ANTHONY WINDEBANK: Good afternoon, and welcome to CTSA Grand Rounds. It's a great pleasure today to be able to introduce my colleague, Dr. James Kirkland.

Jim is a native of Canada, from Montreal. And who did his basic medical and research training in Canada, predominantly in Toronto, and also spent time in England as well. And has really become one of the leaders internationally and nationally in, first of all, the whole area of adipose tissue as an organ and the incredible importance of adipose tissue as an organ, as it relates to the areas of aging, senescence, and metabolism in fat cells. And I think it's always good to see a full house. And there's nothing like aging and adipose tissue to bring everybody in.

So Jim, we're looking forward to it very much. Thank you.

JAMES KIRKLAND: Thank you, Tony. And thank you very much for coming today. What I'm going to talk about is, obviously, aging fat tissue and cellular senescence. You'll notice that I cough periodically as this goes on. I've got a bit of asthma. And my asthma tends to be audience induced.

[LAUGHTER]

So I don't want to take steroid inhalers. Being a physician, I shy away from that. So occasionally, I'll drink a bottle of water-- some of the water-- if I start coughing and hacking.

So aging has become an extremely interesting field, particularly over the last three years or so, after the first paper came out in *Nature* in 2009, indicating that there are pharmacologic approaches which can delay aging processes and age-related diseases as a group. We've known that things like caloric restriction and around 500 single-gene mutations can delay some of these processes. But now, there are actual pharmacological approaches. And at last count, there are several dozen potential pharmacological approaches in various species, which can alter this process, at least five of which are in mammals. And some of which might, with any luck in the next five or 10 years, come close to the point of clinical translation.

I was always taught when I was in medical school that aging is the biggest risk factor for virtually every chronic disease. If we talk about Alzheimer's disease; or talked about atherosclerosis; or talked about cancer, most kinds of cancers; if we talked about diabetes; and so forth, aging was always the biggest risk factor. In fact, for many of these chronic diseases, aging is a bigger risk factor than every other risk factor combined.

But then we skipped down to the next risk factor, like managing cholesterol, or managing glucose, or blood pressure, or whatever, because there was nothing you could do about aging. So now that paradigm is beginning to shift. And it's still science fiction to say this, but only just. It might be possible to delay age-related diseases as a group by targeting fundamental aging processes.

If we could do this, it would be really important. Because if you got rid of all cancers, for example, you might add three to four years to median life expectancy. If you got rid of all atherosclerosis, again it's two to three years. If you got rid of diabetes, a similar sort of numbers, or Alzheimer's disease. So we can take bites at these chronic diseases one at a time or we can choose to attack them all as a group and maybe have a more substantial impact, possibly. Science fiction, but it's only just science fiction.

So in lower animals, for example, through caloric restriction, which isn't something that's going to be easily translatable into humans. Or it is possible, at least in rodents, to delay the onset of age-related diseases as a group, it seems, by upwards of 20%. So it's becoming attractive to ask there are other aspects of the aging process we can target, that would affect health care generally?

Now if you look at various tissues, and predisposition to chronic disease, there are five or more things which tend to occur with aging, sort of broad categories of things, which might contribute to both chronic disease and aging phenotypes. And these things are highly interrelated, as I'll talk about as we proceed through this discussion. Chronic, low-level, sterile tissue, pro-inflammatory states occur across most cell types and tissues with increasing age and predispose to many other chronic diseases that we're thinking about.

They predispose to things like inflammatory cytokine release, but also changes in cell subtype abundance within tissues, fibrosis, and all kinds of other things. Progenitor and stem cell dysfunction generally occur with aging. And some of these are related to changes in the tissue microenvironment in older individuals, as well as endogenous changes in progenitor function across a wide variety of types of progenitors.

Something called lipotoxicity contributes to age-related dysfunction. This is when fat is not stored properly. And fat tissue is triglyceride. And instead, gets into all kinds of other tissues. It's highly reactive fatty acids, which wreak havoc with various tissues. So with increasing age, there's increasing lipid deposition in muscle, and liver, and bone marrow, pancreatic beta cells, and elsewhere. And some of these moieties lipid-related moieties, like saturated fatty acid, ceramides, and things, do quite a bit of damage.

Proteotoxicity is a general phenomenon with aging, with protein aggregates appearing in various tissues and contributing to age-related chronic diseases, most notably some of the neurodegenerative diseases, like Alzheimer's and Parkinson's. There may be other things going on that could cause or contribute to these events, like changes in the microbiome, that are only just beginning to become unraveled. So we'll consider what happens with aging and fat tissue as sort of a paradigm. And also, fat tissue is an attractive target for interventions that might affect age-related processes for some reasons that I'll talk about in the next slide.

Fat tissue is the largest organ in most humans, not every person, but most people. For example, a woman with a BMI above 35 is more than half fat tissue. A man with a BMI above 45 or so is more than half fat tissue.

Fat tissue has a huge progenitor pool. Upwards of 50% of the cells in fat tissue are multipotent progenitors. This is why fat tissue is being used as the source of adult-derived stem cells. Because fat tissue turns over throughout life and is a highly plastic tissue, it's also involved in wound repair, immune responses, and so forth. So it's a highly plastic tissue, with a lot of progenitors.

