoxical mixed bag of reprogramming-induced senescence (RIS).

And there are even narrowly cellular forms of aging damage that you can't or wouldn't want to "repair" using reprogramming. Yes, you can reverse cellular senescence by reprogramming, and with a few additional tricks you can even reverse reprogramming-induced senescence, but is that a good idea? Remember, the cellular senescence machinery is a kind of emergency brake, which the cell pulls when it is in danger of careening out of control, such as by progressing to become a cancer or by laying down excessive collagen after an injury, leading to fibrosis.

Link: https://www.sens.org/wouldnt-cellular-reprogramming-be-enough/

Grip Strength Remains a Decent Biomarker of Aging

November, 2022

Of the various simple measures that correlate with mortality and risk of age-related disease, grip strength remains a relatively good option, even in this modern era of epigenetic clocks. Illustrative of this point, researchers here show a correlation between grip strength and epigenetic age data in a sizable study population. The degree to which an individual suffers from the chronic inflammation of aging may be an important determinant of this relationship. Inflammation disrupts tissue function throughout the body, and maintenance of muscle mass and strength is one of the aspects of health negatively affected by unresolved inflammatory signaling.

Researchers modeled the relationship between biological age and grip strength of 1,274 middle aged and older adults using three "age acceleration clocks" based on DNA methylation, a process that provides a molecular biomarker and estimator of the pace of aging. The clocks were originally modeled from various studies examining diabetes, cardiovascular disease, cancer, physical disability, Alzheimer's disease, inflammation, and early mortality. Results reveal that both older men and women showed an association between lower grip strength and biological age acceleration across the DNA methylation clocks. The real strength of this study was in the eight to 10 years of observation, in which lower grip strength predicted faster biological aging measured up to a decade later.

Past studies have shown that low grip strength is an extremely strong predictor of adverse health events. One study even found that it is a better predictor of cardiovascular events, such as myocardial infarction, than systolic blood pressure - the clinical hallmark for detecting heart disorders. Researchers have previously shown a robust association between weakness and chronic disease and mortality across populations. This evidence coupled with the recent findings shows potential for clinicians to adopt the use of grip strength as a way to screen individuals for future risk of functional decline, chronic disease and even early mortality.

Future research is needed to understand the connection between grip strength and age acceleration, including how inflammatory conditions contribute to age-related weakness and mortality. Previous studies have shown that chronic inflammation in aging - known as "inflammaging" - is a significant risk factor for mortality among older adults. This inflammation is also associated with lower grip strength and may be a significant predictor on the pathway between lower grip strength and both disability and chronic disease multimorbidity.

Link: https://labblog.uofmhealth.org/body-work/muscle-weakness-new-smoking

Non-Dividing Neurons Do In Fact Become Senescent, Impairing Brain Function

December, 2022

Cellular senescence is generally thought of as a characteristic of replicating cells; it is an end state reached when telomeres, reduced in length with each cell division, become too short. This is followed by programmed cell death or destruction by immune cells. When senescent cells linger, as is increasingly the case with age, they contribute to degenerative aging via their pro-growth, pro-inflammatory signaling, disruptive of tissue structure and function. Researchers have suggested that non-dividing,

post-mitotic cells such as neurons can also exhibit a form of senescence, and here evidence is provided for this to be the case. Senescence in supporting cells in the brain, such as microglia and astrocytes, is known to contribute to neurodegeneration. If some neurons are also senescent, producing similar harmful signaling, then these cells will also contribute to the aging of the brain.

As cells age, they can undergo cellular senescence, which contributes to tissue dysfunction and age-related disorders. Senescence is also thought to play a role in cellular stress, molecular damage, and cancer initiation. However, scientists previously believed that senescence primarily occurred in dividing cells, not in neurons. Little was known about the senescence-like state of aging human neurons.

In this study, researchers took skin samples from people with Alzheimer's disease and converted those cells directly into neurons in the lab. They tested these neurons to see if they undergo senescence and examined the mechanisms involved in the process. They also explored senescence markers and gene expression of post-mortem brains from 20 people with Alzheimer's disease and matched healthy controls. This allowed the team to confirm that their results from the lab held true in actual human brain tissue.

The team found that senescent neurons are a source of the late-life brain inflammation observed in Alzheimer's disease. As the neurons deteriorate, they release inflammatory factors that trigger a cascade of brain inflammation and cause other brain cells to run haywire. Additionally, the gene KRAS, which is commonly involved in cancer, could activate the senescent response. The consequences of even a small number of senescent neurons in the aging brain could have a significant impact on brain function. This is because a single neuron can make more than 1,000 connections with other neurons, affecting the brain's communication system.

In addition to these findings, the authors also administered a therapeutic (a cocktail of Dasatinib + Quercetin) to the patient neurons in a dish. Both drugs are used to remove senescent cells in the body in conditions such as osteoarthritis, so the authors wanted to see if they were effective in senescent cells in the central nervous system as well. They found that the drug cocktail reduced the number of senescent neurons to normal levels. Targeting senescent cells could thus be a useful approach for slowing neuroinflammation and neurodegeneration in Alzheimer's disease.

Link: https://www.salk.edu/news-release/deteriorating-neurons-are-source-of-human-brain-inflammation-in-alzheimers-disease/

Details on the LEV Foundation's First Study of Combined Interventions in Mice

December. 2022

The recently launched Longevity Escape Velocity (LEV) Foundation will, initially at least, focus on testing combinations of interventions. This work is informed by the SENS view of aging, in that degenerative aging is produced by a limited number of forms of cell and tissue damage that result from the normal operation of metabolism. These include the accumulation of senescent cells, cross-linking of the extracellular matrix, mitochondrial DNA damage, and so forth. Each form of damage produces its own contribution to a complex web of interacting downstream consequences, so while repairing any one form of damage should be beneficial, repairing more than one should be better.

Unfortunately the research and development communities operate under incentives that strongly discourage earnest work on combinations of therapies, these incentives largely resulting from the way in which intellectual property and regulation of medical development interact. Research into combined therapies is necessary to achieve the end goal of a comprehensive toolkit of rejuvenation therapies, but it is almost entirely ignored as an aspect of this area of medical research. Thus philanthropic efforts are required to fill in the gap and light the way. Combined interventions are on the roadmap of the rejuvenome project, for example. And now they are a focus at the LEV Foundation.

Robust Mouse Rejuvenation - Study 1

LEV Foundation's flagship research program is a sequence of large mouse lifespan studies, each involving the administration of (various subsets of) at least four interventions that have, individually, shown promise in others' hands in extending mean and maximum mouse lifespan and healthspan. We focus on interventions that have shown efficacy when begun only after the mice have reached half their typical life expectancy, and mostly on those that specifically repair some category of accumulating, eventually pathogenic, molecular or cellular damage. The first study in this program is starting in January 2023.

Our ultimate goal in this program is to achieve "Robust Mouse Rejuvenation". We define this as an intervention, almost certainly multi-component, that: (a) is applied to mice of a strain with a historic mean lifespan of at least 30 months; (b) is initiated at an age of at least 18 months; (c) increases both mean and maximum lifespan by at least 12 months.

In each study in this program, we will examine the synergy of (typically at least four) interventions already known individually to extend mouse lifespan when started in mid-life. We will determine not only the ultimate readout of lifespan, but also the

interactions between the various interventions, as revealed by the differences between the treatment groups (receiving different subsets of the interventions) in respect of the trajectories with age of cause of death, decline in different functions, etc. In this way we will add greatly to the understanding of which benefits these interventions confer and how they synergize, or possibly antagonize.

There are two key motivations for this program. One is purely biomedical: as with all mouse work with a biomedical end goal, we hope to generate data that will inform the development of therapies to let humans live longer in good health. The other could be called rhetorical, societal, political - it is to demonstrate a definitive proof of concept that aging is much more malleable than society currently insists on thinking it is, and thus must be viewed as a tractable medical problem, rather than a fact of life.

Interventions are chosen on the basis that they 1) act systemically and 2) have individually shown some lifespan-extending effect in naturally aged mice. In this way, we are specifically selecting rejuvenation therapeutics, as opposed to those which are purely preventive and/or require early life intervention. Therapies are also selected to have minimal mechanistic overlap, based on our current understanding of their mechanisms of action. The first four interventions selected for the initial study are rapamycin, hematopoietic stem cell transplant, telomerase upregulation via TERT gene therapy, and senolytic treatment.

Link: https://www.levf.org/projects/robust-mouse-rejuvenation-study-1

A Look Back at 2022: Progress Towards the Treatment of Aging as a Medical Condition

December, 2022

At the end of 2022, we can reflect on the fact that we are steadily entering a new era of medicine, one in which mechanisms of aging are targeted rather than ignored. It is a profound change, one that will change the shape of a human life and ultimately the human condition by eliminating the greatest sources of suffering and death in the world. Year after year, we see increased funding, ongoing progress towards therapies capable of slowing aging or reversing aspects of aging, and a growing taxonomy of such potential therapies and their target mechanisms.

The view of aging in the medical community and public at large is changing, slowly, in the face of this, shown, for example, by the recognition that the long-established practice of dividing aging into many different diseases and treating them one by one isn't working. To fight aging one must tackle the causes of aging, and each cause contributes to multiple conditions. One day in the not-so-distant future, the average person in the street will see aging the same way that he or she presently sees cancer, meaning that it is obviously a research priority, something that should be treated and cured.

The Longevity Industry and Associated Non-Profit Initiatives

The longevity industry continues to grow and diversify, and there are now far too many companies and too many venture funds for any one observer, and certainly not this one, to keep up with new teams, new funding, and new projects. Much the same could be said for the non-profit space. The organizer and volunteers at AgingBiotech.info are certainly doing their best to maintain a useful, up-to-date resource, however!

That said, I will note a few items, starting with one sizable fund, Kizoo Technology Ventures, that was profiled earlier this year. It is an important fund because its principals specifically focus on the SENS view of aging: the importance of molecular damage, and the point that rejuvenation will only be achieved by repairing that damage. The SENS Research Foundation released its annual reports a few months ago, and it is, as always, interesting reading for those interested in the science of rejuvenation. Aubrey de Grey has launched a new non-profit, the Longevity Escape Velocity Foundation that will focus on similar work to that conducted at the SENS Research Foundation, with an emphasis on repairing the cell and tissue damage that causes aging.

Fundraising activity was quite energetic prior to the recent market downturn. While many of these companies are actually working on drug discovery platforms or next generation dietary supplements or other low-hanging fruit rather than bold new therapies, there are nonetheless exciting biotechnologies under development as well, true means of rejuvenation. We'd always want to see a larger portion of the industry undertaking that sort of work, but it is what it is. As growth occurs, it is interesting to see the rush to moderation in messaging. It is a longevity industry, yes, but one in which the larger players are quick to reassure the world that they are not in fact trying to produce longevity.

Cellular Senescence

Cellular senescence is an important contributing cause of aging, in that the burden of senescent cells rises with age, and these cells disrupt tissue and organ function with their pro-growth, pro-inflammatory signaling. Researchers are optimistic regarding the potential of therapies targeting senescent cells, even the cautious types, and so is the popular science press. In just the last year, many studies have reported slowing or reversal of specific aspects of aging via clearance of senescent cells, or otherwise implicated senescent cells in disease progression. A partial list: neurogenesis; neuronal function; Alzheimer's disease, liver disease; kidney aging; T helper cell function; atrial fibrillation; reducing pain but not cartilage damage in osteoathritis; loss of

microvasculature; atherosclerosis; fibrosis and inflammation in NASH; diabetic macular edema; particularly senescence in vascular smooth muscle; pulmonary fibrosis, amyotrophic lateral sclerosis; chronic obstructive pulmonary disease, and age-related loss of pulmonary function via a range of mechanisms; failure of organ transplants; loss of regenerative capacity in the heart; cardiovascular disease in general; cognitive function and brain aging in late life; amyloid aggregation in the vasculature; sarcopenia via reduced stem cell function; osteoporosis was frequently discussed; disc degeneration; vascular calcification; Parkinson's disease, such as via removing senescent microglia; improving ischemic stroke recovery; accelerated aging due to induction of cellular senescence by chemotherapy and radiotherapy; abdominal aortic aneurysm; immune function in the brain; gum disease.

Clearing senescent cells should be synergistic with stem cell therapies and partial reprogramming, both combinations expected to provoke regeneration. Senolytics should also be synergistic with cancer therapies, providing better patient outcomes with fewer long-term side-effects. These combinations should receive more attention! Further, some research suggests that combinations of senolytics may synergize to improve on the results of any single drug.

New clinical trials continue to be launched for established senolytics, including one for Alzheimer's disease, and senolytics as a class are approaching clinical use. Novel approaches to reducing the burden of senescent cells continue to emerge as the wheels of drug development begin to turn in earnest. Those mentioned in the last year include the following: use of 25-hydroxycholesterol; improving the efficiency of natural killer cells via several approaches; mitochondrial transfer; applying partial reprogramming to senescent cells, something that continues to seem a bad idea; MCL-1 inhibition; control of viral infection; YAP upregulation; mitochondrially targeted tamoxifen; glutaminase inhibition; overexpression of GPNMB; inhibiting the BDNF-TrkB interaction; p53 upregulation; SFRP4 inhibition; overexpression of DDIT4 and HDAC4; PD-L1 checkpoint inhibition; GATA4 inhibition; USP16 inhibition; reducing mitochondrial dysfunction to prevent the onset of some cellular senescence; targeting antoxidants to telomeres; nintedanib, a similar drug to dasatinib; derivatives of FOXO4-DRI.

