



# The Mitochondriacs

*Is a drug maker hooked on treating mitochondrial dysfunction a loony, an oddball, a kook, a mitochondriac? Ten years ago there weren't so many who would have said no. But more recently, consensus against the economic value of mitochondrial medicines, based on a two-part argument, has eroded. The first objection: That the organelles -- which are the principal cellular energy source -- were too important to risk manipulating with drugs. Side effects would be intolerable. The second objection simply noted that while inherited mitochondrial diseases may be legion, they are also rare, so treatments would serve only miniscule markets.*

*Well, clinical evidence gathered over the last several years demonstrates that, so far, the side effects of mitochondrial drugs seem no worse than for other types of drugs. Moreover, mitochondrial dysfunction is part and parcel of more than just inherited diseases. Mitochondriacs -- including the four companies discussed in this article -- have mainstream missions, targets as diverse as cancer, hepatitis C, angina and osteoarthritis. Hardly small markets.*

By Tom Hollon  
Special To Signals



To the connoisseur of bizarre afflictions, mitochondrial diseases are meat and drink. Take this oddity, for instance: Imagine finding it harder and harder to move your eyes, until one day you cannot read this article, or look up or down or gaze across the room unless you turn your head. That's PEO -- progressive external ophthalmoplegia -- where the muscles that move the eyes gradually deteriorate until following an object is impossible without moving head and neck.

A recently discovered cause of PEO is a non-lethal mutation in polymerase gamma, the DNA polymerase responsible for replicating our other genome -- the 16.5 kb mitochondrial chromosome. The mutation cuts the fidelity of mitochondrial DNA replication in half. Because mitochondrial chromosomes can be present in cells in hundreds or thousands of copies, depending on the cell type, the mutation takes decades to make itself felt, sometimes until middle age. But eventually, accumulated miscopied genes build to a tipping point, and mitochondrial energy production (in the form of ATP, adenosine triphosphate) falls below what cells demand. Shortages register earliest where energy requirements are highest and sensitivity to decline greatest -- eye muscles like the lateral rectus, where mitochondria make up 60 percent of cell volume. Eye paralysis is not the end, either; PEO heralds energy brownouts to come in the brain, kidneys and other tissues requiring high levels of ATP.



Electron micrograph of a mitochondrion. From NIGMS publication *Inside The Cell*.  
Photo credit: Friend, D.S., Brigham and Women's Hospital.

So much has been unclear about how drugs work, and remains unclear about mitochondria, that it often escapes notice that medicine joined hands with mitochondria a long time ago. What better drug to make the point than nitroglycerin, with a mitochondrial connection unmasked only this summer, after more than 130 years treating angina. Nitroglycerin is inactive until converted into a bioactive form by a nitrate reductase. The summer's news was the reductase's identity -- mitochondrial aldehyde dehydrogenase.

The final tally of mitochondrial diseases, whenever it arrives, will likely list hundreds, most prominent among them the inherited childhood tragedies, manifested variously by muscle weakness, deafness, blindness, seizures and dementia. Drug development programs here are as rare as the diseases. But mitochondriacs will tell you, if that's all you think mitochondrial medicine concerns itself with, you're missing the boat.

A big one.

## Mitochondrial To The Max



At [MitoKor](#) Inc., commitment to mitochondria is total. The San Diego company, founded as Applied Genetics in 1992, was renamed in 1996 after new investors broadened its mission from Alzheimer's disease diagnostics to mitochondrial therapies. MitoKor has unmatched resources for illuminating the murky world of inherited and acquired mitochondrial disease. Its database of human mitochondrial DNA sequences -- the world's largest, containing complete sequences from over 1,200 subjects -- is used to genotype inherited mitochondrial disorders. Its proteomics program has characterized some 300 proteins, gathering a head of steam for cataloging the estimated 2,000 proteins in the mitochondrial proteome.

It's natural to assume that therapeutics for rare diseases are the heart of the company's business. Actually, therapeutic focus is elsewhere, on "degenerative conditions associated with aging where mitochondrial dysfunction plays a role," says Walter H. Moos, chairman and CEO. In other words, MitoKor focuses on familiar territory: Alzheimer's disease, Parkinson's disease, stroke, diabetes, obesity, glaucoma and osteoarthritis.