And it appears to be closely related to aspects of health, and healthspan, and lifespan. For example, if you surgically remove visceral fat in younger experimental animals, you can quite substantially increase maximum lifespan. Nir Barzilai showed this. Incidentally, he's coming to give a talk here on the 12th of November, at the Aging Center seminar series.

FIRKO mice-- Ron Kahn was recently here a couple of times from Harvard-- showed that knocking out fat cell-specific insulin receptors increases maximum lifespan in experimental animals. So insulin receptors being knocked out specifically to fat tissue in mice extends maximum lifespan substantially.

The mutations in experimental animals-- and it looks like humans-- that have the biggest effect on healthspan and lifespan involve the growth hormone and IGF-1 signaling pathways. So growth hormone receptor knockout mice, for example, have a 40% increase in their maximum lifespan and a substantial delay in age-related diseases as a group. And there are a number of other animal models related to this, some of which we'll talk about in a few slides from now. These mutations predominantly affect fat tissue, as well as other tissues, of course. But they have an impact on age-related fat tissue redistribution and a number of aspects of fat cell function, which I'll talk about.

It looks like, at least in experimental animals, that fat tissue is the main source of circulating inflammatory cytokines in old age. This was worked on by Simin Meydani at Tufts. So the IL-6 that is a correlate of the frailty syndrome in humans mainly originates from fat tissue.

Obesity and lipodystrophies that are associated with fat tissue dysfunction result in accelerated appearance of age-related diseases and dysfunction, including diabetes, cancers, including nonendocrine cancers, atherosclerosis, and dementias. People with obesity can have up to a 10-year earlier onset of Alzheimer's disease than non-obese individuals. For example.

So dysfunctional fat tissue appears to be associated with features somewhat like accelerated aging in a way, and at least accelerated onset of age-related diseases, as well as inducing chronic inflammation on a systemic basis. Caloric restriction, as you are aware, has the opposite effect. There's some debate about whether and how caloric restriction works in primates. But at least, it appears to extend healthspan. In one study, it appeared to extend lifespan. In another recent study, it at least tended to improve healthspan, depending on the control animals that were used.

But across other species, right from single-celled organisms, there's something about food supply, fat tissue, and aging that are linked. And you can see why this would be important. If food supply is insufficient, it's important to have animals that outlive a period of food deprivation and remain fertile, so they can continue dividing after food becomes available again.

So there are a number of signals that are given out by the plant world when food restriction is occurring. For example, various plant flavonoids that appear to have some impact on some processes that are related to aging events through the sirtuins, for example.

So we pick up signals from the plant world that a famine is about to occur. And we alter some processes that are related to the rate at which aging changes occur. And also, if there's a period of caloric restriction, we up-regulate processes to allow us to eat garbage basically. Because there are signals from the plant world that tell us that a famine is approaching, we're more likely to eat bad things during a famine. And so we up-regulate xenobiotic defenses. And these things, combined, appear to have an effect on maximum lifespan, which is quite profound, and the age of onset of age-related diseases.

So with aging, as many of us are becoming painfully aware, you tend to develop less fat where it should be and have more fat where it shouldn't be. So we'll talk about some of the underlying mechanisms responsible for both of these things and how cellular senescence fits into this.

With aging in experimental animals-- this shows what happens in rats. But it could be mice or it could be humans as well. Fat tissue tends to expand in most depots through middle age. After middle age, the absolute amount of fat tissue declines in traditional fat depots. So it declines at different rates in different depots.

You begin to lose retro-orbital fat first. You get a sunken appearance to the eyes beginning in the 40s. Then you begin to lose subcutaneous fat over the distal extremities. You start getting spindly legs and backs of the hands have very little subcutaneous fat.

Then the subcutaneous fat loss proceeds more and more centrally. Visceral fat is relatively preserved. So that by the 70s and 80s, there is a profound redistribution of fat, from subcutaneous to visceral regions, which is associated with a high incidence of metabolic syndrome. And then after that, even visceral fat starts to become lost. And you accumulate fat in the liver, the bone marrow, muscle, and other places where it shouldn't be, atopically.

These changes in fat cell mass are not associated with a decrease in fat cell number. In fact, fat cell number increases in many depots with increasing age. What decreases is fat cell size. And this occurs coincident with changes in some of the transcription factors that mediate differentiation of fat cells from preadipocytes and are responsible for fat cells being insulin responsive. So fat cells become smaller and less insulin responsive, and less able to accumulate lipid with increasing age. And this is associated with lipotoxicity, with storage of reactive lipids outside fat tissue, where they shouldn't be, where they wreak havoc.

The progenitor cells in fat tissue, their numbers actually increase with increasing age in relation to the abundance of fat cells. And this indicates that there's some kind of a block, which I'll talk about in a minute, in the capacity of these progenitor cells to turn into fully differentiated fat cells.

So I mentioned that fat cell has a large pool of multipotent mesenchymal progenitors that are capable of, through a process of commitment, becoming preadipocytes that are committed to the fat cell lineage. But this is highly reversible.

These committed fat cell progenitors, or preadipocytes, are capable of dividing or of differentiating into fat cells, whereupon they lose the capacity to replicate. Around 2,500 genes are switched on during this process of differentiation.