Mitochondrial Dysfunction

Mitochondrial dysfunction is a prominent feature of aging, and is accompanied by a decline in mitophagy, meaning the cell maintenance processes of autophagy targeted to mitochondria. Attempting to upregulate mitophagy is a popular topic, with approaches mentioned this year including urolithin A (and a clinical trial), BNIP3 upregulation, and iterations on spermadine. Unfortunately none of the easier, supplement based approaches appear to be any better than exercise or calorie restriction when it comes to improve the operation of autophagy in older individuals. There are other stress responses that can be triggered to improve mitochondrial function, such as the unfolded protein response, influenced via protein import mechanisms.

A range of other potential paths to reversing mitochondrial dysfunction are under development. Reprogramming is one of the more prominent, followed by the various groups developing the basis for mitochondrial transfer therapies. Researchers recently demonstrated editing of mitochondrial DNA in vivo, though it seems challenging to use this technology to deal with stochastic mutational damage. The role of mitochondrial DNA mutation remains much debated, both for and against. Other approaches mentioned this year include: enhancing mitochondrial fission to restore the imbalance in mitochondrial dynamics; magnetic fields used to improve mitochondrial function; use of antifibrosis drugs to improve mitochondrial metabolism; use of mitochondrially derived peptides; sirtuin upregulation; upregulating NAD levels via NMN supplementation and CD38 inhibition; partially inhibiting complex I of the electron transport chain; and mitocondrial uncoupling via newer, safer means than in past decades. Too few of these approaches seem likely to have large effect sizes, unfortunately.

Clocks for Aging

The number of clocks that assess biological age, derived from omics data and other measures, is proliferating rapidly. Even only considering epigenetic clocks the number is steadily increasing. Fortunately, at least some effort is being put into comparing clocks in order to winnow out the less helpful ones. New clocks noted this year incuded: a retinal image clock; a metabolomics clock; a clock for naked mole-rats; and a lipid clock.

This broadening of work on clocks is taking place because a consensus measure of aging would revolutionize the field, enabling rapid, directed progress towards the best approaches to treat aging as a medical condition. This point is widely recognized. We are not there yet, however, as the clocks developed to date are not yet well enough understood. Despite a few inroads, the connections between epigenetic aging and the rest of aging are not yet well mapped.

There appear to be some odd blind spots in many clocks, such as inflammatory status and biases in centenarian epigenetic age. Reduced epigenetic age does not prove slowed or reversed aging, we should probably be suspicious of clocks that use few data points, and all studies measuing clock data must also measure other metrics of health and disease. This state of affairs is not preventing speculation as to whether the existence of cross-species clocks should mean that therapies that actually address root causes of aging should perform equally well between species, or that epigenetic clocks will point the way to a true identification of the causes of aging.

Partial Reprogramming

There is so much money flowing into the exploration of partial reprogramming with Yamanaka factors and potential alternatives, in order to restore more youthful epigenetic patterns and thus more youthful cell behavior, that we're going to see a great deal of coverage, even in the popular press, in the years ahead. Hopefully the obstacles are overcome, such as the inclination of natural killer cells to attack reprogrammed cells, and the techniques improved, and this turns out to be a viable path to rejuvenation at the end of the day. It can't fix everything, but we can hope that it will positively influence many aspects of aging.

The fundamental research in animal models is getting funded as well, and continues apace. Progress in understanding and capabilities is quite rapid. If interested in the present state of play and the road to the clinic, there were a number of popular science overviews published following the large-scale funding of Altos Labs and other companies. Alternative approaches to reprogramming beyond Yamanaka factors are being considered. Is it possible to reprogram effectively with small molecules? Time will tell. Researchers are starting to think about the specific conditions they might treat with reprogramming approaches. Some of those metioned in the last year include: disc degeneration; T cell exhaustion resulting from cancer; and liver injury.

Neurodegeneration

Neurodegeneration is connected to near all of the mechanisms and environmental factors studied in the context of aging. Novel approaches to the treatment of neurodegenerative conditions are constantly suggested, and existing approaches mentioned while under development. A selection from the past year includes the following items: the use of disaggregases to clear amyloid, while noting that amyloid is still the primary focus in Alzheimer's treatment, despite continued failures in clinical trials; forcing microglia into the anti-inflammatory M2 polarization, or clearing them entirely; TREM2 antibodies to prevent the incapacity of microglia in the aging brain; transfer of cerebrospinal fluid from younger donors; a combination of rapamycin, acarbose, and phenylbutyrate; adjusting the aging gut microbiome; greater control of viral infection, particularly those involving herpesviruses capable of persistent infection; whole blood exchange to encourage amyloid-β to leave the brain into the vasculature; reprogramming astrocytes into neurons in vivo; drugs to decrease microglial inflammation and slow neurodegeneration in early stages; influenza vaccination correlates with a 40% reduced Alzheimer's risk; transcranial direct current stimulation; provoking greater activity in neural progenitor cell populations; Atoh1 gene therapy to regenerate hair cells in the inner ear; activating or somehow expanding the limited supply of precursor neurons; immunization against amyloid-β; use of plasmalogens; GM-CSF treatment improves memory in mice; enhancing neurogenesis to treat Alzheimer's, such as via upregulation of oxytocin.

The good news is that manifestations of neurodegenerative processes, such as cognitive impairment, are in decline, and those on the path to dementia can now be screened early, years in advance of symptoms. This lowered risk is likely the result of improved cardiovascular health, while individually reduced risk is offset by the aging and growth of the population, leading to higher absolute incidence of disease. Being fitter correlates with better late life cognitive function, and exercise with improved synaptic function, as well as improved brain function more generally. Physical fitness also clearly reduces dementia risk and improves brain function, perhaps largely via lowered blood pressure, with as much as 40% of dementia cases being the result of lifestyle choices.

Aging of the Immune System

Chronic unresolved inflammation is a key feature of aging. Immune system aging is these days described as a mix of immunosenescence and inflammaging; every year a few review articles discuss the definitions and relationship between them. Important areas of declining function include the thymus and hematopoietic populations in the bone marrow, though every aspect of the immune system shows loss of function. The thymus atrophies with age, a sizable contribution to the decline of the adaptive immune system. Modest calorie restriction in humans has been shown to produce a surprisingly large regrowth of active thymus tissue, a surprising result that suggests the thymus is more dynamic in adults than suspected. KGF overexpression and introduction of subpopulations of thymic cells produces an enlarged thymus in animal studies, but would need a clever delivery mechanism to get the therapy into the thymus in humans.

Various approaches are under consideration to reverse immune aging, either generally or focused only on narrow issues within the broader set of problems. Lowering the lifetime burden of infection will likely slow hematopoietic aging. Upregulation of autophagy might help slow immune aging. A gene therapy delivering a BPIFB4 variant improved immune function in old mice. PGD2 inhibition can help with the slowdown in some narrow aspects of immune cell communication, improving the immune response. Trained immunity is an interesting phenomonen in which some challenges can improve innate immune function in late life. For example, vaccination with mycobacterium vaccae suppresses chronic inflammation. Calorie restriction has similar effects on innate immune system activation. Lowering inflammation may be a useful treatment for frailty. Targeting the inflammasome may be an improvement on current very blunt methods of suppressing inflammatory signaling, and clearing senescent cells to remove their inflammatory signaling should also help greatly. Other approaches to lowering inflammation mentioned recently include resistance training in older adults and reduced SPARC levels in fat tissue.

The Gut Microbiome in Aging

The balance of populations in the gut microbiome changes with age in detrimental ways, such as via increasing inflammatory signaling, producing harmful metabolites, or a diminished production of beneficial metabolites. This impacts health, and numerous correlations can be drawn between the activity of the microbiome and various manifestations of aging. Adjusting the gut microbiome in lasting ways to restore youthful populations may or may not achieve that much more than choosing a good program of exercise and diet, but it can be accurately measured. One can determine exactly what happened following any given intervention via the sequencing of microbes from a stool sample. Various approaches move the needle, and those in the spotlight this past year include: calorie restriction and methionine restriction; fecal microbiota transplantation was shown to improve function in mice, and this approach was a particularly popular topic in the lead in to FDA approval of one implementation and a few results in aged humans; oral administration of Akkermansia muciniphila; and heterochronic parabiosis, the latter obviously more interesting than useful.

Cardiovascular Disease

Atherosclerosis and consequent cardiovascular disease is the largest single cause of human mortality, and should be a high priority in research and development. A great deal of evidence points to the inflammation of aging as a major driver of atherosclerosis. At the core of the condition, there is macrophage dysfunction where cholesterol overwhelms cells in artery walls. Calcification of vascular and heart tissue occurs in parallel with atherosclerosis, and is known to raise the risk of stroke as well as other cardiovascular events.

Numerous approaches have been suggested in the past year as treatments for cardiovascular diseases and their consequences: upregulation of autophagy; TRPM2 inhibition; targeting matrix vesicles to reduce pro-calcification signaling; CCL17 inhibition reduces inflammation in cardiac hypertrophy; an oligodendrocyte cell therapy, the cells responsible for generating myelin, improves stroke recovery, as does PTPσ inhibition; influenza vaccination can reduce inflammation to reduce stroke risk. Cholesterol continues to be the primary focus of therapeutic development in cardiovascular disease, such as via upregulation of reverse cholesterol transport. Atherosclerosis is in principle highly preventable, and early detection of atherosclerotic lesions might encourage greater success on this front.

Vascular stiffening is a major feature of aging. It is driven in part by degeneration of elastin, but has a broad range of contributing mechanisms. It contributes to many pathologies, even only considering the downstream effects of resulting hypertension, such as vascular restructuring and pressure damage to delicate tissues. Controlling hypertension greatly reduces risk of stroke. Targeting the inflammasome in vascular tissues or the use of SGLT2 inhibitors may reduce the aforementioned vascular dysfunction. Inhibition of piezo1 signaling may block the connection between hypertension and vascular hypertrophy.

Cancer

Cancer is the second largest cause of mortality in our species. It is a numbers game: damage and cell replication versus the odds of the wrong combination of mutations occurring in a cell, leading to unfettered replication. We might imagine that any narrow rejuvenation therapy that improves regeneration and increases cell activity in later life, such as improving mitochondrial function, for example, is going to increase cancer risk. Still, it is clear that a better lifestyle more than halves cancer risk, even just considering exercise.

We might argue that targeting therapeutics to cancer cells is the true key to success in treating cancer. Prodrugs are one way to achieve that goal, ensuring that the drug is only active in cells with certain chemical characteristics. One of the reasons why immunotherapies are an improvement over the older approaches of chemotherapy and radiotherapy is that immune cells inherently provide the basis for a targeted approach. Early CAR-T therapies are looking good; long-term remission has occurred in a significant number of patients. Since then, many potential approaches to improve immunotherapies have emerged, such as via engineering T cells to reduce exhaustion in the face of exposure to cancer cells, replacement of checkpoint inhibition with new varieties of T cell therapies, or mRNA cancer vaccines.

Regenerative Medicine

Cell therapies and extracellular vesicle therapies of various sorts are under development for many conditions, including those in which cells are made universal to allow transplantation between individuals, though arguably we don't well understand the more widely used forms of stem cell therapy that presently exist. Some of the stem cell and vesicle therapies mentioned in the past year follow: cell therapies for degenerative disc disease and spinal cord injury; stem cell derived vesicles can reduce epigenetic age; cells sourced from the peripheral nervous system can be used to treat neurodegenerative conditions; brain regeneration might be achieved through suitable cell therapies; first generation stem cell and exosome therapies can upregulate neurogenesis; improving muscle regrowth with vesicles, and using exosomes to treat ventricular arrhythmia.

Beyond cell therapies, growing and then implanting organoids may have utility, augumenting aged organs with new and functional tissue. Additionally, the use of scaffold material implants continues to be developed, such as for regrowth of dental pulp. Further, organ replacement may benefit from the advent of engineered pig organs; the first such heart transplant was performed this year, but the challenges encountered suggest that a longer road than hoped lies ahead before widespread use.

Is it possible to manipulate native cells to induce regeneration? Targeting fibroblasts to alter their behavior may enable scarless healing in mammals, for example, or reprogramming fibroblasts to cardiomyocytes in the heart. Engineering regrowth of organs in adults is potentially possible, given that highly regenerative species are capable of it, and non-regenerative species can perform much the same feats of regeneration during embryonic development. Enhancer sequences from zebrafish can be used to spur heart regeneration in mice. Further, researchers managed to imperfectly regrow frog limbs using a cocktail of growth factors; the result was not a fully formed limb, but that it worked at all suggests that this line of research may have potential.

