MITOCHONDRIAL TRANSPLANTATION

Summary, scientific critique and tidied transcript

Conversation with Dr Colwyn A. Headley • approximately 80 minutes

Source: https://www.youtube.com/watch?v=WbDYhkbK0iU

Executive summary

Bottom line. The interview presents mitochondrial transplantation as a potentially lower-risk, more controllable route to functional rejuvenation than partial epigenetic reprogramming. The strongest evidence discussed is not whole-body rejuvenation, however: it is proof-of-concept rescue of aged CD4+ T-cell metabolism and immune function, including improved infection control after adoptive transfer in mouse models. The platform is scientifically interesting, but it remains preclinical for ageing and far from demonstrating durable organism-level rejuvenation in humans.

What Headley argues

- Age-related mitochondrial dysfunction can be approached as an "engine failure": instead of only repairing damaged components, functional mitochondria can be introduced into impaired cells.
- Exogenous mitochondria can enter aged CD4+ T cells, reduce mitochondrial oxidative stress, alter the mitochondrial proteome and restore activation, IL-2 production and proliferation.
- Mito-transferred aged T cells protected immunodeficient mice better against influenza A and Mycobacterium tuberculosis; later work also reported improved effector/memory differentiation and lower exhaustion/senescence markers.
- Antibody conjugation to the mitochondrial outer membrane could convert mitochondria into cell-targeted delivery vehicles—an "Uber" system—potentially avoiding inefficient, non-specific uptake.
- The approach might be extended from ex-vivo cell treatment to in-vivo targeting, vascular ageing, abdominal aortic aneurysm, spaceflight-associated mitochondrial injury and perhaps engineered delivery of other therapeutic payloads.
- Compared with epigenetic reprogramming, mitochondrial transfer is proposed to offer a lower oncogenic risk and a transient, degradable intervention.

Evidence hierarchy

Claim level	What is shown	Interpretation
Published, strongest	Aged mouse and elderly human CD4+ T cells improve on metabolic and functional assays after mitochondrial delivery. Treated aged T cells improve infection outcomes after adoptive transfer in mice.	Credible proof of principle for immune-cell rescue; not evidence of human rejuvenation.
Published, supportive	A 2024 study reports enhanced protective effector/memory responses and reduced exhaustion/senescence markers in ageing/TB models.	Extends the phenotype, but is still cell- and disease-context specific.
Platform concept	Antibody-coated mitochondria are proposed for receptor-specific uptake and possible in-vivo targeting.	Promising delivery logic; biodistribution, dose control and manufacturing remain decisive.
Preliminary/unpublished	Marmoset and NASA-linked work, vascular targeting and broader anti-ageing applications are discussed.	Hypothesis-generating until full methods, controls and results are peer reviewed.
Speculative	Engineered mitochondria as transient reprogramming or payload-delivery vehicles.	Creative but substantially beyond the evidence presented.

Scientific critique

What is persuasive

- The intervention is mechanistically aligned with a measurable defect: aged T cells have altered mitochondrial metabolism, redox state and signalling, and the studies test functional rescue rather than relying only on molecular ageing markers.
- Protection against influenza and tuberculosis in recipient mice is more meaningful than a change in ATP or membrane potential alone; it connects cell treatment to organism-level immune performance.
- Targeted delivery is a genuine translational advance if its high uptake and specificity reproduce in vivo. It addresses one of the field's central problems: getting intact, functional mitochondria to the intended cell type.
- A transient organelle intervention could, in principle, be easier to stop or clear than permanent nuclear genome modification.

Where the argument overreaches

- "Rejuvenation" is used too broadly. Restoring selected functions in isolated aged T cells, followed by adoptive transfer into mice, does not establish reversal of biological age, durable tissue rejuvenation, lifespan extension or healthspan improvement.
- The early ROS–mtDNA mutation "vicious cycle" is presented as if it were a settled master mechanism of normal ageing. ROS are also signalling molecules; mild mitochondrial stress can be adaptive, and the causal importance of accumulated mtDNA point mutations in ordinary ageing varies by tissue and context.
- The interview often moves from autologous ex-vivo transfer to systemic, targeted in-vivo therapy without pausing over the much harder pharmacology: circulation stability, tissue penetration, endosomal escape, intracellular trafficking, fusion, mitophagy and persistence.
- Comparisons with partial epigenetic reprogramming are premature. Lower theoretical oncogenic risk is plausible, but mitochondrial transfer has its own poorly quantified risks and lacks comparable dose–response, durability and large-animal evidence.
- Some claims rely on unpublished slides and verbal descriptions. Without sample sizes, randomisation, blinding, exclusions, complete statistics and negative results, the strength of those findings cannot be judged.

Key unresolved risks and experiments

1. Identity and fate: quantify how many donor mitochondria enter each target cell, whether they remain intact, fuse, replicate, transfer mtDNA, or are rapidly degraded.
2. Mechanism: separate effects of intact respiration from mitochondrial proteins, lipids, metabolites, mtDNA or innate immune stimulation using respiration-deficient and membrane-disrupted controls.
3. Compatibility: test autologous versus allogeneic sources, mtDNA haplotype mismatch and nuclear–mitochondrial incompatibility.
4. Safety: monitor extracellular mtDNA/DAMP signalling, complement and coagulation activation, cytokine release, autoimmunity, ectopic tissue uptake, arrhythmia and tumour-supporting metabolic effects.
5. Dose and persistence: establish a therapeutic window, repeat-dose behaviour and whether benefit survives normal mitochondrial turnover.
6. Translation: use aged, immunocompetent large animals with clinically relevant administration, prespecified endpoints and long follow-up before describing the platform as close to general clinical rejuvenation.

Overall assessment

Novelty: high at the delivery-platform level; moderate at the biological premise. Intercellular mitochondrial transfer and experimental mitochondrial transplantation predate this work, but applying extracellular mitochondrial delivery to aged CD4+ T-cell rescue—and developing antibody-directed organelle targeting—is distinctive.

Evidence grade: promising preclinical proof of concept. The interview is strongest as a research programme and weakest when it implies that mitochondrial transplantation is already a near-clinical alternative to epigenetic rejuvenation. A fair description is “a plausible platform for targeted functional rescue that now needs rigorous in-vivo pharmacology and safety testing.”

Interview map

- 00:00–05:30 — Mitochondrial dysfunction, ROS and the “engine replacement” analogy.
- 05:30–20:00 — Ageing of CD4+ T cells; ex-vivo mito-transfer; influenza and tuberculosis mouse experiments; elderly human T-cell assays.
- 20:00–35:00 — Broader immune-ageing interpretation; fibroblast sources; mitochondrial quality, lipids and possible clinical development.
- 35:00–45:30 — Natural intercellular transfer; rationale and workflow for antibody-conjugated, cell-targeted mitochondria.
- 45:30–55:00 — mtDNA depletion, uridine, uptake efficiency, receptor choice and mitochondria as delivery vehicles.
- 55:00–65:00 — Candidate tissues/diseases, vascular ageing, abdominal aortic aneurysm, spaceflight injury and marmoset work.
- 65:00–73:00 — Translation, funding, dosing and safety; autologous versus allogeneic transfer; systemic inflammation concerns.
- 73:00–79:45 — Mitochondrial quality control, exercise comparison and speculative combination with transient epigenetic reprogramming.

Sources used for the critique

Video
https://www.youtube.com/watch?v=WbDYhkbK0iU

Headley et al., 2023 — Extracellular Delivery of Functional Mitochondria Rescues the Dysfunction of CD4+ T Cells in Aging
https://pubmed.ncbi.nlm.nih.gov/37990641/

Headley et al., 2024 — Mitochondrial Transplantation Promotes Protective Effector and Memory CD4+ T Cell Response…
https://pubmed.ncbi.nlm.nih.gov/39039808/

Headley & Tsao, 2023 — Building the case for mitochondrial transplantation as an anti-aging cardiovascular therapy
https://pubmed.ncbi.nlm.nih.gov/37229220/

Stanford profile and publication list
https://profiles.stanford.edu/259923

ClinicalTrials.gov example: autologous mitochondrial transplant for cerebral ischaemia
https://clinicaltrials.gov/study/NCT04998357

Tidied transcript

Editorial note. This is a readability edit of the supplied automatic transcript, not a court-verbatim transcription. Filler words, repeated starts and caption line breaks have been removed; obvious scientific terms and names have been corrected. Timestamps are approximate. Ambiguous phrases are retained conservatively, and speaker labels are omitted where the automatic transcript does not support reliable attribution.