Fat cells have an average lifespan of the order of 10 years. But it might be less. But they can appear very rapidly after a period of high-fat feeding. Mike Jensen here did some studies, for example, in collaboration with us, where younger subjects were high-fat fed, in the CTSA actually, for a couple of months. And we looked at fat cell number before and after the high-fat feeding. And in the thighs particularly in people, there were huge increases in the number of fat cells within two months of high-fat feeding in 20-year-olds, where they only gained about five pounds or something like that.

So new fat cells can form very quickly under conditions of nutrient excess. Preadipocytes can be gotten rid of through apoptosis or senescence, which we'll talk about. And fat cells can be gotten rid of through a sort of hybrid apoptosis/necrosis kind of process.

So we'll focus on the inflammation, which originates from fat tissue, remembering that preadipocytes are very much like macrophages. Their gene expression profiles are almost indistinguishable. There are very few markers that will distinguish a macrophage from a preadipocyte.

Preadipocytes, like macrophages, or motile. They can engulf bacteria. And they're capable, upon exposure to cytokines, of mounting a pronounced innate immune response. So these cell types are very, very, very closely related. And this may contribute to the pro-inflammatory state that can occur in fat tissue with obesity and diabetes.

And then the other process we'll consider a bit about is differentiation. And this process is required to protect us against lipotoxicity.

So with increasing age, the inherent capacity of preadipocytes to turn into fat cells declines. This shows, if you culture preadipocytes from young, compared to middle age, compared to old rats, and you put these cells in culture for several weeks, and then you expose them to differentiation-inducing media-- so you've got cells from young, middle-aged, and old animals growing in parallel in an incubator. Expose them to differentiation-inducing media. And you make sure that there are only preadipocytes in the cultures by excluding other cell types.

You find that the preadipocytes from the young animals are able to accumulate lipid. Whereas, with increasing age, there's less capacity to accumulate lipids. The same is true in humans.

So with aging, there's some kind of an inherent decline in capacity of these cells to accumulate lipid. You find this at the clonal level as well. But you do find individual preadipocytes from older individuals that behave like younger individuals, and vise versa. But, on average, at the clonal level, you find this same thing occurring, even after being in culture for many months.

So to get at the mechanism of this, I'll just briefly go through what happens during adipogenesis. When cells are exposed to IGF-1 in particular, but also fatty acids and other nutrients-- remembering that preadipocytes do not respond to insulin. They don't have insulin receptors. They only acquire insulin responsiveness after they've started to differentiate-- these cells have an immediate up-regulation of CCAAT, and hence are binding protein beta, a transcription factor. It turns on peroxisome proliferator-activated-receptor-gamma, which is the target of the thiazol anti-diabetic drugs, like pioglitazone.

This is a nuclear transcription factor, that's ligand activated. So if there are lipid moieties around this transcription factor will transactivate a couple thousand genes. Amongst these is C/EBP alpha, another C/EBP family member. It turns PPAR gamma on, in a sort of autocrine loop. It turns itself on. And PPAR gamma turns itself on.

So these two things come on. And they have to stay on for a fat cell to become a fat cell and to remain insulin responsive. You need both of these things on for a fat cell to remain a fully functional fat cell. Together, they turn on or off around 2,500 genes.

With increasing age, if you take preadipocytes from younger individuals compared to older individuals, the ability to turn on C/EBP alpha declines. And the same way with PPAR gamma isoforms. And this is in cells in culture, maintained in parallel for many weeks. So this is a cell-autonomous kind of event.

If you overexpress C/EBP alpha in preadipocytes from older individuals, you largely restore their capacity to accumulate lipid. And to some extent, this occurs with PPAR gamma in the presence of a ligand, but not to the same extent because they're-- I won't go into the details. But they're co-activators of PPAR gamma, which change with age as well.

So the block in adipogenesis that's inherent is somewhere between C/EBP beta being switched on within minutes after induction of adipogenesis and PPAR gamma being switched on several hours later. And I'm not going to go through them all. But there appear to be at least six or seven interrelated pathways that do this, that get activated with increasing age.

One of the common features of these pathways is that inflammatory cytokines will induce them. So if there's an increased abundance of TNF-alpha around, for whatever reason, it tends to induce a lot of these stress-related pathways, and pathways that ultimately interfere with insulin function.

So I mentioned before that preadipocytes are very closely related to macrophages. They're quite capable of producing a lot of TNF-alpha, as our macrophages if they're activated. Preadipocytes taken from younger individuals, that are just put into culture, in parallel with preadipocytes from older individuals, produce a lot less TNF-alpha. In fact, they produce very little.

But preadipocytes from older individuals can produce as much TNF-alpha as an activated macrophage. This is at the protein level. So they begin to pile out this stuff. And you can see that that would interfere with adipogenesis and insulin responsiveness. If you treat preadipocytes from older individuals, you get some restoration and capacity for adipogenesis.

When we do genome-wide expression profiling and look at the RNA species that are up-regulated with aging in preadipocytes from different depots, we find first of all that different depots have different patterns of gene expression changes with increasing age. But, in general, inflammatory cytokines, chemokines, and extracellular matrix proteases tend to get up-regulated in preadipocyte, cell-autonomously, with increasing age of the donor. And one of the things which could initiate or be upstream of this is a process called cellular senescence. So by definition, cellular senescence means irreversible loss of replicative potential of normally replicating cells. That's the old definition of it.