Regulation of Medical Development

Early in the year, I noted a charitable view of the problems at the FDA regarding the development of drugs to treat aging. Meanwhile the wrangling continues over the question of whether largely unaccountable international bodies will decide to classify aging as a disease, something that is of importance to the regulation and funding of medicine and medical research, but irrelevant to the science itself. Longevity industry companies involved in developing therapies to treat aging are ignoring this circus in favor of picking specific diseases of aging and proceeding through the regulatory gauntlet as-is, in expectation that widespread off-label use will result. Still, comparatively few trials of genuinely age-targeted therapies have yet taken place. These are still early days.

Cryonics and Cryopreservation

That there should be more support for cryonics research and development is a popular viewpoint in that side of the longevity community. Until recently, cryonics was largely a non-profit industry. It has been proposed that a path to for-profit cryonics might involve first starting a hospital (or a veterinary clinic), and then adding cryonics services to that business, rather than starting with dedicated cryonics providers. Some new capabilities may pay off more than others when it comes to generating greater funding and growth for the cryonics industry, such as reversible vitrification of organs for use in transplantation. At some point in the future, a tipping point will be reached, and cryonics will have its time in the sun, just as the once-fringe field of rejuvenation research is enjoying that time in the sun today.

Rewarming tissue for use without damaging cells, structures, and function is arguably the real challenge in cryopreservation; in the last year use of magnetic nanoparticles has shown potential as a solution that might at least be applied to organs intended for transplantation.

Thoughts and Short Essays

I occasionally set down a few thoughts on topics relevant to longevity. Here are the few times that happened in the past year:

- Be Extraordinary or Be Dead
- Request for Startups in the Rejuvenation Biotechnology Space, 2022 Edition
- A Hypothetical Project: the Fast Track to Partial Reprogramming in Human Volunteers
- Should I Actually Be Working on Cryonics Rather than Rejuvenation?
- Understanding Anencephaly as the Start on the Road to Building Replacement, Youthful Bodies
- Is Reversing Paracrine Senescence a Useful Approach to Alleviating the Age-Related Burden of Senescent Cells?
- Distributed Full Disclosure Medical Development
- A Short Commentary on Why We Advocate for Aging
- Reporting on a Study of One with Khavinson Peptides and Melatonin for Thymic Regrowth
- Two Year Update on a Study of One with Flagellin Immunization to Adjust the Gut Microbiome
- Notes from the Rejuvenation Startup Summit, Held in Berlin in October 2022
- Year End Charitable Donations to Help Advance Rejuvenation Research

Onwards!

Things move slowly when you look at the world a year at at time. They run quite fast when comparing today with ten years ago, or ten years from now. There was no such thing as a longevity industry ten years ago, for example. Ten years from now, doctors will be widely prescribing the first rejuvenation therapies, and it will be the common wisdom that one can and should be treated to slow and reverse processes of aging. We live in interesting times, a great transition over decades from a world in which aging was thought of as immutable to a world in which aging is just another medical condition to be addressed.

Send email to Reason at Fight Aging!: reason@fightaging.org

Start preparing your

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Alcor makes available to every member and patient, without charge, one acid free Memory Box about the size of a standard banker's box (H10" x W12" x L15") for memorabilia to be stored underground at a commercial storage site called Underground Vaults and Storage (UV&S) in Kansas. Additional Boxes are a one-time charge of $250 each for perpetual storage.

Some of the most popular items that have been placed into storage are such things as letters, cards, photographs, diaries, journals, notebooks, books, clippings, army records, directories, recipes, video tapes, cassettes, medical records, flash drives, and external drives.

If you would like to begin working on your own Memory Box, or perhaps contribute items to a Box for an Alcor Member already in stasis, or if you have any questions, please contact **Linda Chamberlain at linda.chamberlain@alcor.org**.

Asset Preservation Trusts for Alcor Members

Would you like to have access to your assets when you are revived?

Would you like to talk to someone who understands cryonics as well as trusts and estate planning?

There are two unique revival trusts that have been developed to help accomplish those goals. The Asset Preservation Trust is an individual trust for Members who can place a minimum of $500,000 into it, and the pooled Multi-Investor Future Income Trust (MIFIT), which requires a minimum investment of $25,000.

Want to learn more? Contact Linda Chamberlain at linda.chamberlain@alcor.org

Revival Update

Scientific Developments Supporting Revival Technologies

Reported by R. Michael Perry, Ph.D.

Ion-tunable antiambipolarity in mixed ion–electron conducting polymers enables biorealistic organic electrochemical neurons

Padinhare Cholakkal Harikesh, Chi-Yuan Yang, Han-Yan Wu, Silan Zhang, Mary J. Donahue, April S. Caravaca, Jun-Da Huang, Peder S. Olofsson, Magnus Berggren, Deyu Tu, Simone Fabiano *Nature Materials*, 12 Jan. 2023, https://www.nature.com/articles/s41563-022-01450-8, accessed 16 Jan. 2023.

Abstract

Biointegrated neuromorphic hardware holds promise for new protocols to record/regulate signalling in biological systems. Making such artificial neural circuits successful requires minimal device/circuit complexity and ion-based operating mechanisms akin to those found in biology. Artificial spiking neurons, based on silicon-based complementary metal-oxide semiconductors or negative differential resistance device circuits, can emulate several neural features but are complicated to fabricate, not biocompatible and lack ion-/chemical-based modulation features. Here we report a biorealistic conductance-based organic electrochemical neuron (c-OECN) using a mixed ion–electron conducting ladder-type polymer with stable ion-tunable antiambipolarity. The latter is used to emulate the activation/inactivation of sodium channels and delayed activation of potassium channels of biological neurons. These c-OECNs can spike at bioplausible frequencies nearing 100 Hz, emulate most critical biological neural features, demonstrate stochastic spiking and enable neurotransmitter-/amino acid-/ion-based spiking modulation, which is then used to stimulate biological nerves in vivo. These combined features are impossible to achieve using previous technologies.

From: Artificial nerve cells – almost like biological

Mikael Sönne, Linköping University News, 13 Jan. 2023, https://liu.se/en/news-item/konstgjorda-nervceller-nastan-som-biologiska, accessed 16 Jan. 2023.

Researchers at Linköping University (LiU) have created an artificial organic neuron that closely mimics the characteristics of biological nerve cells. This artificial neuron can stimulate natural nerves, making it a promising technology for various medical treatments in the future.

Work to develop increasingly functional artificial nerve cells continues at the Laboratory for Organic Electronics, LOE. In 2022, a team of scientists led by associate professor Simone Fabiano demonstrated how an artificial organic neuron could be integrated into a living carnivorous plant to control the opening and closing of its maw. This synthetic nerve cell met 2 of the 20 characteristics that [closely mimic] a biological nerve cell.

In their latest study, published in the journal *Nature Materials*, the same researchers at LiU have developed a new artificial nerve cell called "conductance-based organic electrochemical neuron" or c-OECN, which closely mimics 15 out of the 20 neural features that characterise biological nerve cells, making its functioning much more similar to natural nerve cells.

"One of the key challenges in creating artificial neurons that effectively mimic real biological neurons is the ability to incorporate ion modulation. Traditional artificial neurons made of silicon can emulate many neural features but cannot communicate through ions. In contrast, c-OECNs use ions to demonstrate several key features of real biological neurons", says Simone Fabiano, principal investigator of the Organic Nanoelectronics group at LOE.

In 2018, this research group at Linköping University was one of the first to develop organic electrochemical transistors based on n-type conducting polymers, which are materials that can conduct negative charges. This made it possible to build printable complementary organic electrochemical circuits. Since then, the group has been working to optimise these transistors so that they can be printed in a printing press on a thin plastic foil. As a result, it is now possible to print thousands of transistors on a flexible substrate and use them to develop artificial nerve cells.

In the newly developed artificial neuron, ions are used to control the flow of electronic current through an n-type conducting polymer, leading to spikes in the device's voltage. This process is similar to that which occurs in biological nerve cells. The unique material in the artificial nerve cell also allows the current to be increased and decreased in an almost perfect bell-shaped curve that resembles the activation and inactivation of sodium ion channels found in biology.

"Several other polymers show this behaviour, but only rigid polymers are resilient to disorder, enabling stable device operation", says Simone Fabiano.

In experiments carried out in collaboration with Karolinska Institute (KI), the new c-OECN neurons were connected to the vagus nerve of mice. The results show that the artificial neuron could stimulate the mice's nerves, causing a 4.5% change in their heart rate.

The fact that the artificial neuron can stimulate the vagus nerve itself could, in the long run, pave the way for essential applications in various forms of medical treatment. In general, organic semiconductors have the advantage of being biocompatible, soft, and malleable, while the vagus nerve plays a key role, for example, in the body's immune system and metabolism.

The next step for the researchers will be to reduce the energy consumption of the artificial neurons, which is still much higher than that of human nerve cells. Much work remains to be done to replicate nature artificially.

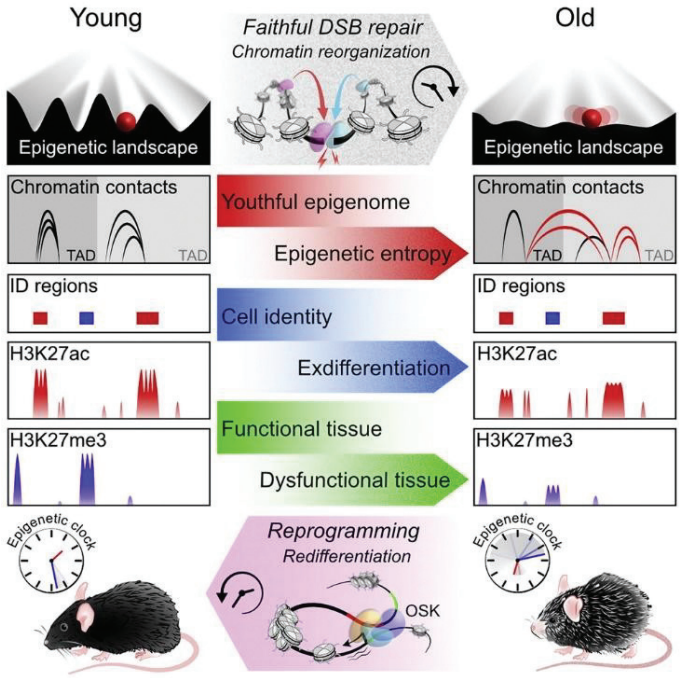
Loss of epigenetic information as a cause of mammalian aging

Jae-Hyun Yang, Motoshi Hayano, Patrick T. Griffin, João A. Amorim, Michael S. Bonkowski, John K. Apostolides, Elias L. Salfati, Marco Blanchette, Elizabeth M. Munding, Mital Bhakta, Yap Ching Chew, Wei Guo, Xiaojing Yang, Sun Maybury-Lewis, Xiao Tian, Jaime M. Ross, Giuseppe Coppotelli, Margarita V. Meer, Ryan Rogers-Hammond, Daniel L. Vera, Yuancheng Ryan Lu, Jeffrey W. Pippin, Michael L. Creswell, Zhixun Dou, Caiyue Xu, Sarah J. Mitchell, Abhirup Das, Brendan L. O'Connell, Sachin Thakur, Alice E. Kane, Qiao Su, Yasuaki Mohri, Emi K. Nishimura, Laura Schaevitz, Neha Garg, Ana-Maria Balta, Meghan A. Rego, Meredith Gregory-Ksander, Tatjana C. Jakobs, Lei Zhong, Hiroko Wakimoto, Jihad El Andari, Dirk Grimm, Raul Mostoslavsky, Amy J. Wagers, Kazuo Tsubota, Stephen J. Bonasera, Carlos M. Palmeira, Jonathan G. Seidman, Christine E. Seidman, Norman S. Wolf, Jill A. Kreiling, John M. Sedivy, George F. Murphy, Richard E. Green, Benjamin A. Garcia, Shelley L. Berger, Philipp Oberdoerffer, Stuart J. Shankland, Vadim N. Gladyshev, Bruce R. Ksander, Andreas R. Pfenning, Luis A. Rajman, David A. Sinclair, *Cell*, 12 Jan. 2023, https://www.cell.com/cell/fulltext/S0092-8674(22)01570-7, accessed 15 Jan. 2023.

Summary

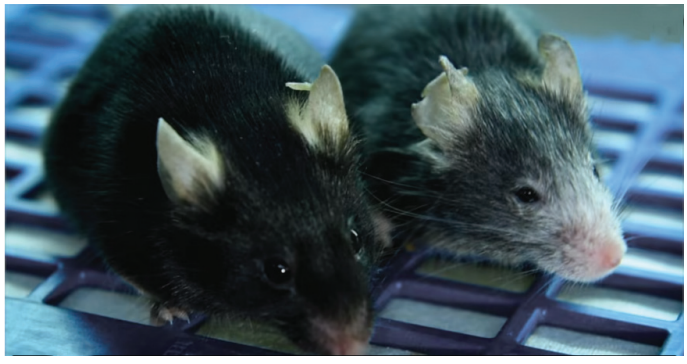
All living things experience an increase in entropy, manifested as a loss of genetic and epigenetic information. In yeast, epigenetic information is lost over time due to the relocalization of chromatin-modifying proteins to DNA breaks, causing cells to lose their identity, a hallmark of yeast aging. Using a system called "ICE" (inducible changes to the epigenome), we find that the act of faithful DNA repair advances aging at physiological, cognitive, and molecular levels, including erosion of the epigenetic landscape, cellular exdifferentiation, senescence, and advancement of the DNA methylation clock, which can be reversed by OSK-mediated rejuvenation. These data are consistent with the information theory of aging, which states that a loss of epigenetic information is a reversible cause of aging.