[0:00] In terms of human rejuvenation, although most people are focusing on epigenetic reprogramming, there may be a better approach and one that is closer to clinical use. With that in mind, today's guest is Dr. Colewin Headley, who has used mitochondrial transplantation to improve cellular function. So, welcome Coin, and let's get into the presentation. I'm excited for this. So, I call my presentations yap sessions cuz it's really just me yapping most of the time.

[0:26] It takes a village to get all the stuff done and I like to just get this out of the way because I always forget sometimes and this is probably one of my most up-to-date technology pages. It's and you know this is stuff I started at Ohio State and sort of transition from Texas to now Stanford. Mitochondrial dysfunction to me I kind of I view it as engine failure. Check engine light comes on. You take your car to mechanic and you can put a new engine in that vehicle keep using it for another couple you know thousand maybe 10,000 miles.

[0:51] And I think in principle this is something that we can do for the body. But before I start talking about the engine, I think or talking about transplantation, I like to just review just quickly touch on how the engine breaks or some of the modalities that influence the mitochondria's function. The mitochondria electron transport electron transport chain contributes to ATP production. as the electrons kind of get go through this roller coaster, some fall off unfortunately or they're necessary.

[1:22] So from complexes one and three, you have some leak of electrons and because you've got oxygen sort of everywhere, these react and you get a superoxide, but we're just going to say reactive oxygen species for you know, for simplicity. I consider ROS to be kind of, you know, it's like a drunk guy at a bar, maybe too much alcohol and he's ready to fight and they're so they're super reactive. They like to attack anything that they can which is proteins, lipids, DNA, RNA, whatever they can attack to. And within the matrix, you've got copies of mitochondrial DNA. You've got couple hundred to a couple thousand depending on the cell type. And under typical conditions, you also have the bouncers which are like the antioxidants. You've got catalase, superoxide dismutase, glutathione peroxidases that are they're really helping in nullifying or in keeping a proper ratio of ROS to cell activity. And you also have these these repair enzymes, the polymerase complexes that when ROS levels are you know normal or within a proper threshold, you don't see a lot of damage being introduced by polymerase DNA polymerase. But when ROS are it becomes more like a mosh pit, there's so much more oxidative stress. Everything gets hit. Everything is getting like knocked out. The enzymes are supposed to help with it, but detoxifying the mitochondrial DNA. The stuff that's supposed to repair it also increases it sort of like error rate and you have accumulation of mutated mitochondrial DNA. And this is just some of the flavors that contribute to mitochondria dysfunction from I guess the purview of mitochondrial DNA mitochondrial DNA mutation accumulation. This is the perpetual ROS sort of debt cycle that I like to use because it's it's really interconnected the amount of if there's an increase in ROS within mitochondria typically there's a shift there's a pathogenic shift towards more mutated mitochondrial DNA which then ultimately influences the electron transport chain or another way I think people call this like heteroplasm and pathogenic heteroplasm really where is this like continual shift of as you get older if you consider your cells to be cars the engine is always running like you never turn that stuff off. So over time that's just it's just the mat starts the mat and you can't outrun you can't outrun a bad engine. And so another way I think about it is in terms of the Pac-Man. You can think of ROS as the little balls and then the antioxidants are like you know the Pac-Man. And because mitochondria is such a is probably one of the major contributors of oxidative stress inside the cell. If there's increased mitochondrial dysfunction, there's going to be increased oxidative stress. And aging to me or you know how I think about it is a young versus an old cell is really just this misbalance of ROS being produced versus eliminated. And then sort of chronic conditions pushes you over the edge. And if it becomes too if the this sort of bias becomes too great, it's like game over. That's it. And so mitochondrial transplantation again this idea that you can kind of just the same way that you can go to a mechanic swap an engine in and keep using that vehicle. I think it's it's a it's been an area of growing interest where one has been organically shown by well I get it today. So there's a couple methods by which been by which this transplantation stuff has been kind of characterized like there's the tunneling nano tubes where cells will make these kind of protrusions and they'll siphon or it seems to be going both ways but they'll trade mitochondria. There's also encapsulation either in the form of mitochondria in microvesicles or we have the ability to make lipid nanop particles in the liver as well. And then we also noticed that mitochondria are kind of they have this I call it kind of like a secret handshake because we don't know all of the nitty-gritty mechanistic like you know things that happened for internalization but they seem to be taken up by an environment. And then depending on the cell it's like a different handshake. So there's like I said a growing interest in this space because of just the universatality of mitochondria.

[5:30] There's almost every cell except probably red you know mature red blood cells use mitochondria either for energy for it energy portfolio or for some of the signaling modalities. So when I was in grad school, this really when I started grad school in 20 2015, bam. when I was in grad school, one of my projects was looking at you know the impact of aging on T cells really trying to understand well it was it's kind of already been characterized that with aging there's a decrease in mitochondrial function there's an increase in oxidative stress and this sort of correlates with a decrease in ability of aged T cells if they're responding to the same sort of cognitive antigen those aged T cells don't express the same markers of activation they don't produce the same magnitude cytokines and it has a bias towards inflammatory cytokines and they don't proliferate to the same capacity as a younger T- cell responding to the same cog antigen and I like simple stuff I you know we got to this idea where it was like why don't we just replace the mitochondria instead of trying to dose with antioxidants or different or you know trying to modulate one or two pathways within the you know mitophagy or biogenesis if we can replace it then we'd hope the cell is smart enough to figure out how to get rid of the bats stuff and so and we the method that we came up with is we figured out that centrifugation is simple enough to do it. You can isolate your mitochondria, mix them in culture with whatever cell type, put them in a centrifuge for 5 minutes and that's it. Like to me that was goal mine because I like simplicity and I like reproducibility. You never want to be in a situation where someone's like oh man this protocol don't make any sense. You literally just need a centrifuge. like centrify that's that's the most expensive part probably like so and it's easy to scale because we can just change the ratio of mitochondria to cells within suspension and so we're able to scale it to you know almost 100% for per transplantation and so we could really do experiments using this sort of framework and so it was like this you culture cells isolate their mitochondria and then centrifuge them with your immune cells and so we narrow down on CD4+ T cells particularly because my PhDs was in immunology and we're looking at sort of the consequences of adaptive immune aging particularly with you know T cells in context of tuberculosis one of the major bugs that I studied again this sort of like you know the ROS debt loop that I mentioned and so in after sort of optimizing the protocol we just went to work we would take mitochondria from fiberblast from for the my studies they were coming from msbranic fiberblast We essentially made like a sort of a calibration curve to standardize how much mitochondria we'd isolate from specific amount of fiberblast and then just you know standardize that for all of our studies. And so what you're seeing here is the young CD4+ T cells and then versus the old and then the old after mito-transfer micron transplantation. We saw a significant increase in oxygen consumption. And again, I like the seahorse because it really does, you know, it's a it's a bonafideed way to measure oxygen tension in within like a culture. but because oxygen consumption and oxygen utilization like you know you can it's oxfos activity is sort of coupled to raw production. So if you measure an increase in oxygen consumption, you kind of have to also make sure that this is not related to a significant increase in reactive oxygen species production. So we I learned this technique in undergrad called electron paramagnetic resonance. it's probably the most sensitive way to detect free radicals because you know kind of like trap them and gives you like an extended time to characterize them and then based off of the intensity of the signal of the of the spin trap we can we can sort of infer how much based off the intensity but and also based off