A lot of the original work on this was done by Hayflick and Moorhead back in the 1950s. They found that if you put human embryonic fibroblasts in culture and repeatedly subcultured them, there was a fixed number of population doublings that human cells could undergo before they'd stop dividing. They don't die.

In fact, you can keep them in an incubator for 10 years. They don't die. In fact, they're resistant to apoptosis and cell death. They're resistant to low serum.

And they remain metabolically active. They become enlarged, around three times larger than normal cells. They produce a lot of protein. They have a single large nucleolus within their nucleus.

And they acquire a number of features which are distinct with respect to gene expression and epigenetic patterning than nonsenescent cells. They exhibit activity of an enzyme, senescence-associated beta-galactosidase, which is a modestly sensitive and modestly specific indicator of cellular senescence. So it's lysosomal beta-galactosidase that reacts at pH 6, instead of pH 4, because of glycosylation of the lysosome beta-galactosidase.

They exhibit DNA damage responses with DNA damage foci, as evidenced by gamma histone 2AX staining. So they get at least four foci or more. So there are persistent DNA damage responses going on in these cells. And they produce a lot of things which damage cells around them.

So this figure below here was made by Jan van Deursen, my collaborator. And what it shows is stuff that Judy Campisi at Buck Institute had first found, that senescent cells produce a lot of inflammatory cytokines that damage cells around them. So a few senescent cells in a tissue will damage cells around them and cause widespread tissue dysfunction.

Now, cellular senescence may have evolved originally as a way of protecting against cancer. So DNA damage from such things as mutations or alkylating agents, radiation, telomere shortening will induce cellular senescence. So potentially oncogenic insults will induce a cell to become senescent. The replicative senescence that occurs is largely related to telomere shortening or also adverse culture conditions. Those are the two reasons why you get replicative senescence in culture.

But this is an antioncogenic kind of defense mechanism. So you can imagine if a cell has a precancerous lesion to its DNA, by becoming senescent first of all, it stops dividing. And second of all, it kills all the cells around it. So cancer tends to develop as nests. And this is a way of killing the cancer cells around-- the potentially precancer cells around a cell that's become senescent.

Furthermore, these senescent cells activate the immune system. They call in macrophages. And it's macrophages that clear senescent cells. They're resistant to cell death themselves.

So it's sort of like what happened during the Vietnam War. If a fire base was under attack and overrun by the enemy, they would call in an order called Rolling Thunder. And that meant to the artillery bases nearby, fire on my position. I'm dead anyway. Kill as many people as you can around me. That's what senescent cells do. They're a defense against cancer.

But there are other things which will activate senescent processes. Reactive oxygen species will do it, ceramides, fatty acids, high glucose. There are maybe 15 or 20 things now that have been found to induce cellular senescence. It seems to be a cell fate much like differentiation, replication, or apoptosis.

So these signals can come in, converge on a cell. The cell will make a decision to become senescent or not. It will activate one of at least three transcription factor pathways, that result in a phenotypic change, with changes in heterochromatin, especially over a 7- to 14-day period, and large changes in gene expression of over a couple of thousand genes.

So the three pathways that could become activated are the p16 related pathway, the p53 related pathway, and then another pathway that involves some inflammatory cytokines. It's not very well characterized yet. This can result in this replicative arrest and this pro-inflammatory state.

The pro-inflammatory state is reinforced by intracellular autocrine loops involving IL-6-- and I'll come back to why this is important-- as well as the DNA damage response through ROS and cross-talk with mitochondria, and TGF-beta, and the NF-kappa-B system. These things lead to a senescence-associated secretory state, where there are well over a hundred proteins that get produced by senescent cells, that will tend to damage cells around them. So they're things which will stop stem cells from working properly, TGF-beta family members; inflammatory cytokines; chemokines; extracellular matrix proteases, like MMPs.

This results, if there are enough senescent cells, in tissue dysfunction. And our hypothesis was that this could contribute to aging phenotypes, frailty, and chronic disease.

So senescent cells accumulate in fat tissue with aging. This is work that we did back in the early 2000s. And they also accumulate in obesity. This shows what happens in experimental animals, in fat tissue.

You can see the senescent cells follow a linear pattern. They tend to follow blood vessels. That's where preadipocytes and endothelial cells are, the two cell types that tend to become senescent in fat tissue. In obesity, there's massive generation of senescent cells, especially if there's inflammatory obesity associated with diabetes.

Preadipocytes are amongst the cell types that become senescent in fat tissue. This shows preadipocytes from young, compared to older individuals. You can see there are senescent cells here, staining for senescent-associated beta-galactosidase. The same thing happens in humans with increasing age.

This shows fat tissue from a leaner young female subject. This shows, from an even younger subject, who's obese, senescent cells forming along blood vessels. So with obesity in humans, you find this happening as well. And in humans, if you take preadipocytes from obese humans, you find increased senescent cell abundance.

Senescence can occur at any phase during the lifespan. This is work by Dean Morbeck and his group here. It occurs in blastocysts. So if you treat blastocysts the right way, you can induce cellular senescence, even during embryogenesis.

So senescent cells can appear at any phase during the lifespan. But they tend to accumulate with old age. They tend to accumulate in a variety of age-related diseases, not just obesity. But it's turning out, Alzheimer's disease, Parkinson's, atherosclerosis, various cancers, and a long list of other things, chronic obstructive lung disease, kidney disease. Associated with old age, you find an accumulation of these senescent cells.