Graphical abstract



From: Loss of epigenetic information can drive aging, restoration can reverse it

Stephanie Dutchen, Harvard Medical School News, 12 Jan. 2023, https://hms.harvard.edu/news/loss-epigenetic-information-can-drive-aging-restoration-can-reverse, accessed 15 Jan. 2023.



An international study 13 years in the making demonstrates for the first time that degradation in the way DNA is organized and regulated — known as epigenetics — can drive aging in an organism, independently of changes to the genetic code itself.

The work shows that a breakdown in epigenetic information causes mice to age and that restoring the integrity of the epigenome reverses those signs of aging.

"We believe ours is the first study to show epigenetic change as a primary driver of aging in mammals," said the paper's senior author, David Sinclair, professor of genetics in the Blavatnik Institute at Harvard Medical School and co-director of the Paul F. Glenn Center for Biology of Aging Research.

The team's extensive series of experiments provide long-awaited confirmation that DNA changes are not the only, or even the main, cause of aging. Rather, the findings show, chemical and structural changes to chromatin — the complex of DNA and proteins that forms chromosomes — fuel aging without altering the genetic code itself.

"We expect the findings will transform the way we view the process of aging and the way we approach the treatment of diseases associated with aging," said co-first author Jae-Hyun Yang, research fellow in genetics in the Sinclair lab.

The authors say that because it's easier to manipulate the molecules that control epigenetic processes than to reverse DNA mutations, the work points to new avenues that focus on epigenetics rather than genetics to prevent or treat age-related damage.

First, the results need to be replicated in larger mammals and in humans. Studies in nonhuman primates are currently underway.

"We hope these results are seen as a turning point in our ability to control aging," said Sinclair. "This is the first study showing that we can have precise control of the biological age of a complex animal; that we can drive it forwards and backwards at will."

Re-formation of synaptic connectivity in dissociated human stem cell-derived retinal organoid cultures

Allison L. Ludwig, Steven J. Mayerl, Yu Gao, Mark Banghart, Cole Bacig, Maria A. Fernandez Zepeda, Xinyu Zhao, and David M. Gamm, ed. John G. Flannery, PNAS 120 (2), 04 Jan. 2023, https://www.pnas.org/doi/10.1073/pnas.2213418120, accessed 06 Jan. 2023.

Abstract

Human pluripotent stem cell (hPSC)-derived retinal organoids (ROs) can efficiently and reproducibly generate retinal neurons that have potential for use in cell replacement strategies. The ability of these lab-grown retinal neurons to form new synaptic connections after dissociation from ROs is key to building confidence in their capacity to restore visual function. However, direct evidence of reestablishment of retinal neuron connectivity via synaptic tracing has not been reported to date. The present study employs an in vitro, rabies virus-based, monosynaptic retrograde tracing assay to identify de novo synaptic connections among early retinal cell types following RO dissociation. A reproducible, high-throughput approach for labeling and quantifying traced retinal cell types was developed. Photoreceptors and retinal ganglion cells—the primary neurons of interest for retinal cell replacement—were the two major contributing populations among the traced presynaptic cells. This system provides a platform for assessing synaptic connections in cultured retinal neurons and sets the stage for future cell replacement studies aimed at characterizing or enhancing synaptogenesis. Used in this manner, in vitro synaptic tracing is envisioned to complement traditional preclinical animal model testing, which is limited by evolutionary incompatibilities in synaptic machinery inherent to human xenografts.

From: Lab-grown retinal eye cells make successful connections, open door for clinical trials to treat blindness

Chris Barncard, *UW Communications*, 04 Jan. 2023, https://www.waisman.wisc.edu/2023/01/04/lab-grown-retinal-eye-cells-make-successful-connections-open-door-for-clinical-trials-to-treat-blindness/, accessed 06 Jan. 2023.

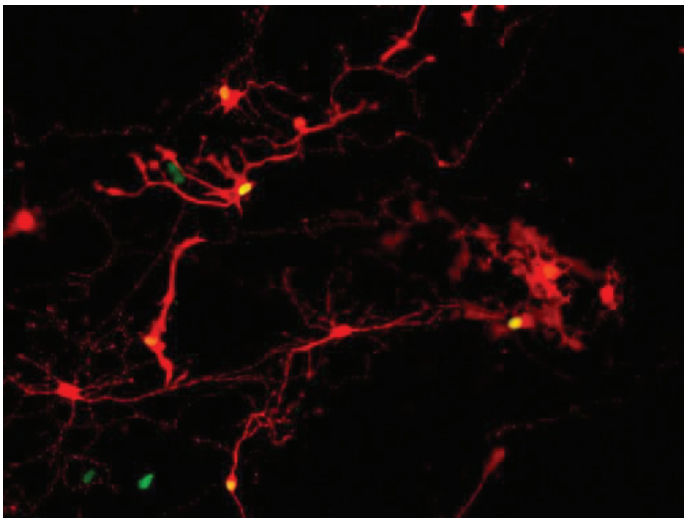
Retinal cells grown from stem cells can reach out and connect with neighbors, according to a new study, completing a "handshake" that may show the cells are ready for trials in humans with degenerative eye disorders.

Over a decade ago, researchers from the University of Wisconsin–Madison developed a way to grow organized clusters of cells, called organoids, that resemble the retina, the light-sensitive tissue at the back of the eye. They coaxed human skin cells reprogrammed to act as stem cells to develop into layers of several types of retinal cells that sense light and ultimately transmit what we see to the brain.

"We wanted to use the cells from those organoids as replacement parts for the same types of cells that have been lost in the course of retinal diseases," says David Gamm, the UW–Madison ophthalmology professor and director of the McPherson Eye Research Institute whose lab developed the organoids. "But after being grown in a laboratory dish for months as compact clusters, the question remained — will the cells behave appropriately after we tease them apart? Because that is key to introducing them into a patient's eye."

During 2022, Gamm and UW–Madison collaborators published studies showing that dish-grown retinal cells called photoreceptors respond like those in a healthy retina to different wavelengths and intensities of light, and that once they are separated from adjacent cells in their organoid, they can reach out toward new neighbors with characteristic biological cords called axons.

"The last piece of the puzzle was to see if these cords had the ability to plug into, or shake hands with, other retinal cell types in order to communicate," says Gamm, whose new results on



Proof of synapses connecting pairs of retinal cells derived from human pluripotent stem cells comes from the red coloring of infection by a modified rabies virus passed from one cell with a yellow nucleus across the synapse to a cell that glows only red. UW–Madison image courtesy Gamm Laboratory.

successful connections between the cells was published today in the Proceedings of the National Academy of Sciences.

Cells in the retina and brain communicate across synapses, tiny gaps at the tips of their cords. To confirm that their lab-grown retinal cells have the capacity to replace diseased cells and carry sensory information like healthy ones, the researchers needed to show that they could make synapses.

Xinyu Zhao, UW–Madison professor of neuroscience and co-author of the new study, worked with the Gamm lab's cells to help study their ability to form synaptic connections. They did this using a modified rabies virus to identify pairs of cells that could form the means to communicate with one another.

The research team, including graduate students and co-first-authors Allison Ludwig and Steven Mayerl, broke apart the retinal organoids into individual cells, gave them a week to extend their axons and make new connections, exposed them to the virus, and then took a peek. What they saw were many retinal cells marked by a fluorescent color indicating a rabies infection had infected one across a synapse successfully formed between neighbors.

Cryopreservation of tissues by slow-freezing using an emerging zwitterionic cryoprotectant

Takeru Ishizaki, Yasuto Takeuchi, Kojiro Ishibashi, Noriko Gotoh, Eishu Hirata, Kosuke Kuroda, *Scientific Reports* 13, no. 37, 02 Jan. 2023, https://www.nature.com/articles/s41598-022-23913-3, accessed 06 Jan. 2023.

Abstract

Cryopreservation of tissues is a tough challenge. Cryopreservation is categorized into slow-freezing and vitrification, and vitrification has recently been recognized as a suitable method for tissue cryopreservation. On the contrary, some researchers have reported that slow-freezing also has potential for tissue cryopreservation. Although conventional cryoprotectants have been studied well, some novel ones may efficiently cryopreserve tissues via slow-freezing. In this study, we used aqueous solutions of an emerging cryoprotectant, an artificial zwitterion supplemented with a conventional cryoprotectant, dimethyl sulfoxide (DMSO), for cell spheroids. The zwitterion/DMSO aqueous solutions produced a better cryoprotective effect on cell spheroids, which are the smallest units of tissues, compared to that of a commercial cryoprotectant. Cryopreservation with the zwitterion/DMSO solutions not only exhibited better cell recovery but also maintained the functions of the spheroids effectively. The optimized composition of the solution was 10 wt% zwitterion, 15 wt% DMSO, and 75 wt% water. The zwitterion/DMSO solution gave a higher number of living cells for the cryopreservation of mouse tumor tissues than a commercial cryoprotectant. The zwitterion/DMSO solution was also able to cryopreserve human tumor tissue, a patient-derived xenograft.

From the Introduction (lightly edited)

Efficient long-term preservation of tissues is a tough challenge, and novel technologies for the long-term preservation of various tissues are in high demand. Cryopreservation is a key technology for long-term preservation and has already been well established for single dispersed cells. Cells are generally cryopreserved in a deep freezer at around −80 °C or in liquid nitrogen at −196 °C. Cryoprotective agents (CPAs) have been developed to prevent physical damage to cells induced by ice crystals formed under extremely low temperature. The most commonly used CPAs are glycerol and dimethyl sulfoxide (DMSO). Although some papers have reported cryopreservation of multicellular spheroids and tissues using these CPAs with a certain degree of cell viability, generally, cryopreservation is inadequate for multicellular systems.

Cryopreservation is categorized into slow-freezing and vitrification. Slow-freezing has a low cooling rate and prevents intracellular ice formation (IIF) by dehydration of cells. Slow-freezing is widely used for dispersed single cells because it works even with low concentrations of toxic CPAs and the amateur skills of the operator. Vitrification has a high cooling rate and prevents IIF by the instantaneous formation of a glass-like structure. Although it requires high concentrations of toxic CPAs and expert skills, it has recently attracted attention because it completely

avoids ice crystal formation. In addition, vitrification leads to low volume change at low temperatures, avoiding compression of the tissues. Based on these advantages, vitrification is recognized to be suitable for the cryopreservation of tissues. Slow-freezing of tissues has not, therefore, been enthusiastically studied in recent years, though recently it was reported that slow-freezing is superior for cryopreservation of ovarian tissues. Kosuke Kuroda of Kanazawa University and colleagues considered that slow-freezing has untapped potential. In their study, the emerging CPAs were applied to harness this potential, because conventional CPAs have already been well studied.

They proposed low-molecular-weight, aprotic synthetic zwitterions as novel CPAs for slow-freezing. Zwitterions have positive and negative charges in one molecule. The synthetic ones that are applicable to cryopreservation possess various cations such as imidazolium, ammonium, and pyridinium and anions such as carboxylate and sulfonate. They have low cytotoxicity. For example, after 24 hours the zwitterion did not kill zebrafish embryos at 5% but the same concentration of DMSO killed most of them. The zwitterions the researchers synthesized are aprotic, thus basically always possess electric charges unlike protic zwitterions such as amino acids. The constant electric charges strongly interact with water, resulting in the inhibition of ice crystallization. Therefore, the zwitterions show a similar effect to that of commercial CPA for single cells. Although synthetic zwitterionic polymers and natural zwitterions as CPAs have been studied in detail, low-molecular-weight synthetic zwitterions have not been well studied.

The synthetic zwitterions are cell-impermeable CPAs and cannot inhibit IIF directly, unlike cell-permeable natural zwitterions; however, they increase the osmolarity of the freezing medium and indirectly inhibit IIF by dehydrating cells due to the high osmotic pressure. Moreover, their cryoprotective effect is improved by the supplementation of cell-permeable DMSO. In a preliminary study, the researchers demonstrated that some aqueous mixtures of zwitterion/DMSO showed a higher cryoprotective effect than a commercial CPA on a variety of single dispersed cells vulnerable to freezing. In the present study, the zwitterion/DMSO aqueous solutions were applied to the cryopreservation of tissues. Tumor tissues were used for this primitive study because of convenience. While there are many zwitterion species, the researchers used the imidazolium/carboxylate zwitterion (called OE2imC3C in their previous works) because it is a promising species.

Base-edited CAR T cells for combinational therapy against T Cell malignancies

Christos Georgiadis, Jane Rasaiyaah, Soragia Athina Gkazi, Roland Preece, Aniekan Etuk, Abraham Christi, Waseem Qasim,

Leukemia 35, 3466–3481, 25 May 2021, https://www.nature.com/articles/s41375-021-01282-6, accessed 25 Dec. 2022.