of the probe that we're using can infer how much superoxide is being produced by mitochondria in T cells. And so this is a comparison of a young C4 T cells. are the old then the old plus mitochondria. So we saw a increase in oxygen consumption but a concurrent decrease in superoxide production. we also did some proteomics and looked at some of the most uprelated genontology pathways related to mitochondri and the cell itself. But what we noticed was things related to the architecture. So the MICOS complex and cristae formation were some of the top hits and so we did electron microscopy to take a look at the mitochondria be no look at the m look at the mitochondrian site T cells that didn't get any transplantation and then these are after transplantation and I kind of you know it the difference is like going to the gym and you can see your abs like that's you know look at that it's just it was amazing the first time I saw it and again because it's such a simple thing like we did like I every time I present I tell people that this is not it's not rocket science for the for the fact of how simple this was to just do and but the magnitude of impact that it had is exciting and so we were able to show that you could improve OXPHOS reduce oxidative stress and we went on to sort of compare the functionality of a of a sort of the OT cell or the OT cell after it's sort of been biogenically reprogrammed after transplantation Would that change activation marker expression? Would that have an impact on cytokine production? And would that lead to improved proliferation? And so again, because the mitochondria is such a you know, it's a engine, but it's also a CPU, nothing really happens without its permission. I already anticipated that because we saw these sort of changes in redox activity, we would suspect we would we would see changes in activation markers and stuff like that. And that's exactly what we saw. We saw improve increased expression of CD69 CD20 co-expression of CD69 CD25 on CD4+ T cells after transplantation. So this is these are experiments meaning that the cells came from the same animal and then they were either just given mitochondria or not. We looked at cytokine production particularly IL-2 which is known to go down with aging and we saw an increase in that and then we also looked at proliferation and so to me again this is all xvivo but if you can augment the activity of a cell that easy then the obvious step is to is to go in vivo and so one of the experiments that we did two experiments we did we did a lot of experiments but I'll talk just try to be brief we wanted to show that at least I wanted to show that this is like a disease agnostic sort of stuff. If you fix the T cells, it doesn't matter what the what the condition is, it's going to have a benefit. And so we chose two models, a viral infection, influenza, which all folks get a lot too, and then tuberculosis, which you know, tuberculosis is a aerobic what's the word?

[12:50] it's a it's a it's a bacterium that you know it's in the air and you get infected that way. But typically most competent people will not develop a hardcore infection until later in life. The bacteria goes latent and then when you're older because of your immune system being all wonky and your engine crapping out. Chronic inflammation tends to reactivate the bacteria and so it's a it's another aging associated pathology. not being in America, but I'm from the Caribbean. So, so when we looked at influenza, well, maybe I'll give a little bit more context, but so for the influenza, we particularly looked at mortality. Look, we either gave them younger mitochondria or didn't. And then after infection influenza, we just track primary mortality, but then with tuberculosis, we looked at the CO burden in some of the organs that it's we expect to see TB. So lung, spleen, I think and liver. For influenza, we saw that there was a significant increase in the survival of the of the RAG-knockout of the RAG-knockout mice that got the old CD4+ T cells after transplantation versus old CD4+ T cells with nothing or then RAG-knockout mice didn't get any CD4+ T cells at all. And then for tuberculosis, we saw a better control of microbacterial burden in lung and spleen in the mice that got in the RAG-knockout mice that got the old plus mito combo in tuberculosis and I mean other conditions as well. There's there's exhaustion. So exhaustion happens when sort of like your immune system is constantly fighting and it just can't can't deal with the it's getting beat up like you are not like you know you're trying to get up and it's like getting punched Mike Tyson that's really what I think about and beyond exhaustion there's this sort of sort of senescence how I kind of look at it is or another way that I think about it is like exhaustion is like your car dies on the highway senescence is like you never actually able to leave the parking a lot like you either way you're going to have a bad day, you're going to have to call for some help. So with tuberculosis, exhaustion is one of those things that the longer you're infected the more you have a profile of exhausted immune response and some of those markers are PD-1 and TIM-3 and from some of the data that we had we just screened for change in exhaust exhaustion associated proteins and so we compared the old versus the old plus mito and we saw this there was this shift and to me that was okay let's let's go a little further. And so we did another mouse study where we took TCR-knockout mice this time. So we can it's they don't control the infection long term because if they if they don't have any TCR if they don't have any T cells but it's a good way to show to sort of phenotype because we know that the T cells that we're putting are the ones that are functional and we can really look to see whether there's a change in their surface marker expression which sort of infers like functionality. And so we similar to the rag knockout gave them about two I think it was 2 million was it 2 million maybe it was 2 million adopted transfer 2 million T cells and then we tracked infection 30 days and 60 days post-rplantation and post infection TB and in the lung and spleen what we saw is like some populations there were significant there was a decrease in exhaustion so CD4 Tim 3 expression on CD4+ T cells suggesting that you know the mice are putting up a better fight. The mice that received the old plus matter are putting up a better fight. We then did a follow-up experiment with elderly CD4+ T cells.

[16:48] So the average age from these are these were just commercially procured. I think the average age was 60 65. we just chronically stimulated them with CD3, CD28 crossing antibodies to because this is this was in vitro to sort of stimulate or to show that this would have an impact also in you know beyond just the mouse and so we looked at we simulated them continuously for seven days and this is the response that we saw at day seven. So we saw improved activation reduced markers of exhaustion in the LT cells. Again, all we did was we just gave him some mitochondria. Five minutes in a in a centriuge, you know. So, this whole time I'm doing these things, I'm like, man, this is so simple. Like, this is the this is some of the simp simplest science of I've I've had to do it. So, but it's again the impact that it's had. We also took a look at senescence. again we looked at the proteium that we had and we saw that there was some shifts and so we then went to again some LC4 cells and did the just the typical stimulation that you would do for activating them and then just followed them at for 7 days. And again here we looked at CD69 expression so as a proxy of activation that went up CD57 which is associated with senescence went down and then beta gal p16 double positive t cell significantly decreased. So at this point I was like man maybe there's something here that we can use you know because this is just for T cells but again because the you know mitochondria they're different quantities per cell type but they truly do the same things to different magnitudes. So if you could really scale this to try on different diseases or different you know conditions I think it would be amazing. And this is just a quick recap. really just saying that the mitochondrial transplantation reprograms AHT cells to function better. And I talked a little bit about you know the improving immunity. It reduced exhaustion and senescence. I showed a little bit of the influenza and tuberculosis data. And now here at Stanford we've been looking at you know just different other conditions. So can we use it for glioblastoma as a sort of a dual strategy targeting both the immune cells but also you know trojan horsoing the cancer cells with pathogenic mitochondria and so when I got to Stanford and hold on just that I was trying to wait for the right time to interject because the story is so great that I didn't want to jump in too soon and now it looks like we've got a second chapter going so I think it's a good time for me to All right. So just to add a little bit more context because I think when people hear CD4+ T cells their eyes gloss over they really don't know I don't know average people right or people who are unfamiliar with the immune system right so if you take lymphosytes for people who don't know if you go to if you do a blood draw you'll get white blood cells now white blood cells can be further subdivided into neutrfils monocytes lymphosytes eocinophils basopils of the lymphosytes now you can subdivide them into CD4 CD8 NK cells B cells so just to add a little bit more context you know the CD4 4 T cells. are you familiar with Allesio Lana's work, the preprint on Tieumir Rivers?

[20:00] Man, look that I am really interested in that paper. like it's very interesting. I don't I think I saw the preprint, but I haven't seen whether it's been officially sort of, you know, given the final peer review. Yep. Yeah, it I'm inclined to believe that there's there's some like stuff there that is true. So, so he said that it's taken four an average of four years for his papers to be accepted. He gets tremendous push back. You know, just people just don't buy it. It seems too good to be true.

[20:32] But for people who don't know, in his study in that preprint, which I'm sure will be accepted at some point, they rejuvenated CD4 and CD4 T cells and is a big part of that story. mitochondrial beta oxidation, fatty acid oxidation, ATP production was central to that story. So for people who don't know who haven't seen the video you know basically rejuvenating CD4+ T cells led to dramatic lifespan extension like greater

than every lifespan extension in any animal study calorie restriction rapomy you name it. So when considering the emphasis on mitochondrial function as being a key part of that story, your data ties beautifully into that where you know instead of them giving the antigenic stimulus to donate you know so the APCs donate the tie to CD4 and then the CD4 donate the tieumirs everywhere else that's that gives you systemic rejuvenation. You're coming at it from let's give the old and you know exhausted and maybe even senescence CD CD4 T cells let's give them mitochondria and that restores their functionality. So then the big question even though the you know in infection and boosting the immune system is amazing because you know if you look at and I know I'm going on a long no you this is good podcasting debate.