So we began to ask in the case of preadipocytes, what are the kinds of things that they produce, using proteomics approaches, which were easier, and the lab is doing? And basically, as in fibroblasts and other sort of cell strains, these primary cells from humans have up-regulation of pro-inflammatory cytokines, chemokines, and extracellular matrix proteases. They have a functional effect. So if you proculture senescent preadipocytes with normal preadipocytes you abrogate adipogenesis. So you inhibit adipogenesis. This shows nonsenescent cells cultured with normal preadipocytes.

So you can use conditioned medium or do co-culture experiments. They have an impact on adipogenesis. This just shows that if you expose preadipocytes to senescent cell-conditioned medium, you get impaired lipid accumulation in these cells compared to controls.

The patterns of adipogenic transcription factor expression, their downstream targets, are what you see with aging. So if you co-culture preadipocytes from younger individuals that are differentiating with senescent cells, you replicate the pattern that you see if you take tissue from-- or preadipocytes from older individuals and look at what happens in primary cells.

These senescent cells also produce things which call in macrophages, as well as other immune cell types. So this shows what happens with monocyte chemoattractant 1-protein production by preadipocytes from lean younger individuals compared to age-matched obese individuals. So there's more MCP-1 produced. With cultured replicating senescent-induced cells from these lean subjects, more MCP-1 gets produced. Or if you take lean aged subjects and compare them to the young lean subjects, you find increased MCP-1.

And these senescent cells have a functional effect. If you look at macrophage migrations through membranes, you find that preadipocytes from young individuals that are obese cause more macrophage migration than from lean young subjects. And the same with elderly lean subjects compared to lean young subjects. So these cells have functional effects.

We asked back in around 2003, whether some of the age-related-- I mean some of the mutations that delay aging in mice are associated with delayed accumulation of senescent cells? And the answer appears to be yes. So this shows fat tissue from an Ames mouse, which is a mouse that has deficient growth hormone production, amongst other things, and lives 40% longer than other mice, compared to an age-matched wild type animal, stained for blue, for senescence-associated beta-galactosidase. So there are many more senescent cells in the age-matched wild type animal, than the Ames animal, which lives longer.

Adipogenesis was preserved in the Ames compared to wild type older animals. The same thing is true of Snell dwarf mice, where you find senescent cell accumulation in the wild type animals is greatly decreased in the Snell dwarf mice, which also have growth hormone deficiency.

And the same with growth hormone receptor knockout mice. Here are wild type mice. You see senescent cells following blood vessels. And you don't see them in the growth hormone receptor knockout mice. This is the same mutation as you see in the Laron dwarfs in Ecuador, these people who don't get cancer and appear to have greatly delayed age-related disease. That was described recently by Longo and others.

So one of the key regulators of cellular senescence is p16. We found that in the Snell dwarf animals and the growth hormone receptor knockout animals there's much less p16 in their preadipocytes than in cells cultured in parallel from wild type animals. And the same with IL-6. IL-6 is not only produced as part of the senescent-associated secretory phenotype, it modulates through that autocrine loop that I showed you, the acquisition of that phenotype.

So a number of years ago, Tamara, who's in the audience, and myself, came up with this mad hypothesis, that there are abnormal cells in fat tissue that produce inflammatory cytokines with increasing age, that cause problems. We began to think, around 2003, that these might be senescent cells. And we found that, indeed, they are senescent cells.

Ned Sharpless, in 2004, found that p16 increases in a number of tissues with aging and that caloric restriction decreases p16 positivity in a variety of tissues with aging and enhances healthspan. So we began to wonder whether there is some kind of link between cellular senescence and healthspan. We first noted in some publications that aging results in an increase in fat tissue senescent cells. A bit earlier, people had found that this was probably the case in skin. A collaborator, when we were at Boston University, found that IGF-1 can induce senescent cells in vitro through at least three pathways.

And so we began to wonder if these mutations, where you knock out growth hormone IGF-1 signaling, could be related to an effect on cellular senescence. And we found that cellular senescence, as I showed you in the previous slides, is delayed in the growth hormone receptor IGF-1-deficient animals.

Judy Campisi found that senescent cells have this senescent-associated secretory phenotype in 2007. So we began to try efforts to target senescent cells to try to improve function. And we fiddled around with diphtheria toxin fusion proteins for awhile. And that was an extremely difficult exercise, and a waste of time.

So we wanted to answer this question basically, do manipulations that enhance healthspan, that also are associated with decreased senescent cells and reduced features of the senescent-associated secretory phenotype, do these kinds of manipulations, like caloric restriction or these growth hormone receptor knockouts, directly induce increased healthspan, or through some other mechanism? Or is cellular senescence involved as a waypoint in these processes?

So we began to think about could we create a construct in mice with a senescence-activated promoter and some kind of a suicide gene that would kill the cells? And through our links with Phil Scherer, we thought of using the ATTAC construct, which is caspase-8 FKBP fusion protein, which gets myristolated, goes to a cell membrane as a monomer. But can be activated with a drug, AP20187, that cross-links these monomers, activates caspase-8, and drills a hole in the cell membrane, killing the cell.