"Clearly in some cases mitochondria are primary in these disease processes and secondary in others," says Jim Dykens, the company's director of business and drug development. "However, mitochondria play such a central role -- driving energy and metabolism, calcium regulation, cell

death pathways -- it may not matter whether mitochondria are primary or secondary. If you intervene at the level of the mitochondrion, you ought to be able to help the patient."

In fact, he believes that the benefits of protecting the organelle that supplies 90 percent of the cell's energy could be enormous. In Alzheimer's disease, for instance: "If you can delay the onset by five or 10 years, [for many] you've effectively cured the disease, because the patient will die of something else at the age that they normally would come down with Alzheimer's."

An example of how MitoKor views disease through a mitochondrial prism is osteoarthritis, estimated to affect 16 million Americans over the age of 60. Cartilage is considered an avascular tissue, so it has been assumed that chondrocytes, which are isolated in little islands within cartilage, have minimal ATP requirements. "It turns out that's all wrong," says Dykens. "If you repress the function of mitochondria in chondrocytes by as little as 30 percent, they stop supporting cartilage and actually start to calcify it."

MitoKor's preclinical drug candidate MITO-402 "tries to modify the progression of osteoarthritis, as opposed to just treating the inflammation that results from cartilage degeneration," Dykens says. "By targeting molecules that allow mitochondria to regain their normal level of function, we're restoring normality." Chondrocytes start repairing cartilage; calcification, a hallmark of osteoarthritis, ceases; and "response to inflammatory agents normally active in the joint is much less robust."

## Calcium Bucklers



MitoKor's research on the dramatic differences between mitochondria from different tissues ties directly into its drug development goals. Take calcium challenge, for instance, says Dykens: "Mitochondria in the brain collapse from a calcium challenge that mitochondria in skeletal muscle or myocardium tolerate with no problem." Even in the heart, though, too much calcium can be deadly. "In acute diseases like stroke or myocardial infarction mitochondria fail generally because calcium entering the cell undermines mitochondrial integrity." Mitochondrial membranes become leaky, mitochondria fail and cells die. "In chronic degenerative diseases it's more a question of long-term senescence of mitochondria and their inability to bounce back after a calcium exposure. It's the same kind of process -- calcium flooding the mitochondria, causing collapse -- just on a different time scale."

MitoKor's most advanced drug development program -- which it inherited as part of its 2001 acquisition of [Apollo BioPharmaceutics](#) Inc. -- centers around estrogens, which protect neurons by stabilizing mitochondria during a calcium load. Estrogen neuroprotection has been known for about 10 years, says Moos. Hormone replacement therapy studies indicate that estrogens either delay the onset of Alzheimer's disease or reduce its severity; a long-term study conducted by the NIH seeks to confirm the observations. More recently, "people have shown that estrogen can be changed so that it no longer has significant affinity for estrogen receptors and lacks feminizing activity. These non-hormonal estrogens are as neuroprotective or better than the feminizing hormones," he adds.

In the presence of MitoKor's less-feminizing estrogens like MITO-4509, mitochondria tolerate more calcium. The company's preliminary data suggest that these drugs may also lower calcium entry into cells. MITO-4509, targeted for neurodegenerative diseases, has completed a Phase I trial in the U.K. Once the first indication is decided (probably mild cognitive impairment, Moos says) follow-up surrogate marker trials will take place. NeuroStat, a natural feminizing estrogen and a component of marketed estrogen mixtures, is a pre-IND product for stroke and heart-

attack protection. "We hope that paramedics will use NeuroStat upon showing up at the site of someone with suspected stroke," says Dykens. "There's a very short window of opportunity to save brain cells that will otherwise die. If we can change from getting the patient to the hospital for treatment to getting the ambulance paramedics to treat the patient, we'll save critical time during this short period."

The change Moos and Dykens hope to see one day in stroke treatment is mirrored in the way they see others regarding their field. "It's interesting," says Dykens, "a number of years ago I was giving a talk at the [Society for Neuroscience](#) meeting on mitochondrial dysfunction and neurodegenerative disease and there were maybe 10 or 15 people in the audience. The last time one of my colleagues gave a talk at Neuroscience, it was standing room only."