[21:46] I appreciate that. Cool. So when you look at the leading cause of death or one of the leading cause of death in centinarians top three sure cardiovascular cancer but pneumonia. So the immune system and imunosiness is a big part of you know mortality at extreme older ages. So if you can rejuvenate CD4 and other immune cell types whether it's through mitochondria transfer or whatever I mean this is potentially a huge ROI on health span and longevity. So that's exactly it. I mean that's why I think this is why I get excited doing this kind of show because it's again it's very simple reproducible and the impact magnummonious impact and even when I was in grad school I remember cuz you know I did this I was reading about senescence and stuff and there was a clear link towards like mitochondrial function contributing to that like to that to that signaling cascade and I started thinking about you know telmir therapy and timmerase and I you know I remember I was having a conversation with one of the other grad students and I told him I was like man look someone's going to eventually make the connection between mitochondria and timmerase I'm not going to waste my time someone's going to do it but so it seems like it's coming along it's it's coming along towards like that trajectory and yeah yeah to me it makes sense because we've seen it now with different senescence both in from cancer because we're doing studies with that and then also from infections like we see this in improvement in the in immune cell function. So I think this is it's a no-brainer to me to me like I'm sold. I drink the Kool-Aid. So wait, so not just I mean basically all of the immune cell types their function the clients are engaging in. I don't know if mitochondria function is a part of NK cells being less able to you know engulf pathogens and B cells to produce antibodies. So no dude it's all yeah like again even without having you know I tell people like okay I have a background I have a PhD in immunology so I understand the networks understanding how mitochondria work is like a passport to understanding all the other cell types because it's the same circuit in just different magnitudes and so NK cells have a you know a different function than a T- cell per se but the sort of the signaling circuit is really the And so you can make I don't have to study all these other cells to know to have like a sort of a predictive inference of how transplantation would benefit them. And I think what's interesting is also because the know there's this and I'm I think I have a some talk some slides on at the end but there's this I'm interested in also seeing how to decouple oxfos from the signaling modality because I think that's a beautiful opportunity to really control like sulfate like it's clear that metabolites are super integral to that and if you can control how much mitochondria you give and the sort of like the circuit that's within a mitochondria that's going to completely change. Anyway, get excited myself talking about stuff, but this Yeah, exactly it. And because of the simplicity of this of transplantation, at least vivo, you know, one of the things that I've been working at Stanford is how do I expand this to since I started here, you know, for my post phase, I did a cardiovascular fellowship. So I went from the immune system to trying to understand really how immune dysfunction drives pathology in cardiovascular aging. and so looking at microfares in cells, vascular muscle cells, cardiac cells, all of them in the context of transplantation. What became wait colon hang I don't want to I don't want to I don't want to get too far. I got some more questions before you jump. Sorry. Sorry. So you're good. You're good. All right. Just on the details. So in is it optimal to get the so how so I guess the fiberblast you got them from skin cells or what so the mouse studies the fiber blast came from meth like primary mouse and fiberblast but then for the human studies they came from ATCC like dermal fibroblast cell line primary cells so skin so if people if this is translated and I know you're part of or you consult for mitrix or mitrix I don't know. I think they're doing some of this work. So, is it the same setup where it's taking mitochondria from fiberblast? Are you isolating fiberblast from what from skin or where would you get the mitochondria from to do this in people?

[26:28] So, are you talking about in terms of mitrix or in terms of like how I would like if I were to move this? I don't think it matters where the mitochondria come from because if you can isolate them or if you can grow them in culture and if you can mitochondria respond to metabolic cues. So that means that in a cell culture you potentially with the you know with crisper and the things that we have right now you can program any cell to have the quality of mitochondria that you wanted. as far as like you know berogetics or TCA like signaling circuits. I much more interested or I think is going to be a point of concern is the quality of mitochondria DNA that you get like from the cell source and I would say having getting them from younger cells just you know pragmatically makes more sense because you it's kind of anticipated that you're not going to have as many you know mutations and whatnot. But again, because we can we can culture cells xvivo like I don't see at least right now I have not seen anything to say that the that taking a mitochondria from a fiberblast versus a T- cell versus skeletal muscle would dramatically influence would necessarily change the outcome if you can have like a sort of you know standardization after isolation. Does that make sense? Yeah. Yeah. So how so how do you foresee this then? So someone comes into your clinic or Mitrix well I mean granted maybe they've got a different technique the way based on it sounds like you've you would do it differently from them but you know so what that they're just going to take a skin biopsy and then isolate mitochondria from the fibroblast and skin and then so wait along those lines you know what if and you know considering that you know as you mentioned mitochondrial heteroplasm right so would you need to do function I mean granted as you said you can just bowl just inject the whole mitochondria. But the biggest bang for the buck as you as you alluded to with crisper and everything else would be pre-selecting your best mitochondria pre. So would you do the same the same stuff that we do for you know bacteria like this is just bacterial engineering but instead of the bacteria being in solution it's like inside a glove box. So we have to just manipulate that way. That's my perspective on it. I think that it's Go ahead. I was going to say how do you think so how would you pre-seelelect mitochondria ahead of time because once you start hitting you know once you isolate them and you start I mean can you generally after you know you you give like complex one and complex 2 you know I'm going man this I'm it's bringing me back to back in the days but can you can you can you can you isolate mitochondria and then put them through functional assays identify the best ones and then transplant them in but they're already basically used you've used them up.

[29:21] You've consumed you know you know suinate or you know complex one you know things to donate electrons to complex one or complex 2. Can you pre-select the mitochondria ahead of time while t Yeah. Yeah. I mean I think I would more so we have a lot of omix information just scattered everywhere. I think that's a great way to indirectly infer the quality of mitochondria that you have per cell type and then just kind of comp you know do your real functional assessment using seahorse or like flow cytometry. I think that's a I don't see that as being necessarily a major challenge again because if we have the ability to to change how mitochondria respire based off of the metabolites that we you know we put within a cell culture then I don't see it as maybe like UCP1 cuz that's like a super like specific thing that only appears in a specific you know but even with you know crisper you can easily insert that stuff into non-natural places. So, I don't I don't see it as a as a major crux. I would so far I've been using Fireblast because they're cheap. This stuff I mean you see the state of science. We broke. Everybody's broke. So, I designed my experiments from again for simplicity and also because trying to save a couple bucks. And so, fiberblasts are the cheapest youm they grow. You know, they it's pretty obvious when they start to get senescence. So, you stop that culture. So moving forward I think getting someone's you know some of their blood maybe turning it into IPSC's expanding them and then sort of like selecting for the best quality of IPSC's based off their biogenetics and then using that as the seed crystals for you know for future transplantation does not is not implausible to me that seems like a pretty I would try that. All right. So I have some thoughts along those lines. So in studies that we've done on centinarians, are you familiar with dicaroxyic fatty acids?

[31:31] Slightly. Yes. Yeah. So you know, so for people who don't know, you've got fatty acids that have a long carbon tail and then CO at one end. A daroxilic fatty acid has a CO at the other end. All right. So in studies on centinarians, we're we're seeing that levels of DCFAs relative to their parent fatty acid increase during aging all the way up to centinarian status. So when mitochondria when and it's specific to certain carbon chain lengths for fatty acids like mediumchain fatty acids that become dar caroxilic fatty acids. in terms of looking at biomarkers like if you've

got fibiberblasts and you're looking so you know you're just taking an aloquat from you know intracellular aloquat in order to assess functional status potentially mitochondria. The long story short is when there's mitochondria dysfunction the ER and microsomes and even peroxomes dar they the fatty acids undergo omega oxidation which can then target them for excretion rather than having these fatty acids which are going to be cytotoxic in high amounts because your mitochondria can't oxidize them. So I wonder if looking at DCFAs intracellular DCFAs might be a way to pre-seelelect for fiberblasts that have a better mitochondria function. But you'd have to do you said you have omic data all around. If you have you know if you have metabolomic data on fiberblasts that might be one way to pre without having to go through the you know seahorse and you know really you know burning the mitochondria before you would put them into another cell or I mean it would be like in a sort of like a satellite culture of the you know you infer the quality from that.