We thought about ways of accumulating senescent cells. We thought at first of high-fat feeding, if we could ever make these animals. And this was around the time we moved to Mayo. And we were fortunate enough to meet Jan van Deursen at that point, who's an expert at making transgenic animals and is also an expert in p16. So he favored the p16 promoter over the p53 promoter, which I think was a good decision.

Instead of accelerating senescence with high-fat feeding, his view, and Darren's view in his lab, was to cross them with the BubR1 hypomorphic mutation, which Jan had done an awful lot of work on, excellent work, showing that it has an accelerated age-related, aging-like, phenotype. And he favored a transgenic as opposed to an knock-in or other approach because we didn't want to disrupt the endogenous p16 at all, and therefore predispose animals to getting cancer.

So Tamara and Ricky [INAUDIBLE] who, at the time, was in Sundeep's lab, once Jan and Darren had made the first animals, along with others in Jan's laboratory, just double-checked that, in fact, the AP20187 would kill the cells. We were worried because senescent cells have been shown a number of times to be relatively resistant to apoptosis. But it did kill them.

The reason we added Thiazolidinedione is that there's a thiazolidinedione response element in the p16 promoter, that happens to be in the construct, in the transgene. So we just used that as a convenient way to induce p16, and then to see if we could clear the cells. And that worked.

So Darrin, and Tobias, and Bart, and Jan, in the mice, looked in fat tissue, inguinal adipose tissue, for GFP-positive cells because this promoter, this construct, the INK-ATTAC construct, contains a GFP in it. So we could track senescent cells.

And in untreated animals, there were GFP-positive cells after treating exactly genetically identical animals with the AP20187, the senescent cells were removed-- at least the GFP-positive cells were removed that were expressing the transgene.

This shows what happened to senescent-associated beta-galactosidase in those experiments. And this shows what happens in animals where the drug is given to clear senescent cells. And this shows blue staining of senescent cells in these BubR1 INK-ATTAC crosses, where they're getting abundant production of senescent cells in their fat tissue.

So the drug effectively clears SA-beta-gal-positive cells. And Darren did some nice experiments fact sorting GFP-positive, highly p16-expressing cells. And found that IL-6 in fat tissue, that's increased as these animals get older, is largely, if not uniquely, confined to the senescent cell fraction. The same with plasminogen activator inhibitor-1, which is a senescent-associated secretory phenotype component. So it looks like a lot of the inflammatory cytokines and things like PAI-1, which contributes to age-related clotting and atherosclerosis, et cetera, is because of the senescent cells in fat tissue, at least in this particular model system.

One of the dramatic things that occurred on treating the animals with the AP20187 is that-- I mentioned to you before that fat tissue decreases, especially subcutaneous fat tissue, with increasing age. So two subcutaneous fat depots in mice are their inguinal subcutaneous tissue and their subscapular subcutaneous tissue. It's dramatically restored by treating the animals with the AP20187 and clearing senescence cells, both in animals that were treated from young age and in animals that were treated in later life.

And this is work that Darren did a lot of work on. Nathan LeBrasseur and others also helped. But a lot of this work was done in Jan's laboratory by Darren and others.

You can see a dramatic difference between animals that have been treated from early age with the AP20187 compared to animals that were treated with vehicle. And they were able to show that sarcopenia was delayed, and age-related losses in activity and strength were prevented, and age-related cataract was prevented. So that paper created quite a splash. And we're continuing to follow up on that, as I'll show in minute.

We don't yet have senolytic or asenescence for humans. We're trying to develop those, as I'll talk about in a minute. We're trying to develop drugs that will specifically target these cells, that we can give to people. But we're by no means anywhere near there yet.

So an alternative strategy-- what you don't want to do is interfere with pathways that are upstream of cellular senescence. That would cause cancer. If you interfere with p16 or p53, you're going to have all kinds of problems. By getting rid of senescent cells, you're getting rid of cells that harbor oncogene lesions, which is nice. And Jan and Judy Campisi, who's working with us, have some very nice data, beginning to indicate that there may be some beneficial effects on cancer of clearing senescence cells.

But the other place you can target is the things that these cells produce. Now, this wouldn't be a wise thing to do on a lifelong basis. But in sort of acute situations, it might be reasonable to try to target the proteins that senescent cells make in order to ameliorate aging phenotypes or aspects of age-related chronic diseases.

One thing that really caught our attention was Ayalew Tefferi's paper that came out from Mayo, in *New England Journal* in 2010. And he treated-- I think it was around a hundred-- or 80 subjects with and around 80 subjects without a JAK inhibitor. And as you know, both the growth hormone and IL-6 signal through JAK, JAK1, 2.

He was giving them to elderly people, average age around 75, with myelofibrosis. These people felt a lot better. They had a dramatic decrease in their constitutional symptoms. As you probably know from seeing people with myelofibrosis, they get a frailty syndrome.

They feel weak. They stop eating. They lose weight. They get sarcopenia. They get depressed. They have high circulating inflammatory cytokines.

Those symptoms were improved. The underlying hematologic disease wasn't budged a bit, as judged by clonal myeloproliferation assays. But their frailty syndrome was improved.