## Blasting Caps

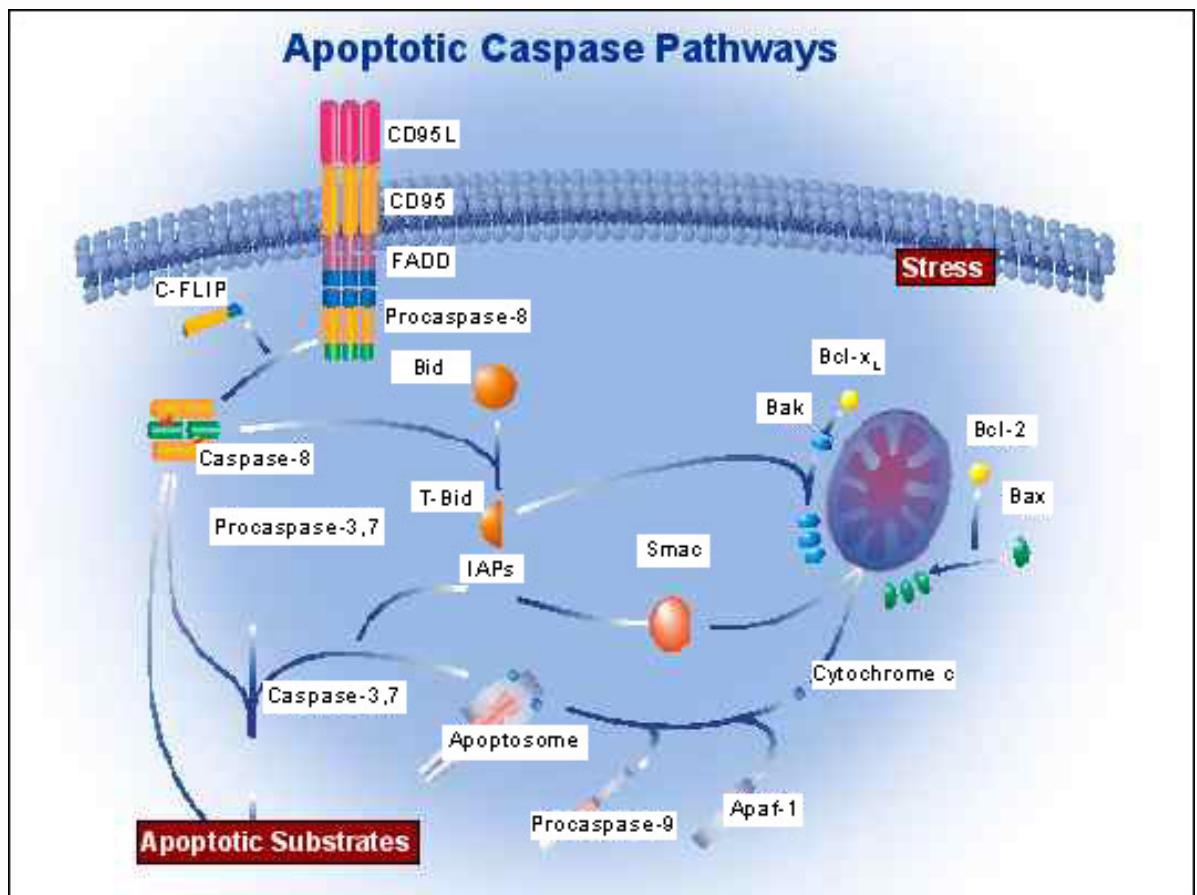
The body is a minefield, explains Kevin Tomaselli, co-founder and VP of discovery research at [Idun Pharmaceuticals](#) Inc., in San Diego. Every cell can kill itself by triggering the "caspase protease bomb" in its cytoplasm that waits to go off. "Cells have a mechanism to be acutely aware of severe cell damage -- from ionizing radiation, chemicals, toxins -- and eliminate themselves for the benefit of all the other cells in the organism." Apoptosis, or programmed cell death, a normal function, detonates caspase bombs each day in cells by the million.

Idun opened its doors in 1994. Tomaselli tells the story that his co-founder, [H. Robert Horvitz](#), who shared this year's Nobel Prize in Physiology or Medicine for identifying the key genes that control apoptosis in *Caenorhabditis elegans*, found the company's name while browsing in a Cambridge, MA bookstore. Iduna, proclaimed a book on Nordic mythology, was the maiden goddess of golden apples, guardian of the food that kept the gods young.