Abstract

Targeting T cell malignancies using chimeric antigen receptor (CAR) T cells is hindered by 'T v T' fratricide against shared antigens such as CD_3 and CD_7. Base editing offers the possibility of seamless disruption of gene expression of problematic antigens through creation of stop codons or elimination of splice sites. We describe the generation of fratricide-resistant T cells by orderly removal of TCR/CD_3 and CD_7 ahead of lentiviral-mediated expression of CARs specific for CD_3 or CD_7. Molecular interrogation of base-edited cells confirmed elimination of chromosomal translocations detected in conventional Cas_9 treated cells. Interestingly, $_3$CAR/$_7$CAR co-culture resulted in 'self-enrichment' yielding populations 99.6% TCR−/CD_3−/CD_7−. $_3$CAR or $_7$CAR cells were able to exert specific cytotoxicity against leukaemia lines with defined CD_3 and/or CD_7 expression as well as primary T-ALL cells. Co-cultured $_3$CAR/$_7$CAR cells exhibited highest cytotoxicity against CD_3+CD_7+T-ALL targets in vitro and an in vivo human:murine chimeric model. While APOBEC editors can reportedly exhibit guide-independent deamination of both DNA and RNA, we found no problematic 'off-target' activity or promiscuous base conversion affecting CAR antigen-specific binding regions, which may otherwise redirect T cell specificity. Combinational infusion of fratricide-resistant anti-T CAR T cells may enable enhanced molecular remission ahead of allo-HSCT for T cell malignancies.

(Editor's note: The following article, applying results of the above study, had no published, peer-reviewed study of its own at time of writing; the above is the closest approach I could find. – RMP)

From: World-first use of base-edited CAR T-cells to treat resistant leukaemia

University College London News (unattributed), 12 Dec. 2022, https://www.ucl.ac.uk/news/2022/dec/world-first-use-base-edited-car-t-cells-treat-resistant-leukaemia, accessed 25 Dec. 2022.

A patient with relapsed T-cell leukaemia has been given base-edited T-cells in a world-first use of a base-edited cell therapy, in a 'bench-to-bedside' collaboration between UCL and Great Ormond Street Hospital for Children (GOSH).

The patient, 13-year-old Alyssa from Leicester, was diagnosed with T-cell acute lymphoblastic leukaemia (T-ALL) in 2021. She was treated with all current conventional therapies for her blood cancer, including chemotherapy and a bone marrow transplant, but unfortunately her disease came back and there were no further treatment options.

Alyssa was the first patient to be enrolled onto the TvT clinical trial and in May 2022 she was admitted to the Bone Marrow

Transplant (BMT) Unit at GOSH, to receive 'universal' CAR T-cells that had been pre-manufactured from a healthy volunteer donor. These cells had been edited using new base-editing technology, which was designed and developed by a team of researchers at UCL, led by Professor Waseem Qasim (UCL Great Ormond Street Institute of Child Health), who is also an Honorary Consultant at GOSH.

She was then fitted with a Chimeric Antigen Receptor (CAR) to allow them to hunt down and kill cancerous T-cells without attacking each other.

Just 28 days later, Alyssa was in remission and went on to receive a second bone marrow transplant to restore her immune system. Now, six-months post-BMT, she is doing well at home recovering with her family and continues her post-BMT follow-up at GOSH. Without this experimental treatment, Alyssa's only option was palliative care.

Researchers are presenting the data for the first time at the American Society of Haematology annual meeting in New Orleans, USA, this weekend.

Professor Waseem Qasim, Professor of Cell and Gene Therapy at UCL GOS ICH and Consultant Immunologist at GOSH said: "We designed and developed the treatment from lab to clinic and are now trialling it on children from across the UK – in a unique bench to bedside approach.

"Alyssa's story is a great demonstration of how, with expert teams and infrastructure, we can link cutting edge technologies in the lab with real results in the hospital for patients. It's our most sophisticated cell engineering so far and paves the way for other new treatments and ultimately better futures for sick children.

"We have a unique and special environment here at GOSH and UCL that allows us to rapidly scale up new technologies and we're looking forward to continuing our research and bringing it to the patients who need it most."

Alyssa

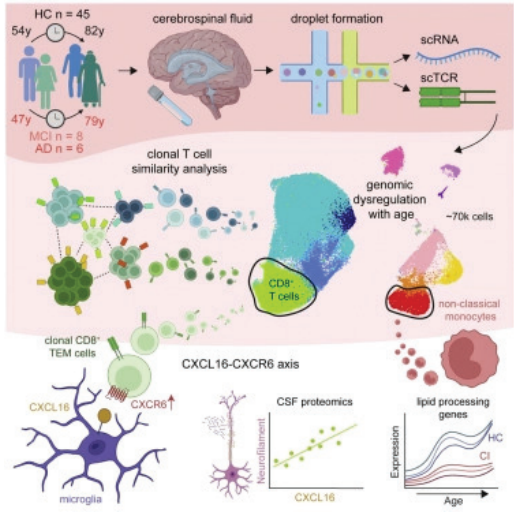
Cerebrospinal fluid immune dysregulation during healthy brain aging and cognitive impairment

Natalie Piehl, Lynn van Olst, Abhirami Ramakrishnan, Victoria Teregulova, Brooke Simonton, Ziyang Zhang, Emma Tapp, Divya Channappa, Hamilton Oh, Patricia M. Losada, Jarod Rutledge, Alexandra N. Trelle, Elizabeth C. Mormino, Fanny Elahi, Douglas R. Galasko, Victor W. Henderson, Anthony D. Wagner, Tony Wyss-Coray, David Gate, *Cell* 185(26), 22 Dec. 2022, https://www.cell.com/cell/fulltext/S0092-8674(22)01463-5, accessed 1 Jan. 2023.

Summary

Cerebrospinal fluid (CSF) contains a tightly regulated immune system. However, knowledge is lacking about how CSF immunity is altered with aging or neurodegenerative disease. Here, we performed single-cell RNA sequencing on CSF from 45 cognitively normal subjects ranging from 54 to 82 years old. We uncovered an upregulation of lipid transport genes in monocytes with age. We then compared this cohort with 14 cognitively impaired subjects. In cognitively impaired subjects, downregulation of lipid transport genes in monocytes occurred concomitantly with altered cytokine signaling to CD8 T cells. Clonal CD8 T effector memory cells upregulated C-X-C motif chemokine receptor 6 (CXCR6) in cognitively impaired subjects. The CXCR6 ligand, C-X-C motif chemokine ligand 16 (CXCL16), was elevated in the CSF of cognitively impaired subjects, suggesting CXCL16-CXCR6 signaling as a mechanism for antigen-specific T cell entry into the brain. Cumulatively, these results reveal cerebrospinal fluid immune dysregulation during healthy brain aging and cognitive impairment.

Graphical abstract



From: New immune culprit discovered in Alzheimer's disease

Marla Paul, Northwestern Medicine News Center, 13 Dec. 2022, https://news.feinberg.northwestern.edu/2022/12/13/new-immune-culprit-discovered-in-alzheimers-disease/, accessed 1 Jan. 2023.

The reason your three-pound brain doesn't feel heavy is because it floats in a reservoir of cerebrospinal fluid (CSF), which flows in and around your brain and spinal cord. This liquid barrier between your brain and skull protects it from a hit to your head and bathes your brain in nutrients.

David Gate, PhD, assistant professor in the Ken and Ruth Davee Department of Neurology, and lead author of the study

But the CSF has another critical, if less known, function: it also provides immune protection to the brain. Yet, this function hasn't been well studied.

A Northwestern Medicine study of CSF published in the journal *Cell*, has discovered its role in cognitive impairment, such as Alzheimer's disease. This discovery provides a new clue to the process of neurodegeneration, said study lead author David Gate, PhD, assistant professor in the Ken and Ruth Davee Department of Neurology.

The study found that as people age, their CSF immune system becomes dysregulated. In people with cognitive impairment, such as those with Alzheimer's disease, the CSF immune system is drastically different from healthy individuals, the study also discovered.

"We now have a glimpse into the brain's immune system with healthy aging and neurodegeneration," Gate said. "This immune reservoir could potentially be used to treat inflammation of the brain or be used as a diagnostic to determine the level of brain inflammation in individuals with dementia."

"We provide a thorough analysis of this important immunologic reservoir of the healthy and diseased brain," Gate said. His team is sharing the data publicly, and its results can be searched online.

To analyze the CSF, Gate's team at Northwestern used a sophisticated technique called single-cell RNA sequencing. They profiled 59 CSF immune systems from a spectrum of ages by taking CSF from participants' spines and isolating their immune cells.

The first part of the study looked at CSF in 45 healthy individuals aged 54 to 83 years. The second part of the study compared those findings in the healthy group to CSF in 14 adults with cognitive impairment, as determined by their poor scores on memory tests.

Gate's team of scientists observed genetic changes in the CSF immune cells in older healthy individuals that made the cells appear more activated and inflamed with advanced age.

"The immune cells appear to be a little angry in older individuals," Gate said. "We think this anger might make these cells less functional, resulting in dysregulation of the brain's immune system."

In the cognitively impaired group, inflamed T-cells cloned themselves and flowed into the CSF and brain as if they were following a radio signal, Gate said. Scientists found the cells had an overabundance of a cell receptor — CXCR6 — that acts as an antenna. This receptor receives a signal — CXCL16 — from the degenerating brain's microglia cells to enter the brain.

"It could be the degenerating brain activates these cells and causes them to clone themselves and flow to the brain," Gate said. "They do not belong there, and we are trying to understand whether they contribute to damage in the brain."

Gate said his "future goal is to block that radio signal, or to inhibit the antenna from receiving that signal from the brain. We want to know what happens when these immune cells are blocked from entering brains with neurodegeneration."

Controlling gene expression with deep generative design of regulatory DNA

Jan Zrimec, Xiaozhi Fu, Azam Sheikh Muhammad, Christos Skrekas, Vykintas Jauniskis, Nora K. Speicher, Christoph S. Börlin, Vilhelm Verendel, Morteza Haghir Chehreghani, Devdatt Dubhashi, Verena Siewers, Florian David, Jens Nielsen, Aleksej Zelezniak, *Nature Communications* 13, 5099, 30 Aug. 2022, https://www.nature.com/articles/s41467-022-32818-8, accessed 28 Nov. 2022.

Abstract

Design of de novo synthetic regulatory DNA is a promising avenue to control gene expression in biotechnology and medicine. Using mutagenesis typically requires screening sizable random DNA libraries, which limits the designs to span merely a short section of the promoter and restricts their control of gene expression. Here, we prototype a deep learning strategy based on generative adversarial networks (GAN) by learning directly from genomic and transcriptomic data. Our ExpressionGAN can traverse the entire regulatory sequence-expression landscape in a gene-specific manner, generating regulatory DNA with prespecified target mRNA levels spanning the whole gene regulatory structure including coding and adjacent non-coding regions. Despite high sequence divergence from natural DNA, in vivo measurements show that 57% of the highly-expressed synthetic sequences surpass the expression levels of highly-expressed natural controls. This demonstrates the applicability and relevance of deep generative design to expand our knowledge and control of gene expression regulation in any desired organism, condition or tissue.

From: AI tailors artificial DNA for future drug development

Chalmers University of Technology news.cision.com (unattributed), 24 Nov. 2022, https://news.cision.com/chalmers/r/ai-tailors-artificial-dna-for-future-drug-development,c3672724, accessed 28 Nov. 2022.

With the help of artificial intelligence, researchers at Chalmers University of Technology, Sweden, have succeeded in designing synthetic DNA that controls the cells' protein production. The technology can contribute to the development and production of vaccines, drugs for severe diseases, as well as alternative food proteins much faster and at significantly lower costs than today.

The principle behind the new method is similar to when an AI generates faces that look like real people. By learning what a large selection of faces looks like, the AI can then create completely new but natural-looking faces. It is then easy to modify a face by, for example, saying that it should look older, or have a different hairstyle. On the other hand, programming a believable face from scratch, without the use of AI, would have been much more difficult and time-consuming. Similarly, the researchers' AI has been taught the structure and regulatory code of DNA. The AI then designs synthetic DNA, where it is easy to modify its regulatory information in the desired direction of gene expression. Simply put, the AI is told how much of a gene is desired and then "prints" the appropriate DNA sequence.

"DNA is an incredibly long and complex molecule. It is thus experimentally extremely challenging to make changes to it by iteratively reading and changing it, then reading and changing it again. This way it takes years of research to find something that works. Instead, it is much more effective to let an AI learn the principles of navigating DNA. What otherwise takes years is now shortened to weeks or days," says first author Jan Zrimec, a research associate at the National Institute of Biology in Slovenia and past postdoc in Aleksej Zelezniak's group.

The researchers have developed their method in the yeast Saccharomyces cerevisiae, whose cells resemble mammalian cells. The next step is to use human cells. The researchers have hopes that their progress will have an impact on the development of new as well as existing drugs.

"Protein-based drugs for complex diseases or alternative sustainable food proteins can take many years and can be extremely expensive to develop. Some are so expensive that it is impossible to obtain a return on investment, making them economically nonviable. With our technology, it is possible to develop and manufacture proteins much more efficiently so that they can be marketed," says Aleksej Zelezniak.