So we began to work with him and others here. And we wondered whether targeting the JAK pathway might be an option, especially based on some of our proteomic data for trying to abrogate the negative effects of the secretory phenotype. And at the same time, Judy Campisi, our collaborator on a program project that Jan, myself, Judy, Nathan, and others are on, was finding similar sorts of things with respect to rapamycin and the TOR pathway.

So the long and short of it is it looks select both of these groups of drugs interfere with the senescent-associated secretory phenotype, with many of the adverse factors that are produced by these cells as a group. So we found that expression of phosphorylated and total JAK1 is increased in fat tissue with increasing age. We've got some evidence that it's increased mainly in senescent cells with increasing age. But that, we're still working on.

But what was interesting was that when we used the kinds of drugs that Dr. Tefferi used in that study in the myelofibrosis patients, we abrogated the [INAUDIBLE]. So this shows undifferentiated perirenal preadipocytes from 30-month-old animals, primary cells that produce a lot of IL-6, and IL-1alpha, and MCP1, and various matrix metalloproteases that destroy tissues.

We found that these JAK inhibitors dramatically reduce expression of these things as a group. And it didn't interfere with generation of senescent cells. So it didn't interfere with upstream mechanisms involving p16, which would be undesirable.

More than any other thing that we've used, that we found so far, with JAK inhibitors we were able to restore adipogenesis in cultures of preadipocytes from old animals. And we did this with multiple different JAK inhibitors that act at different levels, provided they hit JAK1 and 2. This shows that we're able to restore the adipogenic transcription factors in these cells and their downstream targets and get rid of things, as I showed you in the previous slide, like IL-6 and various chemokines that get produced with aging in fat tissue in primary cultures or with cellular senescence.

Tamari and I, together with others in our lab, worked with Nathan to give JAK inhibitors to old mice. And we found that they had restoration of fat tissue. There's also some evidence that they had some degree of restoration of VO2 max, metabolic rate, and perhaps rearing activity, although this isn't significant. And in an early experiment, we found that there was perhaps decreased mortality. But we need to do a lot more work on that.

So based on some of these findings, Jan, Tamara, Darren, myself, Nathan, the others, have come up with sort of a general theory that increasing cellular senescence may be at least one aging mechanism that could contribute to age-related chronic diseases. It looks like cellular senescence is a cell fate. So there are exogenous and endogenous inducers of it, that if they're applied in the right sort of combinations will induce a cell to become senescent. And as the cells accumulate in tissues, our hypothesis is, that we're in these midst of testing, that various age-related diseases may be related to accumulation of senescent cells. And frailty appears to be related to it, at least from the [INAUDIBLE] study.

So the steps that we have underway, or that we feel are needed, are most disease model studies. So Jan is in the middle of testing if what we saw in the accelerated aging models occurs with chronological aging. Our group and Nathan's group, we're looking at obesity and diabetes. So in high-fat fed INK-ATTAC animals, to ask if getting rid of senescent cells or interfering with their secretory phenotype with rapamycin or JAK inhibitors prevents diabetes in the face of obesity?

Jan and our collaborator Judy Campisi at the Buck Institute are working together on whether clearing senescent cells will reduce cancer. And there are some initial very promising results with that regard.

Darren Baker is looking at Alzheimer's disease and neurodegeneration, as well as some people at the Buck Institute. Whether clearing senescent cells in mouse models crossed with the INK-ATTAC animals affects this? Atherosclerosis, Jan has been looking at.

There are other labs around Mayo which are looking at other things. I see Nick LaRusso is here. His lab is beginning to look in liver disease. And there are all kinds of permutations and combinations of interesting studies that can be done with these animals, and also with a parallel animal model that Judy developed.

One of our CTSA MD/PhD students is working on these two projects, Allison Palmer. So she's worked on the obesity/diabetes project. And we're also asking whether by clearing senescent cells, we can improve transplantation success? Transplantation doesn't work as well in elderly individuals as younger individuals, especially stem cell transplantation. And the question is whether that's a seed versus soil issue? So we're testing that by eliminating senescent cells from the cells we transplant, but also from the tissues we're transplanting into.

We need to develop biomarkers of the burden of senescent cells. And this is very early stage. We're doing proteomic studies, as I mentioned before, of the most common cell types that are involved in cellular senescence, to try to find markers or combinations of markers that we can look for in the blood to estimate the burden of senescent cells in patients. We're going to need to develop this so we can do clinical trials.

We're thinking about other bodily fluids. Haven't really started doing that yet. Biopsies where-- we already know that fat biopsies may be at least a marker of the burden of senescent cells. And these are the same kinds of biopsies that you do for testing for amyloid. So they're relatively benign. And we're thinking about and beginning to work on imaging methods to try to figure out where are senescent cells and how many of them are there.

Jan; myself; Judy, in California; Darren; Tamara; and others, we're actively working with various groups around the country to try to develop drugs that will target senescent cells, both using biological approaches, but mostly small molecule approaches, using high throughput screens.

There's some very early preliminary-- extremely preliminary-- data to indicate that we may, in fact, be able to do this. That we may be able differentially targets senescent cells for killing. But that's got an awfully long way to go.

We're beginning to think about how do we do a clinical validation of these drugs, if and when we're able to develop them and if we were able to get the right biomarkers. So obviously, you can't do lifespan or healthspan studies in humans. We don't have the time to do it.