"The pharmaceutical concept," recalls Tomaselli, "was to manipulate this genetically controlled form of cell suicide in the direction we want it to go, using small molecules: Where you have too many cells, to kill cells selectively; when you're losing cells, to spare them."

The biochemical pathway for cell death in human cells is quite similar to the pathway Horvitz worked out for the nematode and offers a number of intervention points. And here is the mitochondria connection, the one that made biologists rethink how they see mitochondria: If caspases are bombs, mitochondria are where the blasting caps are stored.

Normally, caspases reside in the cytosol as inactive precursor enzymes. It is not surprising that their inactivity is tightly controlled, given what activation means: "Their cleavage of protein substrates dismantles a cell within about 30 minutes." Preventing activation is the job of Bcl-2 and its brethren Bcl-X and Bcl-W, residents of the outer mitochondrial membrane.



Graphic courtesy of Idun Pharmaceuticals Inc.

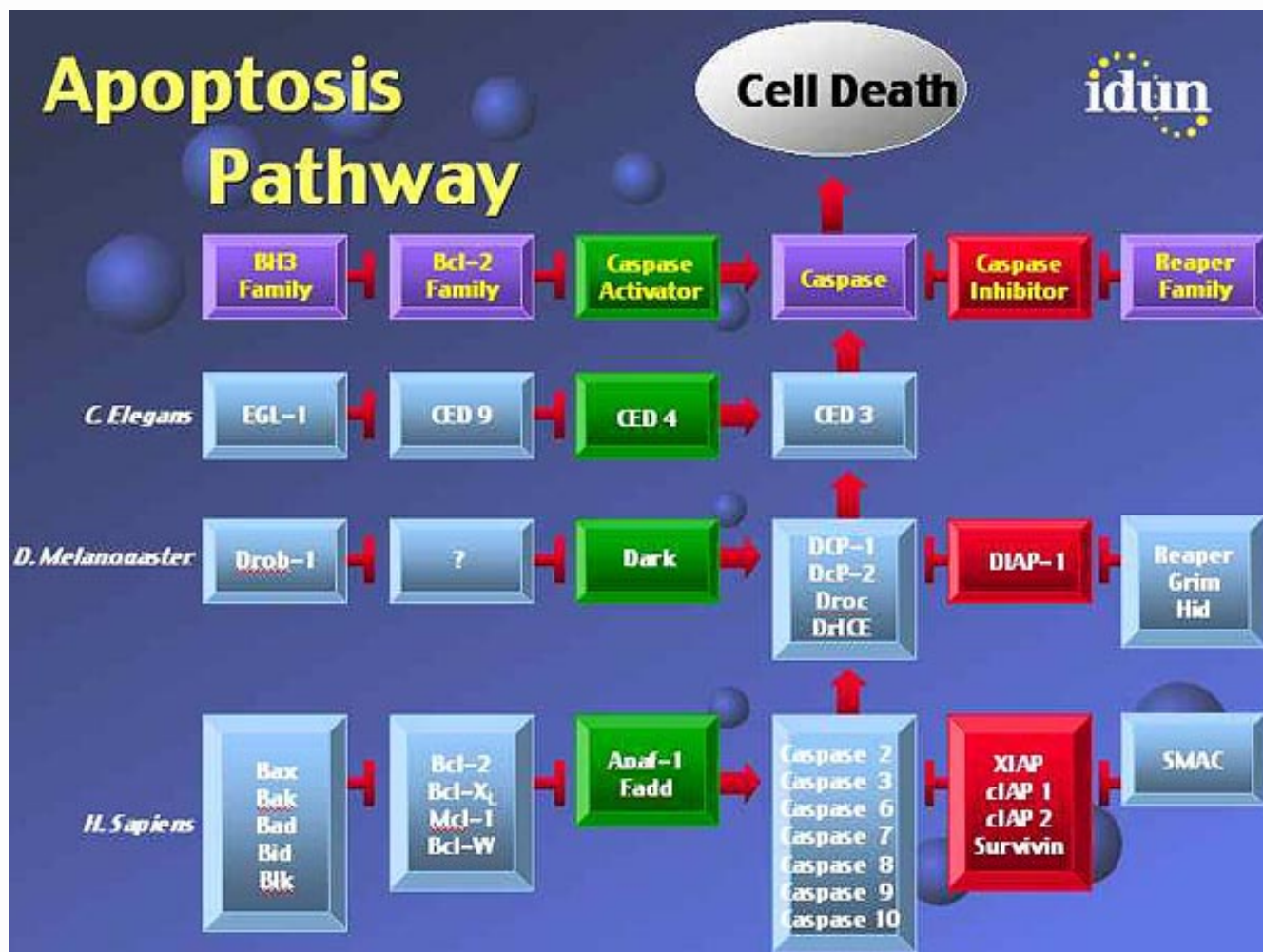
Tomaselli continues: "When cells respond to an injurious stimulus and want to undergo apoptosis, the mitochondrial membrane becomes permeable such that cytochrome C translocates from the intermembrane space of the mitochondria into the cytosol where it binds Apaf 1, which then can activate Caspase 9." Cell death ensues. "What Bcl-2 and Bcl-X do is prevent leakage of the mitochondria and keep the contents, namely cytochrome C, within."