[33:15] I think that's one thing I for sure and then moving forward because I see this moving towards clinical trial you know within the coming years and thinking about sort of the FDA you know QC stuff that needs to be done. I think figuring out the best quality of mitochondria will be a combination of like omix information but then also like functional testing not only looking at the you know because I mentioned ox o oxidative stress and ROS production is coupled to oxygen consumption. So really looking at the antioxidant parameters as well and really the portfolio of mitochondria ATP that is coming from pyuvate versus like amino acids or versus from beta fatty acid oxidation. Having some sort of like you know metric or number or something to that combines that information I think will give a pretty robust range of healthy mitochondria. All right, one more question before going on. So this is great. This is great.

[34:21] Yeah. So, so do when the m two actually I have two questions, man. Sorry. So when the mito, so when CD4 T cells incorporate the new mitochondria, have do they discard some of the old ones? I mean because you if you pack a cell too tight with mitochondria may not be good, right? So do they get rid of some of the old mitochondria? Yeah. So we didn't have the capacity to answer whether because we didn't track we weren't able to track the mitochondrial DNA heteroplasm after transportation. So we don't we don't have that information. I think that some of the some of it is staying and that's based off studies that I've done now based off just the amount of mitochondria can deliver to a cell its impact stability the retention of the liver DNA. And it'll make more sense maybe when I when I show this sort of like stuff in the future. But I think also let me see if in this yeah like right there. So there's also instances like this where we see literally on confocal we can see the cells are trading mitochondria like so I think it's a combination. I think some get deleted, some get traded because I think of a cell as a very intelligent thing because I mean we use them for thinking right and I think the cell has this ability to this to sort of you know QC to say oh these are good but I don't want to delete these hey let me package it and send it you know across the street or something like that. I'm not entirely sure what all the mechanisms but transplantation is not just happening through one modality. I think it's like it's it's a pretty like extensive network and loop and so some get deleted some are probably getting shipped out and then I'm I'm sure that there's like secondary transplantation which also is a benefit for the immune system right because if you think about the cells that are constantly you know kind of driving around the body it's like it's immune cells so if you if you can target the macrofagages or these other cells that are like you know patrolling man then they can do their secondary transplant mentation. That to me is a is a more pragmatic rejuvenation strategy than some you know folks think that you have to deliver mitochondria to every cell in the body to have like a and I'm like dude that is first of all ridiculous like [laughter] that's that's that I mean you can try that I don't I don't think it's necessary because again because if you can bioengineer or customize the engine before putting in the car we can decide how much antioxidants how many mitochondrial DNA copy numbers like TFAM these are things that you can send in the care package So I think that's a beauty of knowing that we can like have like high fidelity transplantation. So then the mitochondria that are I guess donating some additional mitochondria after getting the transplant are they doing that in vesicles? You know they've h how do how do how does CD4 T cells communicate with other CD4 T cells? Are they is it exosomes or they're just butting off some vesicles to do that? I think it's a combination. It's probably buttering off exosomes like mitochondri microvesicles. it's probably true, you know, through the analing as well and also through the non-coded like extrusion into the into the millu. We didn't particularly track the diff the secondary methods but from the literature I mean I wrote a book chapter sort of characterizing T- cell and sort of immune system mito-transfer and it seems like all immune cells just they just be talking like that he's like here you know so I think it's a combination of that's allowing for transplantation nice and then how long does the effect last so after CD4 got mitochondria I think you showed up to 90 days with the infection model or maybe 60 days but 60 days yeah 60 and we had to stop just cuz these were expensive like trying to round out the paper. So we I mean I wish we could have gone longer but like 60 days and we saw durability and then for the immune cells that we did XVivo we looked at we looked at the exhaustion at 7 days and also 14 and we saw the same thing like better so that's like chronically stimulated cells for you know 14 days but also there's this whole selection because of like you know exhaustion so in you know in vitro models for that are not the best but durability looks to be like pretty strong. again we only put two million T cells imagine if you actually put a like a much higher number like so yeah how long do you anticip I mean there's got to be a timeline beyond and I get you know you can't run studies forever but you know where you'd have to do another transplant right I mean is there are there plans to figure out you know how long do you have to go before getting another transplant in order to keep CD4 or other cell type function higher than it would be at a given chronological Yes. And maybe the studies I'll mention at towards the end will kind of give like a you know sneak peek next episode of Dragon Ball Z or something. cool. No more questions for me for now. No, no, this is good. This is good. This this is like we're we're speaking electrons. So nice. like I said like you know I got to Stanford and some of these studies were done as I was transitioning from you know grad school to my to my postoc really started thinking about if it is this simple how can I be like Oprah and give mitochondria to every cell like you get a mitochondria you know with enough specificity but also because some cells the body you really can't you know you can't take them out one thing that was pretty clear was that the other methods that were published on my contrastmentation were kind of very low fidelity. This is this is fancy for 10%. Like I don't know why they wrote it like this but I was like yo 10 to the one is just 10 and these studies got up to you know 40 and 60 up to 40%. At least you know this is when I at the time and then even though our technique you know this not really my technique just the centriugation method is good at increasing like efficiency you can't use this for cells that you really can't take out of the body like and so for transitioning from just looking at the immune system and thinking about you know cardiovascular failure the endothelium dysfunction or dysfunction of the endothelial is like a commonality among most of cardiovascular pathologies and I was thinking about a way to essentially you know use transplantation to help with that. You could do an infusion. You can just you know a systemic bolus of mitochondrian that kind of probably look more like a bus where they would get to the destination but you know maybe not to the right spot. And so we've been looking at a way to kind of make like a Uber system where we can actually tell the mitochondria with some fidelity with some instruction where to go. And so got some inspiration from well honestly tuberculosis you know it hijacks the receptor CD206 on alvula microfars just to internalize and I remember thinking I was like man like mitochondria used to be a bacteria I mean they're they're kind of still are like quasi they're kind of like cats they domesticated themselves into our body right like it's the weirdest thing really think about it but I was like so what if we can make them you know just more permissible to moving And so you know engineering or like devolving mitochondria back into like it's you know ancestor just seemed a little too complex to me and so I was like let me see if there's a simpler way to just make them more internalized and so antibiotic conjugates it's been you know they've been using this for a hot minute and in most of the cases is to deliver a toxic payload to cells. So in cancer picks up the receptor gets internalized kaboui right apoptosis and so we said well why don't we just see if we can do that like can we just put some antibodies on the outside of the mitochondria maybe just maybe it will act like a bacteria but instead of getting you know recognized as foreign because they literally get internalized with you know like I said there secret these we don't know all of the without sort of you know specific receptors we don't understand all of the things necessary to have you know mass internalization but with this because we can choose the receptors then I mean then potentially we can just you know direct them this way. So what we came up with is a simple protocol where we could we have the your donor cell type whatever it is you sprinkle some secret seasoning you isolate the mitochondria and you conjugate them with antibodies and so this is the workflow. So again, my original workflow, you know, was to get cells, isolate, and then spin in. And so now it's just to get cells, sprinkle, and then isolate. And so again, very simple because I'm always thinking about anyone that wants to try this stuff needs to be like, you know, easy again still even if there's internalization like understanding the self like the fate of the of the delivered