Cytidine-containing tails robustly enhance and prolong protein production of synthetic mRNA in cell and *in vivo*

Cheuk Yin Li, Zhenghua Liang, Yaxin Hu, Hongxia Zhang, Kharis Daniel Setiasabda, Jiawei Li, Shaohua Ma, Xiaojun Xia, Yi Kuang, *Molecular Therapy Nucleic Acids*, 11 Oct. 2022, https://www.cell.com/molecular-therapy-family/nucleic-acids/fulltext/S2162-2531(22)00269-4?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2162253122002694%3Fshowall%3Dtrue, accessed 27 Nov. 2022.

Abstract

Synthetic mRNAs are rising rapidly as alternative therapeutic agents for delivery of proteins. However, the practical use of synthetic mRNAs has been restricted by their low cellular stability as well as poor protein production efficiency. The key roles of poly(A) tail on mRNA biology inspire us to explore the optimization of tail sequence to overcome the aforementioned limitations. Here, the systematic substitution of

non-A nucleotides in the tails revealed that cytidine-containing tails can substantially enhance the protein production rate and duration of synthetic mRNAs both *in vitro* and *in vivo*. Such C-containing tails shield synthetic mRNAs from deadenylase CCR4-NOT transcription complex, as the catalytic CNOT proteins, especially CNOT6L and CNOT7, have lower efficiency in trimming of cytidine. Consistently, these enhancement effects of C-containing tails were observed on all synthetic mRNAs tested and were independent of transfection reagents and cell types. As the C-containing tails can be used along with other mRNA enhancement technologies to synergically boost protein production, we believe that these tails can be broadly used on synthetic mRNAs to directly promote their clinical applications.

From: HKUST researchers discover new way to synthesize mRNAs enhancing effectiveness of mRNA drugs and vaccines

Hong Kong University of Science and Technology (unattributed), 11 Oct, 2022, https://hkust.edu.hk/news/research-and-innovation/hkust-researchers-discover-new-way-synthesize-mrnas-enhancing, accessed 27 Nov. 2022.

A team of synthetic biologists at the Hong Kong University of Science and Technology (HKUST) has recently discovered a way that could increase synthetic mRNA's protein production efficiency by up to 10 times, which means the effectiveness of mRNA vaccines and drugs – such as those used against cancer, Covid-19 or other genetic diseases, will be greatly boosted with even less dosage of the mRNAs.

mRNAs can be synthesized to teach our cells in making any kind of proteins, such as antigens, enzymes and hormones which are essential in fighting infections and regulating bodily functions, so mRNA is arguably a preferred option for vaccines and treatment for many different kinds of diseases. However, high dosage and repeated injections are often required for mRNA drugs and vaccines in order to generate sufficient amounts of protein in the body, so enhancing mRNA's effectiveness – such as by increasing its protein production efficiency, is a hot subject among scientists as our immune system, for example, could work better with more of certain antibodies.

Now, a team led by Prof. Becki KUANG Yi, Assistant Professor at the Department of Chemical and Biological Engineering at HKUST, discovered a way that could enhance both the life span and efficiency of mRNA. Having engineered different mRNA's tail sequences, Prof. Kuang's team eventually discovered optimized sequences that could produce 3 to 10 times as much proteins [as] unoptimized tail sequences commonly used for synthetic mRNAs on both human cells and on mice. Duration of protein production is also doubled.

This new technology will not only reduce the amount and the number of injections needed for mRNA drugs and vaccines, but will also potentially lower the cost of treatments. It can also be used along with other mRNA enhancement technologies to synergically boost protein production.

"Increasing the protein production of synthetic mRNA is generally beneficial to all mRNA drugs and vaccines," said Prof Kuang. "In collaboration with Sun Yat-Sen University, our team is now exploring the use of optimized tails for mRNA cancer vaccines on animal[s]. We are also looking forward to collaborating with pharmaceutical companies to transfer this invention onto mRNA therapeutics and vaccines' development pipelines to benefit society."

Temporal optimization of radiation therapy to heterogeneous tumour populations and cancer stem cells

Cameron Meaney, Mohammad Kohandel, Arian Novruzi, *Journal of Mathematical Biology*, J Math Biol 13 Oct. 2022, https://pubmed.ncbi.nlm.nih.gov/36227423/, accessed 29 Nov. 2022.

Abstract

External beam radiation therapy is a key part of modern cancer treatments which uses high doses of radiation to destroy tumour cells. Despite its widespread usage and extensive study in theoretical, experimental, and clinical works, many questions still remain about how best to administer it. Many mathematical studies have examined optimal scheduling of radiotherapy, and most come to similar conclusions. Importantly though, these studies generally assume intratumoral homogeneity. But in recent years, it has become clear that tumours are not homogeneous masses of cancerous cells, but wildly heterogeneous masses with various subpopulations which grow and respond to treatment differently. One subpopulation of particular importance is cancer stem cells (CSCs) which are known to exhibit higher radioresistence compared with non-CSCs. Knowledge of these differences between cell types could theoretically lead to changes in optimal treatment scheduling. Only a few studies have examined this question, and interestingly, they arrive at apparent conflicting results. However, an understanding of their assumptions reveals a key difference which leads to their differing conclusions. In this paper, we generalize the problem of temporal optimization of dose distribution of radiation therapy to a two cell type model. We do so by creating a mathematical model and a numerical optimization algorithm to find the distribution of dose which leads to optimal cell kill. We then create a data set of optimization solutions and use data analysis tools to learn the relationships between model parameters and the qualitative behaviour of optimization results. Analysis of the model and discussion of biological importance are provided throughout. We find that the key factor in predicting the behaviour of the optimal

distribution of radiation is the ratio between the radiosensitivities of the present cell types. These results can provide guidance for treatment in cases where clinicians have knowledge of tumour heterogeneity and of the abundance of CSCs.

From: using math to better treat cancer

University of Waterloo Media Relations (not otherwise attributed), 28 Nov. 2022, https://uwaterloo.ca/news/media/using-math-better-treat-cancer, accessed 29 Nov. 2022.

Researchers at the University of Waterloo have identified a new method for scheduling radiation therapy that could be as much as 22 percent more effective at killing cancer cells than current standard radiation treatment regimens.

While many mathematical studies have examined how to optimize the scheduling of radiation treatment for maximum effectiveness against cancer, most of these studies assume "intratumoral homogeneity" – that is, that all of the cancer cells are the same. In recent years, however, scientists have realized that tumours are made up of many different kinds of cells. Most importantly, they include cancer stem cells, which are more resistant to radiation than other kinds of cells.

"The problem with any calculation involving cancer is that it's super hard to get exact values because things vary from cancer type to cancer type, patient to patient, even within the tumour," said Cameron Meaney, a PhD candidate in Applied Mathematics at Waterloo and the lead researcher on the study.

This new algorithm can generalize the differing radiation resistances of stem cells and non-stem cells, allowing doctors to predict how a tumour will respond to treatment before gathering exact data on an individual's cancer.

The model has limitations, Meaney explained, as tumours contain far more than two kinds of cells. What it does, however, is provide clinical researchers with a better starting point for treatment research.

"The results of the algorithm are important because they shed light on the idea that heterogeneity in tumours matters for planning treatment," Meaney said.

The next step the researchers hope to see is an application of their algorithm to clinical studies: will their suggested therapy schedule outperform existing scheduling practices in a lab trial?

Universal parity quantum computing

Michael Fellner, Anette Messinger, Kilian Ender, Wolfgang Lechner, Phys. Rev. Lett. 129, 180503, 27 Oct. 2022, https://journals.aps.org/prl/abstract/10.1103/PhysRevLett.129.180503, accessed 28 Nov. 2022.

Abstract

We propose a universal gate set for quantum computing with all-to-all connectivity and intrinsic robustness to bit-flip errors based on parity encoding. We show that logical controlled phase gate and R_z rotations can be implemented in parity encoding with single-qubit operations. Together with logical R_x rotations, implemented via nearest-neighbor controlled-NOT gates and an R_x rotation, these form a universal gate set. As the controlled phase gate requires only single-qubit rotations, the proposed scheme has advantages for several cornerstone quantum algorithms, e.g., the quantum Fourier transform. We present a method to switch between different encoding variants via partial on-the-fly encoding and decoding.

Applications of universal parity quantum computation

Michael Fellner, Anette Messinger, Kilian Ender, Wolfgang Lechner, Phys. Rev. A 106, 042442, 27 Oct. 2022, https://journals.aps.org/pra/abstract/10.1103/PhysRevA.106.042442, accessed 28 Nov. 2022.

Abstract

We demonstrate the applicability of a universal gate set in the parity encoding, which is a dual to the standard gate model, by exploring several quantum gate algorithms such as the quantum Fourier transform and quantum addition. Embedding these algorithms in the parity encoding reduces the circuit depth compared to conventional gate-based implementations while keeping the multiqubit gate counts comparable. We further propose simple implementations of multiqubit gates in tailored encodings and an efficient strategy to prepare graph states.

From: New form of universal quantum computers

University of Innsbruck (unattributed), 28 Oct. 2022, https://www.uibk.ac.at/en/newsroom/2022/new-form-of-universal-quantum-computers/, accessed 28 Nov. 2022.

Computing power of quantum machines is currently still very low. Increasing it is still proving to be a major challenge. Physicists at the University of Innsbruck now present a new architecture for a universal quantum computer that overcomes such limitations and could be the basis of the next generation of quantum computers soon.

Quantum bits (qubits) in a quantum computer serve as a computing unit and memory at the same time. Because quantum information cannot be copied, it cannot be stored in a memory as in a classical computer. Due to this limitation, all qubits in a quantum computer must be able to interact with each other. This is currently still a major challenge for building powerful quantum computers. In 2015, theoretical physicist Wolfgang Lechner, together with Philipp Hauke and Peter Zoller, addressed this difficulty and proposed a new architecture for a quantum

computer, now named LHZ architecture after the authors. "This architecture was originally designed for optimization problems," recalls Wolfgang Lechner of the Department of Theoretical Physics at the University of Innsbruck, Austria. "In the process, we reduced the architecture to a minimum in order to solve these optimization problems as efficiently as possible." The physical qubits in this architecture do not represent individual bits but encode the relative coordination between the bits. "This means that not all qubits have to interact with each other anymore," explains Wolfgang Lechner. With his team, he has now shown that this parity concept is also suitable for a universal quantum computer.

Parity computers can perform operations between two or more qubits on a single qubit. "Existing quantum computers already implement such operations very well on a small scale," Michael Fellner from Wolfgang Lechner's team explains. "However, as the number of qubits increases, it becomes more and more complex to implement these gate operations." In two publications in Physical Review Letters and Physical Review A, the Innsbruck scientists now show that parity computers can, for example, perform quantum Fourier transformations - a fundamental building block of many quantum algorithms - with significantly fewer computation steps and thus more quickly. "The high parallelism of our architecture means that, for example, the well-known Shor algorithm for factoring numbers can be executed very efficiently," Fellner explains.

The new concept also offers hardware-efficient error correction. Because quantum systems are very sensitive to disturbances, quantum computers must correct errors continuously. Significant resources must be devoted to protecting quantum information, which greatly increases the number of qubits required. "Our model operates with a two-stage error correction, one type of error (bit flip error or phase error) is prevented by the hardware used," say Anette Messinger and Kilian Ender, also members of the Innsbruck research team.

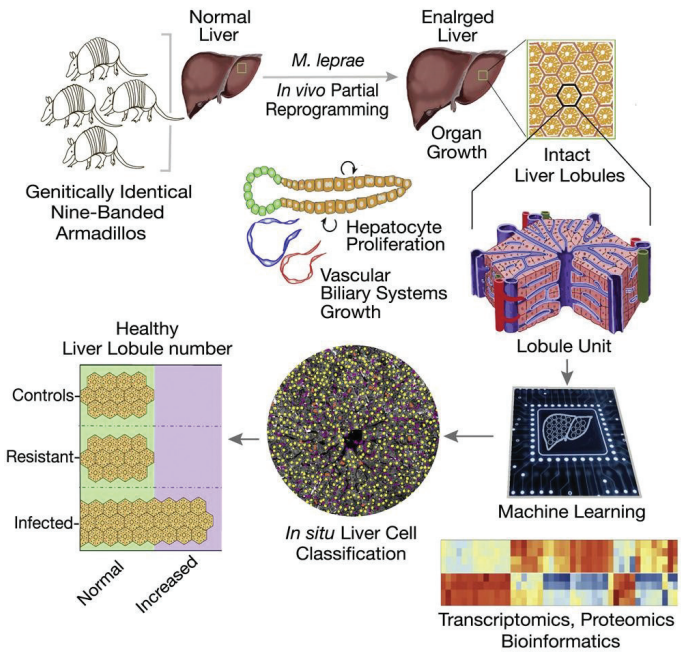
In vivo partial reprogramming by bacteria promotes adult liver organ growth without fibrosis and tumorigenesis

Samuel Hess, Timothy J. Kendall, Maria Pena, Linda Adams, Richard Truman, Anura Rambukkana, Cell Reports Medicine 3(11), 100820, 15 Nov. 2022, https://www.cell.com/cell-reports-medicine/fulltext/S2666-3791(22)00379-2?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2666379122003792%3Fshowall%3Dtrue, accessed 29 Nov. 2022.