And a drug would never be approved, that you would have to give to someone in their 20s to have an effect in their 80s, or something like that. So our view is that if we can go through these age-related chronic diseases at the mouse level, we can select some of the best things to do at the human level. And, at the same time, we can start measuring senescence biomarkers, at least in humans, in various kinds of situations where we have a strong suspicion that cellular senescence may be involved in generation of the particular disease process.

And we're also trying to develop study populations and paradigms where, once we get senolytic agents, we would use them on a first-time basis. And some of the things that we've thought about and are developing the populations for and the tools for measuring-- and you saw the slide for the CTSA core for measuring muscle function. Nathan's in charge of that. This is part of this whole process-- is to look in frail versus nonfrail individuals. And the 48 geriatricians around Mayo are very interested in this as well.

And we're trying to think about outcome prediction, that we could use biomarkers of cellular senescence as a help with. But also, ultimately, use these senolytic agents to try to reduce, for example, side effects after chemotherapy, which induces massive cellular senescence, especially alkalating agents or radiation for cancer, which induces massive cellular senescence.

Can we reduce the side effects that occur after these things, particularly in elderly people, so we can up the dose, or give them full-bore chemotherapy instead of half-dose chemotherapy? Can we predict who's going to respond badly to chemotherapy by estimating the burden of senescent cells in these people, instead of spending two hours doing a complete geriatric assessment with a full Rockwood and Fried index, and only being right a fraction of the time?

Can we predict who's going to do badly or well after elective surgery? Can we predict who's going to do badly or well after myocardial infarction? And again, there are some protocols beginning at Mayo to look at this. The same with cerebrovascular accidents or other events.

In particular, I think some of these agents may have their first use in age-related diseases, especially cancers. We can go directly to IIA, instead of going through phase I. And then, orphan diseases, we're very interested in as well, like some of the progeroid syndromes in children, which are on the FDA list of the 400 orphan diseases. Will that be a valid place to try some of these drugs as a first pass because we can get it through the approval process in a sort of-- in a regulatory process in an expedited manner?

Eventually, we'll need to do longitudinal studies in prefrail people, age-related chronic diseases. And as I mentioned, pogerias. So Nathan's been involved in trying to set up some of these clinical paradigms heavily. So is Joleen Hubbard, who I think is here, and many others. But we really need to get going on this.

So in summary, senescent fat cell progenitors accumulate with aging and obesity. Senescent preadipocytes interfere with adipogenesis by nonsenescent preadipocytes, potentially contributing to age-related systemic lipotoxicity, as well as the inflammation that occurs. Senescent preadipocytes have a pro-inflammatory secretory phenotype and attract macrophages.

Lifespan-extending mutations and also caloric restriction delay progenitor dysfunction in cellular senescence. Eliminating senescent cells reverses age-related lipodystrophy, as well as other features of aging. JAK inhibitors interfere with the fat tissue inflammatory state, restore fat mass, and reduce frailty in experimental animals and possibly humans. And we're currently extending these studies to obesity, diabetes, other age-related diseases, and stem cell transplantation.

So there are a lot of people to acknowledge. There are huge numbers of people in my laboratory, Jan's laboratory, Nathan's laboratory, and others around Mayo. I've mentioned Ayalew Tefferi, who's done a lot of work. Mike Jensen has worked with us a lot. Joleen is working with us.

And in particular, I want to acknowledge Jan, who did a huge amount of work on this. He's director of our cellular senescence program. He's Vita Valley Professor of Cellular Senescence. So you can see where his interests lie. He's been named Mayo Investigator of the Year. He has also recently become chair of the Department of Biochemistry and Microbiology

I want to thank Tamara, who has done a lot of this work over many years. We started having these ideas in the late 1990s. And she remained with me on my move from Boston to here. And she's thought of a lot of the ideas that I talked about.

Darren Baker did a huge amount of work on characterizing the INK-ATTAC animals. And is doing very exciting work on the neurodegenerative disease side. He's got a Brookdale award, which is a major thing. Only two people a year get that in the US. He got an Ellison award for his work. And he's now an independent investigator at Mayo.

Nathan has done a huge amount of work as well. Nathan has been involved in setting up the frailty phenotyping laboratory that we have, that is amazing, for phenotyping aging mice. So we can do clam studies, all kinds of metabolic studies, activity.

We've got very, very advanced CT methods, where we can go down to 40 micron or better resolution in mice. We've got NMR systems, all kinds of ways-- because he's a physiotherapist, as well as a PhD microbiologist-- all kinds of ways of measuring physical function, including shocking the animals, if they don't run on their treadmill.

And he is in charge of the CTSA core, including a mobile core, which we feel will be very important in the translational work we want to do and in the healthspan assessment laboratory, which Nick LaRusso has worked a lot with us, and that Nathan heads, along with people from the Center for Innovation over in Charterhouse, where we can do intensive studies of elderly people. And that could be a valuable resource as we develop these drugs.

I'll just conclude by saying that we've got a conference, which goes into a lot of this stuff in a lot more detail. Jan and I are co-organizing it. But the person who's done all the work is Tamara, not us, as well as Linda and Ben. This is being held the 8th to the 10th of November, at Assissi Heights.

We've got experts in cellular senescence coming in from around the world. So it should be an exciting conference. And you can contact us, if you're interested. So with that, thank you very much.

[APPLAUSE]