mind. Is it going to get eliminated completely? Is it is it going to force the native mitochondria pool to get degraded? Is it going to be sort of fusion and like long-term retention? And don't have all those answers yet, but I'll show you some of the of our data that's suggesting, you know, that there's potential long-term integration. And so, first, I'll I'll show just a comparison of the the unmodified mitochondria versus like the mito or the antibbody conjugated system. And this is comparing the internal the internalization into C-31 positive sub endothelial cells. So we attached the anti-C31 to the outer membrane and then we just looked at uptake and so we can easily again scale to 100% efficiency just by sprinkling the amount of you know antibiot mitochondria conjugates that you need and most techniques that do you know the coinc like 30 minutes in culture and you're good to go the same amount of time that you take to stain your cells with you know for human chemistry or flow cytometry So trying to make it as rudimentary because I know that there's a lot of labs that are that have data that says mitochondrial dysfunction and they're like a what am I going to do with this like you know so this is opportunity to really interrogate and potentially develop I would say therapies that are probably less toxic and multifunctional anyway and so we're able to show that there's better termization we're able to also show there's better specificity so this here is comparing a co- culture of the of CD31 positive endothelial cells which are like the transluc translucent ones and then the green ones are vascular muscle cells that don't express C31 and then blue is a nuclear stain and so this is by confocal zstack and we're able to see that the mitos are very specific to the re to the cells with receptors that they're target so easy to scale spec specificity great I mean at least to me like the initial experiments and then another and some other data I'll show is sort of towards the retention right so in normal cells they have functional OXPHOS urodine is something that's necessary to have a DNA production versus cells that don't have any m that are deficient to mitochondrial DNA they don't produce urine so you have to supplement them and so these roer cells were are kind of like a great model just to test out like stability long-term retention and our I guess like the question that we asked is like well look if [snorts] we can if these cells need urodine to function if these rosio cells these mitochondri cells need urine supplementation to function if we do a transplantation with the mitoapse and we remove the urodine And if there's proliferation and there's an increase in OXPHOS by these rosier cells depending on how long it's suggestive of integration. And so this is just some of that data where we took what type of cells we took the mitochondrial DNA deficient mal cells in gray and then these are the cells that were treated with the mitos or the antibbody mitochondria conjugates. So again, we can restore oxygen consumption from zero to zero to 100. That's crazy. I never noticed that. But that's how like again very very like it does not it's not rocket science. You just sprinkle the mitochondria on your cultures or you know and we can see we can show that there's also restoration in mitochondrial ATP production from the Rosio cells and significant increase in overall ATP. And then we also looked at some of the proliferation and we see there's sustained proliferation. I'm only showing six days but I mean we've got cultures that have been going now for like 6 months off of just one treatment. So again the other question you're asking about you know how much of about retention of mitochondrial DNA and mitochondria. I think it comes down to the number the amount that we deliver and how many how much red dosing we would need to do. But in these experiments that I'm showing you this is just one dose. Like that's it.

[47:50] though some go home for the weekend like [laughter] you come back later. So, question so yeah urine and that's another one that most people probably know nothing about. So I think it's good to highlight urodine. So declines during aging and as you mentioned it can it can what restore restore mitochondrial function in mitochondria that have like the row the row zero cells right so they don't have mitochondrial DNA but urodine can restore mitochondrial function in that situation urrodine bypasses so urodine is made in mitochondria and it supplements DNA replication in the nuclear compartment so you have to fe because if the cells don't have functional mitochondria, they're not going to make urine, so they're not going to divide. And so you give them a urine in culture to sort of bypass their need of mitochondrial activity to continue having proliferation. So once you remove that from the culture, this, you know, they the cells they stop dividing. they'll I don't want to say they become like senescence but they really yeah a weird sort of senescence because mitochondrial DNA deficient cells are really not a natural cell type to begin with but this is just a good first pass to show that one there's if any if there's any system to show that there's integration you know as a first pass I think this is this is the cleanest definitely but couldn't you make the argument that sure row you know row zero cells are the extreme case, but I mean mitochondrial DNA is being oxidized and degraded throughout aging, right? So, couldn't extreme longevity be almost similar to a condition where some fraction of your mitochondria are almost row zero like kind of kind of mitochondria I think. Yeah, definitely.

[49:40] let me see. My first car that I had was a Ford Taurus like engine in a Ford Taurus. Imagine driving a Ford Taurus in 2026. That's how some of yeah some cells in the body are behaving. They're and it is in I think it's row like in the sense of they're the amount of pathogenic heteroplasm that they have is so high that the cells really can't function on OXPHOS. They have to switch over to sort of like you know I consider aerobic glycolysis kind of like credit card debt for cells because it's like you know they turn that on and then it's like hiff one off. Anyway, so so yeah, that's that's my perspective on that. So wait, so along those lines, I mean it seems like urodine can restore mitochondria in this situation where they've got depleted mitochondrial. Well, no, so it doesn't restore it. it only it restores the ability of the cell to produce to replicate DNA but it's independent of like if you if the cell if you can't if the urine is made inside the mitochondria so if the if there's no mitochondrial DNA there's no OXPHOS that means that you can't make any urodine right so you would need you can use urine to help boost or I guess like help in detoxification but I don't think it's the best method because again like you said if you're if you have a card that's already messed up and then you're just providing it with things to help copy stuff that's you're just going to be copying bad templates right so I would not just use urine by itself but also again maybe when you start your intervention if you're like 65 I think maybe urodine is might not be but if you're 35 maybe supplemented and you know have different responses well when you're 35 maybe you see no response because your mitochondria haven't yet hit that threshold old for you know right but that's what I'm saying like you have you so you maintain a better health threshold if you start versus like you're you start later your DNA is going to help with you know proliferation but if your mitochondrial DNA is messed up you're going to be copying messed up copies right because even if we unless I miss something but even when we activate mitophagy it's not like we selectively just degrade the messed up mitochondri I think it's ultimately it's whatever gets deolarized at the time so I think we need some, you know, I always I'm always thinking of like what's the what's the what's the most efficient way to have the cell think less about what it needs to do and just do it. And I think transplantation just fixes that because not only you're delivering the proper machinery to make the urine, you're delivering better copies of mitochondrial DNA again the antioxidant enzymes, better phospholipids, better stable cyrolin, all that stuff. ultimately because you can do you can control how much I think it's a much better way much more elegant way to improve a system.

[52:32] Have you compared the ro with mitochondria transplantation and then with urodine like and is it synergistic where they're working together to boost mitochondria function or the transplant by itself is just the bomb and then urine you know you really don't need it at all. So let's So for example here these cells these cells are in urine like they're cultured in urine right and you still have no oxygen consumption versus the cells that get the transplantation so that's the difference because urine bypasses all need for mitochondrial activity and this why I mentioned earlier about decoupling oxfos from you know the signaling capacity of mitochondria This would be kind of one of those ways if you can decouple oxfos from the ability of the mitochondria to keep producing urine and you can then deliver that to a cell with specificity. I mean, I think that's a powerful thing, right? Definitely. Yeah. All right. Cool. All right. Sorry. No more questions for now.

[53:33] No, no, you're good, dude. This is, like I said, it's much better when it's a Yab session. because I don't know. Anyway, so you know this is the kind of framework I've been thinking about with this technology or like with this you know really I mean it's just antibodies like I call it technology but because we can manipulate because we can get a much higher delivery efficiency like 100% and you can read those because you can choose different receptors I think the sky's is the limit as far as like the potentials but just really manipulating the cellar genome in the mitochondrial genome to either insert things or remove things using crisper or other gene editing tools then isolate those mitochondria.

[54:19] You can even insert synthetic mitochondria outside of the cell. I think that's also a beautiful thing. Repackage them and then get them ready for delivery. I think it's a very powerful way to control cell function because you know you don't have to just rely on one specific kind of mitochondria. If you can if you can really customize and develop different sort of enzyatic circuits, then maybe you can truly control the fate of a