Permeable mitochondria also release a protein called Smac, which binds a protein called IAP. Summing up how these proteins drive apoptosis, Tomaselli talks about driving a car: "The gas is Apaf 1, the brake is IAP. You need to step on the gas and remove the brake to fire up caspase 9. Smac removes the brake. Cytochrome C with Apaf 1 steps on the gas."

Partnered with [Abbott Laboratories](#), Idun is developing Bcl-2 and Bcl-X inhibitors to enhance apoptosis in cancer cells. "It is well known that the cause of non-Hodgkin's lymphoma in humans is up-regulation of Bcl-2," he says. "The cancer is due not to increased cell proliferation but to the lack of death of B cells. The cells can't die because Bcl-2 prevents it." He expects Bcl-2 and Bcl-X inhibitors will have different indications. Hormone refractory prostate cancer, breast cancer and melanoma also over-express Bcl-2. Bcl-X overabundance has been reported in breast, lung and colon cancers. And there is a dual inhibitor under development: A drug inhibiting both proteins will broaden the range of responsive cancers, Tomaselli hopes. Inhibitors are currently being profiled in animal models of human cancer. Abbott and Idun expect to select a clinical development candidate in 2003 and begin phase I trials in 2004.

If too little apoptosis can lead to cancer, too much can lead to tissue and organ damage. Caspase protease inhibitors are the flip side of Idun's drug development coin -- drugs that help mitochondria do a better job keeping cells alive. Indications Tomaselli lists in the acute damage category are stroke, heart attack, acute renal failure, acute liver failure and sepsis, where "an acute, injurious stimulus causes organ cells to undergo apoptosis. If you could prevent the

apoptosis until things resolve -- treating patients one or two or three days after the onset of the injury -- the cells no longer have a reason to die. You could have a better functioning organ and decreased mortality."



Graphic courtesy of Idun Pharmaceuticals Inc.

Treating a heart attack, "caspase inhibitors would reduce cell death during the period that other treatments -- thrombolytics, for instance -- restore blood flow to the heart. The caspase inhibitor buys time for the cells to get through the crisis." Once homeostasis returns to the myocardium and blood flow is normal, caspase inhibitors are no longer needed. "The stimulus that says 'die' is gone."

Idun is developing caspase inhibitors on its own. "Cells activate multiple caspases when they undergo apoptosis," Tomaselli says. "Inhibiting them all is the most effective way to prevent apoptosis." And that is what the company's lead product, IDN 6556, does -- inhibit all of the apoptotic caspases. IDN 6556, now in Phase Ib trials, has been developed for hepatitis C, where "hepatocytes die over time to the point where you have to get a liver transplant." According to Tomaselli, IDN 6556 has been well tolerated up to seven days' exposure and reduces liver-damage markers in mildly liver-impaired patients.

For acute indications, "experimental data support the conclusion that you can broadly inhibit apoptosis in humans for short periods of time, hours to days, without any untoward effects," Tomaselli says. Inhibition in chronic situations is less clear. In Alzheimer's or Parkinson's disease, where neurons are lost over years, "we would predict the need to have the caspase inhibitor on board chronically." And the side effects of chronic treatment? "The only way to address that is to go step wise: First to develop acute therapies, then to experiment first in

animals and ultimately in human clinical trials to look at the tolerability of chronic apoptosis inhibition." If chronic inhibition of apoptosis proves safe, the markets could be colossal.