Summary

Ideal therapies for regenerative medicine or healthy aging require healthy organ growth and rejuvenation, but no organ-level approach is currently available. Using *Mycobacterium leprae* (ML) with natural partial cellular reprogramming capacity and its animal host nine-banded armadillos, we present an evolutionarily refined model of adult liver growth and regeneration. In infected armadillos, ML reprogram[s] the entire liver and significantly increase[s] total liver/body weight ratio by increasing healthy liver lobules, including hepatocyte proliferation and proportionate expansion of vasculature, and biliary systems. ML-infected livers are microarchitecturally and functionally normal without damage, fibrosis, or tumorigenesis. Bacteria-induced reprogramming reactivates liver progenitor/developmental/fetal genes and upregulates growth-, metabolism-, and anti-aging-associated markers with minimal change in senescence and tumorigenic genes, suggesting bacterial hijacking of homeostatic, regeneration pathways to promote *de novo* organogenesis. This may facilitate the unraveling of endogenous pathways that effectively and safely re-engage liver organ growth, with broad therapeutic implications including organ regeneration and rejuvenation.

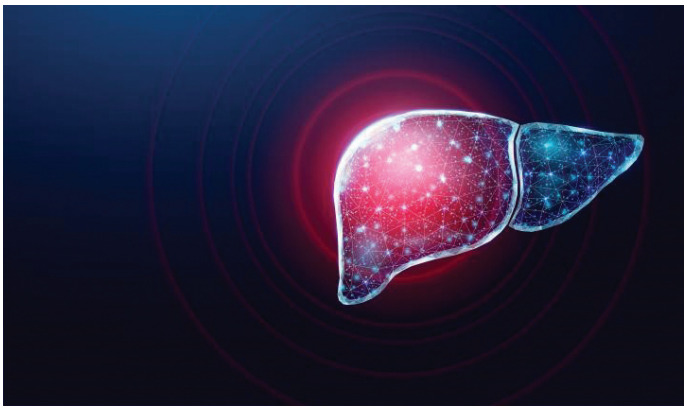
Graphical Abstract



From: Ancient Disease has Potential to Regenerate Livers

University of Edinburgh Research (unattributed), 16 Nov. 2022, https://www.ed.ac.uk/research/latest-research-news/ancient-disease-has-potential-to-regenerate-livers, accessed 29 Nov. 2022 (lightly edited).

Scientists have discovered that parasites associated with leprosy can reprogram cells to increase the size of a liver in adult animals without causing damage, scarring or tumors. The



findings suggest the possibility of adapting this natural process to renew ageing livers and increase healthspan – the length of time living disease-free – in humans. Experts say it could also help regrow damaged livers, thereby reducing the need for transplantation, which is currently the only curative option for people with end-stage scarred livers.

Previous studies promoted the regrowth of mouse livers by generating stem cells and progenitor cells - the step after a stem cell that can become any type of cell for a specific organ – via an invasive technique that often resulted in scarring and tumour growth. To overcome these harmful side-effects, Edinburgh researchers built on their previous discovery of the partial cellular reprogramming ability of the leprosy-causing bacteria, Mycobacterium leprae.

Working with the US Department of Health and Human Services in Baton Rouge, Louisiana, the team infected 57 armadillos – a natural host of leprosy bacteria – with the parasite and compared their livers with those of uninfected armadillos and those that were found to be resistant to infection. They found that the infected animals developed enlarged - yet healthy and unharmed - livers with the same vital components, such as blood vessels, bile ducts and functional units known as lobules, as the uninfected and resistant armadillos.

The team believe the bacteria "hijacked" the inherent regenerative ability of the liver to increase the organ's size and, therefore, to provide it with more cells within which to increase. They also discovered several indicators that the main kinds of liver cells – known as hepatocytes – had reached a "rejuvenated" state in the infected armadilllos. Livers of the infected armadillos also contained gene expression patterns – the blueprint for building a cell – similar to those in younger animals and human fetal livers.

Genes related to metabolism, growth and cell proliferation were activated and those linked with aging were downregulated, or suppressed. Scientists think this is because the bacteria reprogramed the liver cells, returning them to the earlier stage of progenitor cells, which in turn became new hepatocytes and grew new liver tissues. The team are hopeful that the discovery has the potential to help develop interventions for aging and damaged livers in humans. Liver diseases currently result in two million deaths a year worldwide.

XNAzymes Targeting the SARS-CoV-2 Genome Inhibit Viral Infection

Pehuén Pereyra Gerber, Maria J. Donde, Nicholas J. Matheson, Alexander I. Taylor, *Nature Communications* 13, 6716, 16 Nov. 2022, https://www.nature.com/articles/s41467-022-34339-w, accessed 28 Nov. 2022.

Abstract

The unprecedented emergence and spread of SARS-CoV-2, the coronavirus responsible for the COVID-19 pandemic, underscores the need for diagnostic and therapeutic technologies that can be rapidly tailored to novel threats. Here, we show that site-specific RNA endonuclease XNAzymes – artificial catalysts composed of single-stranded synthetic xeno-nucleic acid oligonucleotides (in this case 2'-deoxy-2'-fluoro-β-D-arabino nucleic acid) – may be designed, synthesised and screened within days, enabling the discovery of a range of enzymes targeting SARS-CoV-2 ORF1ab, ORF7b, spike- and nucleocapsid-encoding RNA. Three of these are further engineered to self-assemble into a catalytic nanostructure with enhanced biostability. This XNA nanostructure is capable of cleaving genomic SARS-CoV-2 RNA under physiological conditions, and when transfected into cells inhibits infection with authentic SARS-CoV-2 virus by RNA knockdown. These results demonstrate the potential of XNAzymes to provide a platform for the rapid generation of antiviral reagents.

From: 'Programmable Molecular Scissors' Could Help Fight COVID-19 Infection

University of Cambridge (unattributed), 16 Nov. 2022, https://www.cam.ac.uk/research/news/synthetic-biology-meets-medicine-programmable-molecular-scissors-could-help-fight-covid-19-infection#:~:text=Cambridge%20scientists%20have%20used%20synthetic,new%20generation%20of%20antiviral%20drugs, accessed 28 Nov. 2022.

Cambridge scientists have used synthetic biology to create artificial enzymes programmed to target the genetic code of SARS-CoV-2 and destroy the virus, an approach that could be used to develop a new generation of antiviral drugs.

Enzymes are naturally occurring biological catalysts, which enable the chemical transformations required for our bodies to function – from translating the genetic code into proteins, right through to digesting food. Although most enzymes are proteins, some of these crucial reactions are catalysed by RNA, a chemical cousin of DNA, which can fold into enzymes

known as ribozymes. Some classes of ribozyme are able to target specific sequences in other RNA molecules and cut them precisely.

In 2014, Dr Alex Taylor and colleagues discovered that artificial genetic material known as XNA – in other words, synthetic chemical alternatives to RNA and DNA not found in nature – could be used to create the world's first fully-artificial enzymes, which Taylor named XNAzymes.

At the beginning, XNAzymes were inefficient, requiring unrealistic laboratory conditions to function. Earlier this year, however, his lab reported a new generation of XNAzymes, engineered to be much more stable and efficient under conditions inside cells. These artificial enzymes can cut long, complex RNA molecules and are so precise that if the target sequence differs by just a single nucleotide (the basic structural unit of RNA), they will recognise not to cut it. This means they can be programmed to attack mutated RNAs involved in cancer or other diseases, leaving normal RNA molecules well alone.

Now, in research published today in *Nature Communications*, Taylor and his team at the Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), University of Cambridge, report how they have used this technology to successfully 'kill' live SARS-CoV-2 virus.

Taylor, a Sir Henry Dale Fellow and Affiliated Researcher at St John's College, Cambridge, said: "Put simply, XNAzymes are molecular scissors which recognise a particular sequence in the RNA, then chop it up. As soon as scientists published the RNA sequence of SARS-CoV-2, we started scanning through looking for sequences for our XNAzymes to attack."

While these artificial enzymes can be programmed to recognise specific RNA sequences, the catalytic core of the XNAzyme – the machinery that operates the 'scissors' – does not change. This means that creating new XNAzymes can be done in far less time than it normally takes to develop antiviral drugs.

As Taylor explained: "It's like having a pair of scissors where the overall design remains the same, but you can change the blades or handles depending on the material you want to cut. The power of this approach is that, even working by myself in the lab at the start of the pandemic, I was able to generate and screen a handful of these XNAzymes in a matter of days."

The next step for Taylor and his team is to make XNAzymes that are even more specific and robust – "bulletproof," he says – allowing them to remain in the body for longer, and work as even more effective catalysts, in smaller doses.

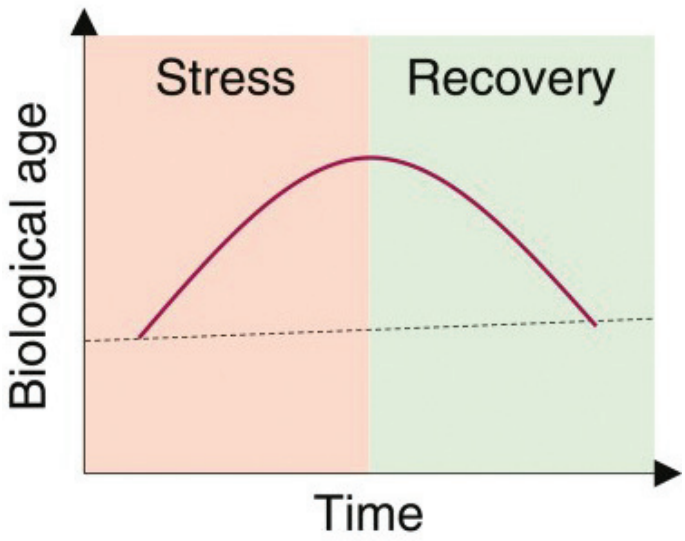
Biological age is increased by stress and restored upon recovery

Jesse R. Poganik, Bohan Zhang, Gurpreet S. Baht, Alexander Tyshkovskiy, Amy Deik, Csaba Kerepesi, Sun Hee Yim, Ake T. Lu, Amin Haghani, Tong Gong, Anna M. Hedman, Ellika Andolf, Göran Pershagen, Catarina Almqvist, Clary B. Clish, Steve Horvath, James P. White, Vadim N. Gladyshev *Cell Metabolism (2023). DOI: 10.1016/j.cmet.2023.03.015*, 21 Apr. 2023, https://www.sciencedirect.com/science/article/abs/pii/S1550413123000931?via%3Dihub, accessed 21 Apr. 2023.

Summary

Aging is classically conceptualized as an ever-increasing trajectory of damage accumulation and loss of function, leading to increases in morbidity and mortality. However, recent in vitro studies have raised the possibility of age reversal. Here, we report that biological age is fluid and exhibits rapid changes in both directions. At epigenetic, transcriptomic, and metabolomic levels, we find that the biological age of young mice is increased by heterochronic parabiosis and restored following surgical detachment. We also identify transient changes in biological age during major surgery, pregnancy, and severe COVID-19 in humans and/or mice. Together, these data show that biological age undergoes a rapid increase in response to diverse forms of stress, which is reversed following recovery from stress. Our study uncovers a new layer of aging dynamics that should be considered in future studies. The elevation of biological age by stress may be a quantifiable and actionable target for future interventions.

Graphical abstract



From: Biological age is increased by stress and restored upon recovery, shows DNA methylation clock study

Cell Press, 21 Apr. 2023, https://phys.org/news/2023-04-biological-age-stress-recovery-dna.html, accessed 21 Apr. 2023.

The biological age of humans and mice undergoes a rapid increase in response to diverse forms of stress, which is reversed following recovery from stress, according to a study publishing on April 21 in the journal *Cell Metabolism*. These changes occur over relatively short time periods of days or months, according to multiple independent epigenetic aging clocks.

"This finding of fluid, fluctuating, malleable age challenges the longstanding conception of a unidirectional upward trajectory of biological age over the life course," says co-senior study author James White of Duke University School of Medicine. "Previous reports have hinted at the possibility of short-term fluctuations in biological age, but the question of whether such changes are reversible has, until now, remained unexplored. Critically, the triggers of such changes were also unknown."

The biological age of organisms is thought to steadily increase over the life course, but it is now clear that biological age is not indelibly linked to chronological age. Individuals can be biologically older or younger than their chronological age implies. Moreover, increasing evidence in animal models and humans indicates that biological age can be influenced by disease, drug treatment, lifestyle changes, and environmental exposures, among other factors.

"Despite the widespread acknowledgment that biological age is at least somewhat malleable, the extent to which biological age undergoes reversible changes throughout life and the events that trigger such changes remain unknown," says co-senior study author Vadim Gladyshev of Brigham and Women's Hospital, Harvard Medical School.