cell and we talk about this in science all the time, right? Like we learn about these pathways and sort of like a linear thing, but it's really like that movie like you know everything all at once. It's really and so to I think to have a much more durable like functional change, you need to have multiple pathways being innovated at the same time and this provides that if you can decouple oxygen consumption from the system. So again, it's really just and how I think about it is reprogramming the bacteria and because you if you for example with these cells like these rosier cells where you they have no mitochondrial DNA, they're not going to they're they have a finite time like you can engineer them so that they're not going to integrate. You can change the ligans on the outside that so that they're more prone to mitophagy. So, it's really, I think, a very like concerted way to have something happen in the cell and then be like, "All right, skaddle." Cuz that's really what that's that's medicine, right? That's what we ultimately try to do with drugs. This is a living drug. I really do think that this is so maybe some people going to think I'm more crazy after this [laughter] presentation but along the lines of then you know realizing that we that I think this is a very efficient way of getting mitochondria into cells it's kind of if you have a Uber then you got to have like a Google maps right and so this is something we've been working on figuring out what's the right receptor combination or like not only receptor combination but like mitochondrial damage threshold necessary or like important to consider to sort of have a picking order for which cells to target. And with this system because it's again because you have you'll have the ability to really it's not going to be you know like 100% specificity because receptors are you going to have some overlap but I do think that because of the you know similar to other ADCs we should be able to have a lot of targeting potential with the system and so I think that this is going to be this could be influential for almost anything that involves mitochondrial dysfunction. Whether it's something that's like aging associated that pops up or something with paralyz disease. I've been focusing on immune aging but and I mean it's all connected anyway. So this would immune aging influences all these other things and these other things also influence immune aging. So targeting mitochondria are you know whatever the cell is I think is a powerful thing. So towards this I was I'm shout out to Burrows man because they're they were the first ones to believe in [laughter] in the craziness but you know through this grant funding I've been able to really kind of build a proper repository or like picking order for which receptors are worth targeting on different cell types. this is for the we're talking about the immune system, right? Like so I we've we've we've shown that we can take immune cells out the body, T CD4 T cells out the body, give them mitochondria and put them back inside, right? Then I think that's great and I think that's safe. But we don't why do we even need to take them out? If we can just target them in vivo. So this is an example of that. We're working on making a immune specific so C1 nasal receptor found in all immune cells. here we're showing that they get like the mitos get internalized by them and this is the efficiency compared to unmodified mitochondria and again this is only after I think this is like 90 minutes to 2 hours so significant 100% internalization and less is more you can use less to get you know a functional benefit another project that we have that is ongoing is looking at aeric anerism development which is smoking and aging associated and there's a lot of gene signatures suggesting mitochondrial dysfunction in both the cardiovascular and the immune compartment are like you know it flares up and so with AAA as they call it's a it's a giant game of who done it nobody really understands what's the you know the first cell to sort of like pop off all the damage and I was like dude you know mitochondrial functions in all of them let's just give them better mitochondria see what happens to the system cuz from the dirt of literature that's out there. You give cells better mitochondria and they just kind of figure it out. I don't think this is going to, you know, again, for people that are listening, this is not going to get you immortality. I don't think, but at least it's a way to save off a lot of the aging associated like, you know, signaling like signaling disregulations that sort of contribute to cardiovascular disease, immune dysfunction, neuropathology, etc. And then this other project here that we got a pilot fund from NASA for looking at everybody wants to go to space you know I think it was Mars for a minute and now maybe it's the moon but anyway like our physiology is just not from the amount of literature that's coming out from NASA our physiology is not supportive for long-term space flight and a major signal a major pathological like signature from space flight is mitochondria dysfunction in all the organs pretty much like they surveyed a bunch of them. Mitochondria are wonky in all of them. And so we said, "Look," or I guess I said, "Look, I mean, this is not a way to again, you can't you can't stop the radiation damage because it's the mats just like you can't if you're in a spaceship and you're out there, you're going to get. But if we have a way to provide astronauts with a sort of like a shielded, you know, mito that they can then resuscitate and, you know, give themselves. I guess this is where people start saying that it sounds like sci-fi, but this is really this is like a next week thing if we really if we really keep moving like they have a way to then just boost their mitochondria health and again because we have the ability to really bioengineer or think tinker and tanker and build better mitochondria for those environments. This is a real opportunity for improving like our physiology beyond natural capabilities. So yeah this is just some pretty cool stuff I guess. and then the you're asking about sort of dosing and so this is a project that we just wrapped up where we had six age marmoset and two six it was a total of eight marmoset two got just saline for eight weeks and then six of them got a weekly dose of the CD1A so this one this conjugate for eight weeks once a week and we've collected serum and we've collected immune cells every two weeks and we've done a PET CT before and after the study to really see what kind of durability this like this transplantation is having. So maybe this answers like you know you know what I've been thinking about towards like the future and moving it forward. But I mean I think we're at the doorstep of something you know something pretty cool. All wait just chime in on the marmoset. So it wasn't a survival study. I mean you're just looking at the impact of the mitochondrial transfer on just basically cellular function or was it what was the end point? What were the end points that you're going to look at? So the bargain or I guess like the way I was able to get this pilot sorry this this pilot funded is that I kind of used like the logic from stuff that I did in ear in grad school/ early postto where we showed that we could take immune cells out and give them mitochondria and put them back inside the body and the cells work better and so the next obvious step would be to give mitochondria directly to the cells inside the body and see what happens longitudinally for this study. the stipulation was that we could not utanize the animals. So we could just really look at kinetics and so again simply designed to understand from a perspective of especially if this you know this is going to be something for like the aging populace the marmoset age group was 10 years or older which is comparable to like 60 65 year old individuals. So it will give us a pretty good understanding of dosing I would say. How much mitoaps can we give per week? How much inflammatory response are we are we anticipating if any like if there's a systemic change in the frequency of circulating immune cells like you know if there's less classical microfasages circulating less infusion ground producing T cells or CD8s that are just like wonky being cytotoxic for no reason just because like they're you know flustered. I think that's a beautiful thing. and so we I'm waiting to get some of the samples or some of the data where we're going to test we're going to try to phenotype the immune cells look at the frequencies it across time to see what we see but this is just the first step in that exploration and then is the plan so you know I know I broached the subject earlier but staying in academia versus doing this you know in a corporate space I mean because I think you got to stay in academia and really bite this fight for you know until we've until you've mapped it out and you know so that so that it becomes a real therapy that can be applied clinically you know especially in aging or even age related disease or any disease. So what are what are your thoughts on that? I mean I man it's it's a jungle first of all. I've benefited from the academic environment for sure. Like I don't think I've been able to do kind of science other places and at the same time getting funding for this imp like it's been ridiculous.

[1:04:25] You know I can't I've I've got more grants calling me stupid like literally like this is some then you know even entertaining the idea for the last four or five years has been ridiculous. And so, the reason I've been able to get these pilots funded is because of, you know, you got to be able to sell the science. And I'm hoping that within because I'm not the only person doing this kind of research. I'm hoping over the next like, you know, maybe year or two that there's more papers that come out that find that provide support towards not only transplantation, but sort of like invivo uses of it and systemic applications. with that said, like I mean it's it's not it's not cheap. Single cell sequencing it costs like two Teslas just to do a couple samples, right? Like it's it's insane. So it really depends on where we can where I can sort of bootstrap to get, you know, funding from. Like if so if I can write more grants and get steady funding. great. But also I have to consider like if it's easier if it's a path of lease you know electron leakage per se if it means got to start a company and go that route. I'm not I'm not opposed to it because I really want to see this get to the to the clinic. So that's the main goal. What are the crowdfunding rules at Stanford? Is that an option at Stanford where you know you can I think you know it's it's definitely an option. I mean, I how I think about it just from the so I know I know for sure that there's there's like an office of philanthropic like donations at Stanford and stuff like that and it's it's easy enough to if that was the case like and there was a

crowdfunding like push I would contact them put the folks in contact and they'd be able to set up a you know it's like a research agreement or whatever and I think there's also you can also provide funding in terms of like a donation. There's some stipulations, but it's it's definitely allowed. especially now with the changing landscape and people realizing that you can't rely on federal dollars for funding that people are really having to universities are really having to rethink how they approach funding and it's going to be towards IP. Like that's to me the clearest thing that's going to happen is like folks are going a lot of universities are going to start spending a lot more time sifting through IP to make sure that they have something that they can pull interest from either from philanthropic but also from industry collaborations. So yeah, if individuals wanted to like donate to your work, so they'd have to contact. Do you have like a link that I can include with the video so that if people wanted to donate to your work or donate to Stanford who would give you a cut of that? Is that is that something that could be possible? Yeah. Yeah, I can I'll email you the link. Let me see if I can find it right now. Yeah. All right. So risks. What about risks? I don't think you And that's basically I that's the last thing I got is what are the risks of mitochondrial transportation? Is there a balance between you inject too much mitochondria or you too many targeted mitochondria maybe you increase cancer risk but then people will say oh cancer fuels is fueled by you know anorobic glycolysis not it's not a mitochondria story so maybe that's a lowrisk proposition but what are some risks for the mitochondrial transportation story so right now based off the literature and you know I can tell you from the experiments I've done all have been allergenic in nature meaning for the marmoset study they're allergenic we haven't seen inflammator response. So far from the data the the experiments with primary mitochondrial disease fibroblasts that we've done are also allergenic and we see long-term re like pretty much engraftment like no no net negative so far but we haven't done extensive enough sort of omix to look at all of the changes in the transatomic profile. I think there's there's hot debate about whether a mitochondria say from me to you would last like if the if it would have like an a long-term impact or would it just like be there for a couple days and then Scooby-Doo? I think it comes down to how much you can like deliver and establish sort of a sort of like a seed crystal, right? Like if you have a establish a network, I think the mitochondria, they're doing some sort of communication. I can't explain it with with enough data, but like they're they're talking they're definitely talking to themselves. and so the major risk that I've that we tend to think about is so healthy non-dysfunctional mitochondria do not seem to induce any sort of pro-inflammatory impact. There's a study that looked at they induced sepsis in in mice and then they just delivered did a tail vin infusion of mitochondria and sepsis in like systemic inflammation completely disappeared which is insane. But I mean the data is there suggesting that it's not it's it's the quality of the mitochondria as far as like the how stable it is. And so we're thinking about like QC processes like how do we make sure that we're isol that we're isol that the isolation process is not in having a lot of damage happen or exposing things from the matrix into the into the outside. so that's those are things I think about. heteroplasm has not been a major I guess like contention point in my head just yet because so far I haven't seen any data to suggest against it but I know there's you know Monovia there's other companies that are doing transplantation and they might have different views on it.