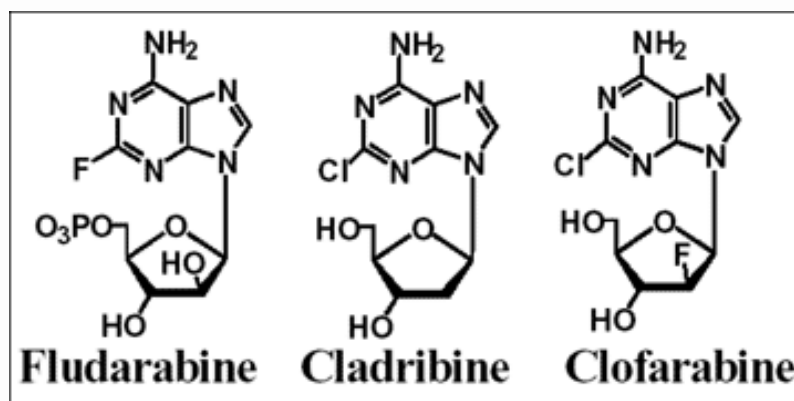
## A View To A Second Kill



Developing a mitochondria-targeted medicine is not always a deliberate decision. Sometimes it's as if the drug decides, as might be said of clofarabine, a second-generation nucleoside analog for treatment of leukemia and solid tumors. Clofarabine is being co-developed by [ILEX Oncology Inc.](#), of San Antonio, TX, and [Bioenvision Inc.](#), of London, England.

The familiar death mechanism of nucleoside analogs is disruption of DNA synthesis, an appropriate means of killing cancer cells proliferating by uncontrolled cell division. It was known, however, that a few nucleoside analogs seemed to have a double punch -- they killed not only dividing lymphocytes, but quiescent ones as well. This suggested a second death mechanism, which researchers at the University of California at San Diego found two years ago. The unusual analogs were disrupting mitochondrial membranes, allowing cytochrome C to escape into the cytosol and initiate apoptosis.

Clofarabine, a rational drug design creation, was not designed to disrupt mitochondrial membranes. That it does is simply good fortune, says Adam Craig, ILEX's director of clinical development. Clofarabine was acquired from the [Southern Research Institute](#) of Birmingham, AL, where Craig says it was designed "on top of fludarabine and cladribine and gemcitabine, which have been used to treat leukemia and some solid tumors for the last 10 years."



Purine nucleoside analogs

Clofarabine's designers succeeded in correcting the major shortcomings of its predecessors. Clofarabine is more resistant to deamination, a primary form of nucleoside analog degradation. It has higher affinity for deoxycytidine kinase, the enzyme that converts inactive nucleosides into active nucleotides. And it resists gastric acid, so "it may eventually be given orally."

But only after clofarabine was designed did its fourth advantage show up like a winning sweepstakes ticket in the mailbox. "The mitochondrial hypothesis was tested because cladribine has activity on the mitochondria," says Craig. "And it became clear that our drug had a similar activity."

Now the question is, which patients will ILEX and Bioenvision help with Clofarabine? Craig is optimistic: "If you look at the group, gemcitabine is a very active in solid tumors, fludarabine is very active in leukemias, and cladribine has some activity in leukemias as well. A drug that incorporates all their good properties could have activity in solid tumors and leukemias. And that

is why we're testing aggressively in both areas." Clofarabine is now in Phase II trials for refractory or relapsed leukemia, indications for which the FDA has granted orphan drug status. One trial is for children with acute lymphoblastic leukemia. Two more are pediatric and adult trials for acute myelogenous leukemia. Animal model experiments suggest that clofarabine may be active in colon and lung cancer, says Craig. This summer ILEX started a Phase I trial of clofarabine in solid tumors.

## Outfoxing Angina



"Ischemic threshold" may be medical gobbledeygook to most folks, but 6 million American chronic angina sufferers live with it as if tethered to an invisible leash, forbidden, on penalty of staggering pain, from simple activities once assumed for granted. "Everyone who has angina knows what that threshold is," declares Brent Blackburn, senior VP of drug discovery and preclinical development at [CV Therapeutics](#) Inc., in Palo Alto, CA. "Can they walk down to the end of the driveway to get the mail? Can they carry in their groceries from the car? Can they play with their grandchildren? They know what they can and cannot do."