To address this knowledge gap, the researchers leveraged the power of DNA methylation clocks, which were innovated based on the observation that methylation levels of various sites throughout the genome predictably change over the course of chronological age. They measured changes in biological age in humans and mice in response to various stressful stimuli. In one set of experiments, the researchers surgically attached pairs of mice that were 3 months old and 20 months old in a procedure known as heterochronic parabiosis.

The results revealed that biological age may increase over relatively short time periods in response to stress, but this increase is transient and trends back toward baseline following recovery from stress. At epigenetic, transcriptomic, and metabolomic levels, the biological age of young mice was increased by heterochronic parabiosis and restored following surgical detachment.

Merged magnetic resonance and light sheet microscopy of the whole mouse brain

G. Allan Johnson, Yuqi Tian, David G. Ashbrook, and Robert W. Williams, edited by J. C. Davis; PNAS 120 (17) e2218617120, 17 Apr. 2023

Significance

We demonstrate the highest-resolution MR images ever obtained of the mouse brain. The diffusion tensor images (DTI) @ 15 μm spatial resolution are 1,000 times the resolution of most preclinical rodent DTI/MRI. Superresolution track density images are 27,000 times that of typical preclinical DTI/MRI. High angular resolution yielded the most detailed MR connectivity maps ever generated. High-performance computing pipelines merged the DTI with light sheet microscopy of the same specimen, providing a comprehensive picture of cells and circuits. The methods have been used to demonstrate how strain differences result in differential changes in connectivity with age. We believe the methods will have broad applicability in the study of neurodegenerative diseases.

Abstract

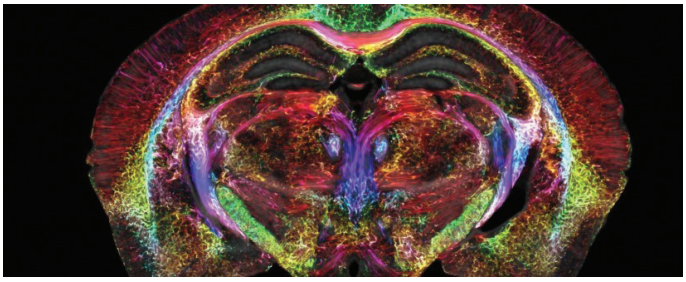
We have developed workflows to align 3D magnetic resonance histology (MRH) of the mouse brain with light sheet microscopy (LSM) and 3D delineations of the same specimen. We start with MRH of the brain in the skull with gradient echo and diffusion tensor imaging (DTI) at 15 μm isotropic resolution which is ~ 1,000 times higher than that of most preclinical MRI. Connectomes are generated with superresolution tract density images of ~5 μm. Brains are cleared, stained for selected proteins, and imaged by LSM at 1.8 μm/pixel. LSM data are registered into the reference MRH space with labels derived from the ABA common coordinate framework. The result is a high-dimensional integrated volume with registration (HiDiver) with alignment precision better than 50 μm. Throughput is sufficiently high that HiDiver is being used in quantitative studies of the impact of gene variants and aging on mouse brain cytoarchitecture and connectomics.

From: Scientists achieve sharpest-ever scan of a brain—64 million times clearer than ever before!

Jace Dela Cruz, *Tech Times* 18 April 2023, https://www.techtimes.com/articles/290497/20230418/scientists-sharpest-ever-scan-brain-64-million-times-clearer.htm, accessed 21 Apr. 2023

Scientists have achieved a breakthrough in medical imaging, producing the sharpest-ever scan of a brain. The scan, which is 64 million times clearer than a typical clinical MRI, was created by a team from Duke's Center for In Vivo Microscopy,

working in collaboration with researchers from the University of Tennessee Health Science Center, the University of Pennsylvania, University of Pittsburgh, and Indiana University.



Scan of mouse brain is 64 million times clearer than a typical clinical MRI. (Photo: Duke Center for in Vivo Microscopy)

The researchers used an incredibly powerful magnet, 100 times stronger than those used in clinical MRI machines, and a high-performance computer that was the equivalent of 800 laptops working together to produce the scan. The images are so detailed that they can reveal microscopic details within the brain, offering a new way to visualize the connectivity of the entire brain at record-breaking resolution. The images were created by the researchers using mice rather than people. Still, the discovery will provide a better knowledge of human conditions, such as how the brain changes with aging, a person's diet, or neurodegenerative disorders like Alzheimer's. The development of the high-resolution MRI has taken nearly 40 years, with the team at the Duke Center for In Vivo Microscopy perfecting numerous components. To photograph one brain, the researchers used an extraordinarily strong magnet, a unique combination of gradient coils that are 100 times more powerful than those used in a clinical MRI, and a high-performance computer equivalent to approximately 800 laptops.

Scientists have yet to repeat the highly detailed scans on human brains, which could in the future help doctors detect diseases earlier and patients survive longer.

A Roadmap to Revival

Successful revival of cryonics patients will require three distinct technologies: (1) A cure for the disease that put the patient in a critical condition prior to cryopreservation; (2) biological or mechanical cell repair technologies that can reverse any injury associated with the cryopreservation process and long-term care at low temperatures; (3) rejuvenation biotechnologies that restore the patient to good health prior to resuscitation. OR it will require some entirely new approach such as (1) mapping the ultrastructure of cryopreserved brain tissue using nanotechnology, and (2) using this information to deduce the original structure and repairing, replicating or simulating tissue or structure in some viable form so the person "comes back."

The following is a list of landmark papers and books that reflect ongoing progress towards the revival of cryonics patients:

Jerome B. White, "**Viral-Induced Repair of Damaged Neurons with Preservation of Long-Term Information Content**," Second Annual Conference of the Cryonics Societies of America, University of Michigan at Ann Arbor, April 11-12, 1969, by J. B. White. Reprinted in *Cryonics* 35(10) (October 2014): 8-17.

Michael G. Darwin, "**The Anabolocyte: A Biological Approach to Repairing Cryoinjury**," *Life Extension Magazine* (July-August 1977):80-83. Reprinted in *Cryonics* 29(4) (4th Quarter 2008):14-17.

Gregory M. Fahy, "**A 'Realistic' Scenario for Nanotechnological Repair of the Frozen Human Brain**," in Brian Wowk, Michael Darwin, eds., *Cryonics: Reaching for Tomorrow*, Alcor Life Extension Foundation, 1991.

Ralph C. Merkle, "**The Molecular Repair of the Brain**," *Cryonics* 15(1) (January 1994):16-31 (Part I) & *Cryonics* 15(2) (April 1994):20-32 (Part II).

Ralph C. Merkle, "**Cryonics, Cryptography, and Maximum Likelihood Estimation**," First Extropy Institute Conference, Sunnyvale CA, 1994, updated version at http://www.merkle.com/cryo/cryptoCryo.html.

Aubrey de Grey & Michael Rae, "**Ending Aging: The Rejuvenation Breakthroughs That Could Reverse Human Aging in Our Lifetime**." St. Martin's Press, 2007.

Robert A. Freitas Jr., "**Comprehensive Nanorobotic Control of Human Morbidity and Aging**," in Gregory M. Fahy, Michael D. West, L. Stephen Coles, and Steven B. Harris, eds, *The Future of Aging: Pathways to Human Life Extension*, Springer, New York, 2010, 685-805.

Chana Phaedra, "**Reconstructive Connectomics**," *Cryonics* 34(7) (July 2013): 26-28.

Robert A. Freitas Jr., "**The Alzheimer Protocols: A Nanorobotic Cure for Alzheimer's Disease and Related Neurodegenerative Conditions**," *IMM Report* No. 48, June 2016.

Ralph C. Merkle, "**Revival of Alcor Patients**," *Cryonics*, 39(4) & 39(5) (May-June & July-August 2018): 10-19, 10-15.

Robert A. Freitas Jr., "**Cryostasis Revival: The Recovery of Cryonics Patients through Nanomedicine**," Alcor Life Extension Foundation, 2022 (https://www.alcor.org/cryostasis-revival/)

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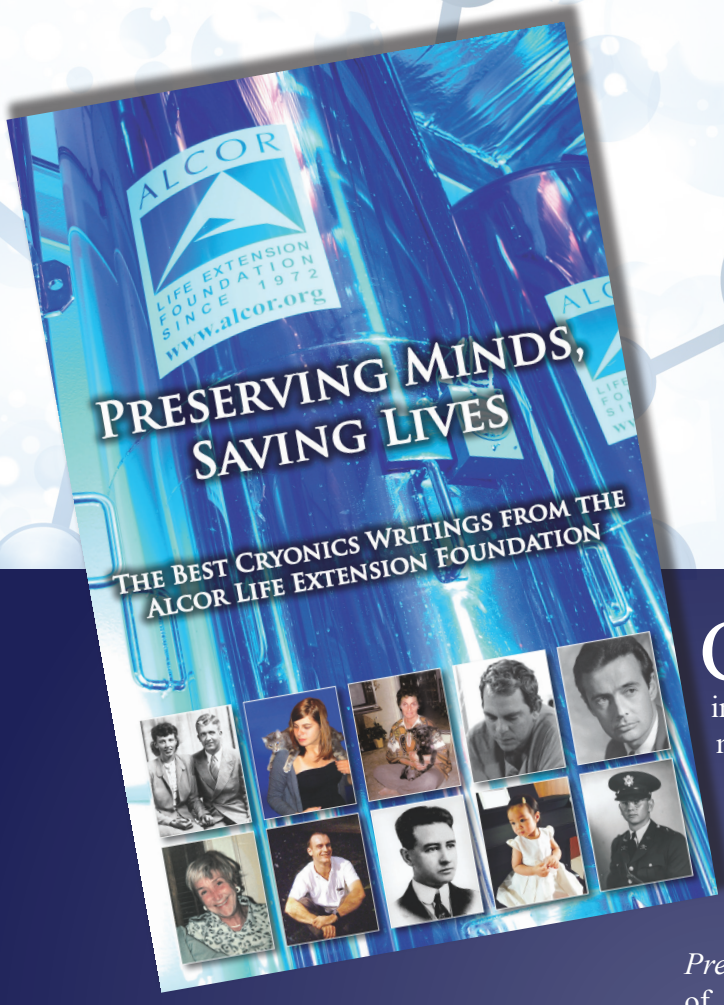

"Cryonics magazine introduced me to Alcor and cryonics at its best back in 1983. The visions and technological breakthroughs that you will read about in this book continue to shape Alcor's mission to preserve life through science."

– Max More, Ph.D.
Ambassador and President Emeritus of Alcor

Cryonics is an experimental medical procedure that uses ultra-low temperatures to put critically ill people into a state of metabolic arrest to give them access to medical advances of the future. Since its inception in the early 1960s, the practice of cryonics has moved from a theoretical concept to an evidence-based practice that uses emergency medical procedures and modern vitrification technologies to eliminate ice formation.

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"Society's failure to take cryonics seriously is a tragedy that is probably costing countless lives. Alcor, notably via its magazine, is leading the fight to change that."
– Aubrey de Grey, Ph.D.
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Stem Cell Scientist and Chief Executive Officer of BioTime, Inc.

What is Cryonics?

Cryonics is an attempt to preserve and protect human life, not reverse death. It is the practice of using extreme cold to attempt to preserve the life of a person who can no longer be supported by today's medicine. Will future medicine, including mature nanotechnology, have the ability to heal at the cellular and molecular levels? Can cryonics successfully carry the cryopreserved person forward through time, for however many decades or centuries might be necessary, until the cryopreservation process can be reversed and the person restored to full health? While cryonics may sound like science fiction, there is a basis for it in real science. The complete scientific story of cryonics is seldom told in media reports, leaving cryonics widely misunderstood. We invite you to reach your own conclusions.

How do I find out more?

The Alcor Life Extension Foundation is the world leader in cryonics research and technology. Alcor is a non-profit organization located in Scottsdale, Arizona, founded in 1972. Our website is one of the best sources of detailed introductory information about Alcor and cryopreservation (www.alcor.org).

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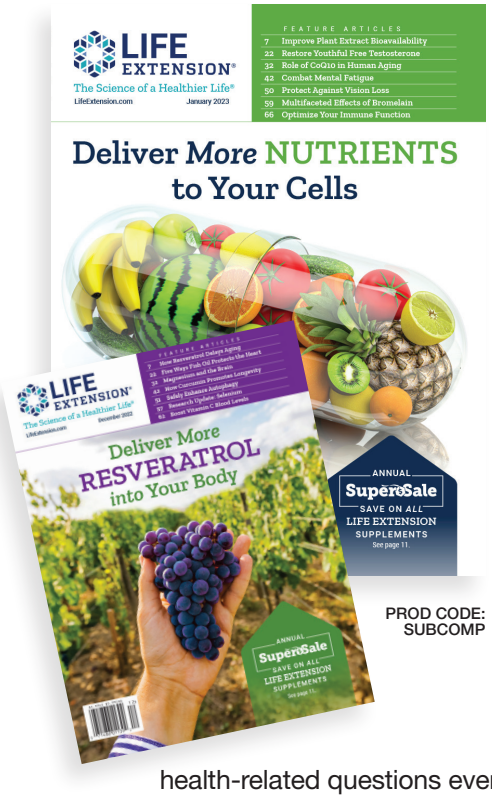

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