[1:10:07] what I think about so towards I was asked once does it need to be autotogus or allergenic and I said I mean we can make both it really it's and it's really not expensive because if you have a cell that's printing your mitochondria like you can standardize that you can take someone's blood and then literally like these rosier cells these mitochondrial DNA deficient cells they kind of in most situations they take up and start proliferating and integrate whatever mitochondria you give them. So in that system you have a printer. That's a printer right there. Just the major difference would be just the sequence of the mitochondria DNA.

[1:10:45] And I think you can you can sort of use like familiar tracing. Maybe take a hloid groups that are similar like to your own lineage and you might not see as much imunogenic impact. But again I'm not a physician so I don't can't answer just yet. I don't I don't see that being the major issue. I think it's more so going to be just the preparation quality. How healthier are the mitochondria like at infusion? That's that's what I've been thinking about. Why would people get mitochondria from someone else? I mean why not just get it from themselves, you know, like you said alo allergenic and you know well so okay. So if you're for example if you it depends on it intrinsically depends on the level like you're if I if I'm a if I've been smoking crack for 20 years right let's extreme example smoking crack for 20 years and I work in a coal mine at 60 I think I' I'd have a better chance finding a familiar mitochondria that is a better quality because the amount of sort of enrichment you need to do and this is all hypothetical Maybe it won't be that much but the amount of enrichment you need to do for your cell population just to find that subset of cells that has you know healthy mitochondria and maybe you can use T cells as a way to you know sort of select for better quality but then a T- cell so the amount of makage in a T cell you need to then think about differentiating them into a different cell that has a much higher capacity right to me it just seems a lot simpler if you can just get in that in that situation but if you're young and you know you're healthy and you want to you know bank some mitochondria, bank some immune cells. Really, there's nothing wrong with that. So, that's what I think is it would be sort of like a case for case per situation. one of the projects we're working on right now, which is really going to be a sort of autogus situation potentially. it's looking at a prime mitochondria disease and there's a mutation in FXBL4. It induces like hyperphagy. and so you know one of the strategies that we're going to do is we're just going to correct the hyperphagy in the patient's own mitochondria and then just redeliver those to see if there's like you know longer retention and better like functionality. So in those cases like you could use your own but yeah it's it's a case by case by case basis but another thing to think about for you know people talk about this the cancer transfer right like they I think it's again I you know I understand cancer but I also understand oxidative stress and I think it's more so that cells have some sensing mechanism for ROS like ROS thresholds and they donate mitochondria because of that. I think that's that's pretty much what it is. And so there was also a study looking at cancer and showing that the you know the heteroplasm in cancer cells is completely different than the heteroplasm in immune cells after they do the whole telling on the the TNT system. So do mitochondria really belong to us? Like are we really like our own mitochondria really ours really? like you know I don't it's a philosophical question too but I haven't spent too much time bidding my head about it. Nice. And then what about man I had another I had another question in there. Where did it go? Doping. So right y dude doping. I that's something I think about a lot because I know I can tell you right now as black market.

[1:14:18] Yeah. People are going to be blood doping. Oh they're gonna be m doping because it's going to be so hard to detect. is like and it's I mean this is something I've literally been thinking about from the moment when we started doing transplantation because we've done studies with looking at perforatory disease and psychopenia and we saw significant recovery after we injected them. I was like oh man people are going like it's going to change a lot of things and I don't know I don't know I don't know if it's going to be necessarily a good or bad thing. if you want to want if you want to run a marathon, maybe you can, you know, train faster, help with recovery in that sense. But I think there it's definitely going to challenge some of the ethical standards that we have right now towards athletics and just like life. I mean, I think I see this being something like, you know, you don't you shouldn't need a prescription to get a mitochondria. You can go get some the same way you can get some ibuprofen because there's such our body literally break stuff down to make mitochondria. It's just to me there's some inefficiencies that we can maybe make better. yeah. Yeah. Blood doping is something that really interesting. So it's going to be unstoppable. Like you said, how would you detect that someone got a bololis? I mean, you'd have to do like a biopsy, pick your muscle of interest, and then look at that, do another biopsy. No athlete's going to want to give a biopsy in the first place, but and it's going to be very hard to differentiate between at least I don't think there's going to be a major difference in showing natural like hypertrophy from working out versus like injecting mitochondria because it's they work on the same sort of pathway.

[1:15:55] So I don't know if you'll be able to differentiate unless you were to take someone else's mitochondria, you have to sequence, but then depending on the amount like you'd have to do a lot of sequencing. It's going to be very tough. It's going to be very tough. So yeah. So in terms of rejuvenation technology in my top three are mitochondria transfer the stuff you're doing Michael Leven's bioelectrical restoration and then the tieumir river story. So, and you know most people I mentioned it you know in the intro that most people are only thinking about epigenetic reprogramming but how do you see like sure you're studying mitochondrial transplantation so maybe you're bi biased in this right but as the objective scientist when it comes to human rejuvenation and restoring youth youthfulness not maybe not totally but at advanced age you know would you put mitochondrial transpl transplantation close to the top better even better than epigenetic reprogramming or how do you see I think

the risk profile is definitely lower for transplantation than epigenetic ep epigenetic reprogramming just because of I think you have to have proper sort of pulsing and time constraint so you don't you know induce too many uncle genes and I was actually thinking about this too like if you can if you can you customize a mitochondria then maybe you can just use that as the transient sort of reprogramming vehicle so they the moment it gets to the cell it like induces it the enzyme circuit produces what it needs to and because it's marked for degradation it doesn't stay long and so I think that's a much better way at controlling reprogram because again I'm not completely expert on reprogramming but from what I understand it's it's it's finding the right sort of range for exposure of the different reprogramming Yamanaka factors on cells and I think because we can program stuff to have mitophagy happen you load the by mitochondria with these things or not even you don't even need to load them. You can just you know co- conjugate them onto the outer membrane and over time the mitochondria either going to fuse but you can also again program them to not fuse and just be headed straight for mitophagy. So you the reprogramming happens the mitochondria you know get gobbled up after and I think that's a durable way to do it. Right now I think there's a lot of issues with specificity like because of this whole the same with like you know with antioxidants every cell has a specific goldilocks kind of kind of range for how much they need and I think it's the same thing with reprogramming and trying to understand those gradients from a you know flooding a system is just is hard right so having specificity mitochondria are literally like our personal bacteria like we should really be thinking about this from this perspective changing how we And the fact that we have so many in us dog like it's so cheap you can grow so many you can really customize them treat them like re-engineer them package them with different things I think it also because our body has an intrinsic they accept them like you don't have to do much like even without these modifications with antibodies like if you were to incubate cells for you know 24 48 hours they would still take up the mitochondria suggesting that there's already you know some original sensing mechanism. This is just trying to make it better. Amazing. This was great. Thanks, Coen. I'm looking forward to I mean, you showed a bunch of studies where, you know, you're I think through 2029, you're funded through 2029. So, I'm looking forward to seeing that data and yeah, thanks for sharing and I'm hopefully I can host you at another point or more points in the future. For sure. We can have a follow-up. This was amazing. This was a I'm I'm I appreciate you also taking, you know, cuz you have to push your schedule back a little bit, but no, this is I like doing this stuff.

[1:19:45] Cool. Thanks, Colin.