The trigger for an angina attack is usually physical exertion or emotional stress. "If you think of what occurs in angina," Blackburn says, "there is an imbalance between the demand that your body places on the heart and the supply of oxygen, due to cardiovascular disease. The most notable angina drugs -- nitrates, calcium channel blockers and beta blockers -- reduce demand. They force your heart to not work as hard by reducing heart rate or blood pressure."

CV Therapeutics, founded about 10 years ago to specialize in cardiovascular medicine, takes a different approach to angina with oral, twice-a-day Ranexa (ranolazine). If approved, Ranexa will be first new class of anti-anginal drugs in more than 20 years -- a partial fatty acid oxidation (pFOX) inhibitor. The company acquired Ranexa several years ago from Syntex Corp., after Hoffmann-La Roche Inc. bought [Syntex](#) and streamlined its pipeline. Instead of following the formula of restraining the heart, Ranexa was developed to "increase cardiac efficiency," says Blackburn. "It's designed to use the oxygen that is available to produce energy more efficiently."

## Trimming The Fat



Cardiac mitochondria burn two fuels to make energy: fatty acids and glucose. Fatty acids make up most of the mix, 60-70 percent usually, and with angina, even more. But when oxygen is scarce, says Blackburn, more fatty acids are just the opposite of what is needed. The heart would be better off burning more glucose, because more ATP is produced per molecule of glucose than per fatty acid. One way to help the heart, then, is to inhibit fatty acid oxidation so that the proportion of glucose rises. As Blackburn puts it, "pFOX inhibitors shift the heart's metabolism to a fuel source which requires less oxygen to generate the same amount of energy."

As the pFOX abbreviation indicates, Ranexa only partially inhibits the mitochondrial beta-oxidation pathway. Partial inhibition, achieved mostly by controlling the dose, "is where we have seen the greatest impact preclinically and clinically." For proprietary reasons Blackburn will not disclose which enzymes are inhibited.

CV Therapeutics has brought Ranexa through two Phase III trials and is preparing an NDA submission for the end of 2002. If it is approved, at first it will probably be prescribed in combination with other anti-anginals. [Innovex](#) Inc., a subsidiary of Quintiles Transnational Corp.,

will build a dedicated sales force for the drug.

The Phase III data indicate that Ranexa slightly improves performance on treadmill stress tests and significantly reduces the number of anginal episodes, as compared to placebo. And some of the patients say they feel like they can be more active with Ranexa. Those unscientific, anecdotal statements won't be submitted with the NDA, needless to say, but they're good to hear. They go right to the whole point of better angina drugs: "So people can do more."

## The Final Mitochondrial Frontier?



Of all the mitochondrial markets, perhaps anti-aging medications will someday be the biggest. Not that they have an anti-aging molecule in the works, but Moos and Dykens at MitoKor find the mitochondrial connection to aging interesting to think about. "Just a few weeks ago I gave a talk at an aging conference in San Francisco," says Moos. "I think about half of the talks mentioned mitochondria."

"It looks as though senescence of mitochondria and human senescence are closely linked," adds Dykens, noting that most of the major mechanistic theories about aging, aside from telomere shortening on chromosomes, have mitochondrial dysfunction at the core. Part of the aging problem is that even in non-dividing cells, mitochondria constantly divide and make new mitochondria. Mitochondrial half-life is a couple of weeks, according to Dykens. Consequently, "over the fullness of a lifetime, genetic errors accumulate in the mitochondrial population. They're not as robust in making ATP, and they become more leaky." Electrons leave the mitochondria to create free radicals in the cytosol. Ninety percent of cellular free radicals originate from mitochondria.

So, can mitochondrial medicines do anything about aging? "Well, yes and no," he says. "We can imagine improving or sustaining mitochondrial function for a longer time. To the extent that the leaky mitochondria produce free radicals responsible for much of the accumulated damage to cells, yes, we think we can develop compounds that will stabilize mitochondria." Of course, Dykens hastens to add, mitochondria aren't the only cause of aging: "We'll bump up against something else in the aging process." Cells will still only undergo a certain number of divisions before they stop dividing.

Not everything gets worse as the years go by: "When I first got involved with this," says Moos, "people thought either mitochondria were too important to cellular function to play around with or they were so small that they were unimportant. People did not know how important mitochondria were for cell death pathways. There's been a change in the respect that mitochondria get from people in academia and industry for their role in health and disease. Mitochondrial diseases are no longer a medical backwater."

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