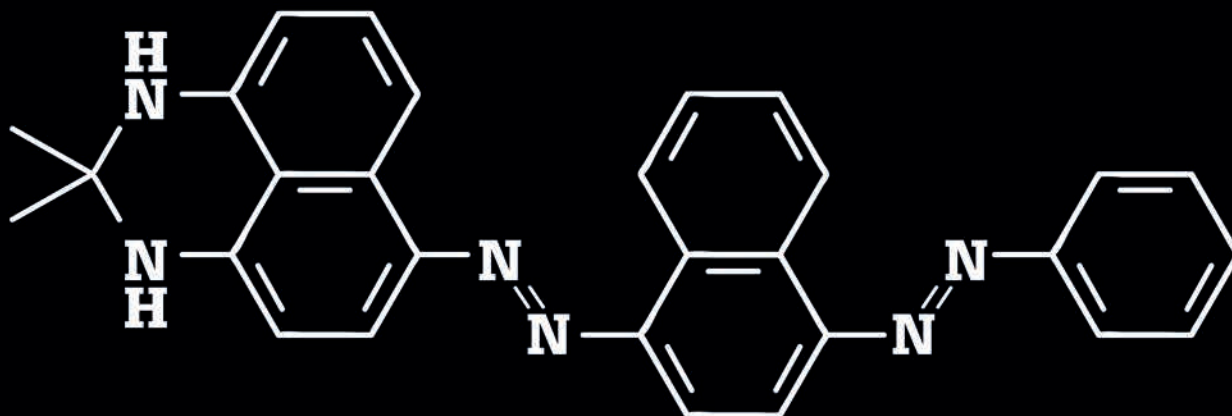


## NextGen

*Research advances  
New technologies  
Future practice*



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The Secrets of Senescence

“Senescence.” A word that sounds so harmless and yet, a cell in this quiet state could turn from a disease barrier into a promoter. Detecting senescent cells has been challenging, but Vassilis Gorgoulis tells us that he and his team might have just found a solution...

## The Secrets of Senescence

**On the back of an old technique – the histochemical detection of lipofuscin by Sudan Black B – we've built a new method for spotting cellular senescence**

*By Vassilis Gorgoulis*

Stemming from the Latin *senex*, meaning “to grow old,” cellular senescence is a key stress response mechanism that preserves cellular homeostasis – which makes it important in normal physiology, embryonic development, and many pathological processes.

Let's imagine a cell in a stressful environment, being subjected to various insults. The cell has various ways of responding: it can die; it can enter arrest; or it can enter a state of senescence. In the latter state, the cell remains metabolically active, but doesn't proliferate. That's why we typically

### *At a Glance*

- *Cellular senescence can tell us a lot about tumor behavior but, until now, we've had no good way of detecting it*
- *Lipofuscin, a byproduct of lysosomal degradation, can identify senescence when detected by Sudan Black B staining*
- *Our new method capitalizes on this, but uses a much purer analog form of Sudan Black B than has been commercially available so far*
- *In the future, we hope to roll the new compound out to pathology labs worldwide – and expand to body fluid analysis as well as tissue staining*

consider it an anti-tumor barrier – how can a cell be cancerous if it is incapable of replicating?

But there is also a dark side. A cell that remains in a state of senescence but isn't cleared from the organism eventually presents what is called the “senescence-associated secretory phenotype (SASP).” It releases cytokines that change the extracellular environment – and can transform the cell from a disease barrier into a disease promoter. How? Changes in expression of secreted factors can cause shedding of normally membrane-bound receptors, cleavage of signaling molecules, and even degradation of the extracellular matrix (1, 2). As a result, it's vital that we are able to detect senescent cells in clinical samples.

*“We're already seeing the new marker's inclusion in clinical trials, even though we only published less than a month ago.”*

An enzymatic answer  
Until now, the scientific community has only had one way of detecting senescent cells: the senescence-associated  $\beta$ -galactosidase assay, which measures the activity of the lysosomal enzyme  $\beta$ -galactosidase. Unfortunately, the method has more drawbacks than benefits. It can only be used in fresh tissue (not archival material), and its

false-positive and false-negative rates are high. Knowing that we needed a better way to spot senescence, we turned to pathology's long history for an answer.

Lipofuscin (derived from the Greek word *lipo*, meaning fat, and the Latin *fuscus*, dark) is a byproduct of lysosomal digestion. A young Danish histologist, Adolf Hannover, first detected it in 1843 in the cytoplasm of nerve cells. Pathologists have been detecting these yellowish-brown granules ever since, but it never occurred to anyone that they could serve as indicators of a stressful condition like senescence. But here's the crux: when a cell is under stress, its bioenergetics can't keep up with demand, so lipofuscin begins to accumulate. We can then detect it using a traditional histochemical stain, Sudan Black B (3) – something that modern pathologists, who rely heavily on immunohistochemistry, may have overlooked.

Like senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) itself, Sudan Black B has its pros and cons. Its key advantage is that it directly detects the cell's aging process via a waste product, rather than relying on enzyme levels. It also improves upon current false-positive and false-negative rates, allows multiple simultaneous stainings, and can identify senescence not only in cell cultures and frozen material, but also in archival material – a major step forward from the  $\beta$ -galactosidase assay. It's a technically challenging protocol, though; you need experienced pathologists to spot the Sudan Black B-stained lipofuscin granules, especially in the presence of background “dirt.” However, we believed we could remove that hurdle entirely by synthesizing our own highly pure Sudan Black B. We performed high-performance liquid chromatography on the commercially available dye, analyzed the spectrum of constituents and isolated the main component. Then we de novo synthesized its chemical analog and added biotin to it, generating GL13 –

a new compound we can finally use in a sensitive and specific hybrid histo/immunochemical method.

#### Probing senescence mysteries

We are very proud of our discovery and its advantages over existing methods. I believe the scientific community will embrace it as the key method for the detection of senescent cells, especially as it can be expanded to other applications – immunofluorescence or flow cytometry, for instance. I'm also pleased that we've been able to provide something the field has needed for over 20 years: a tool for examining senescence in vivo. It's true that there have been biomarkers in the past, but none were specific; for instance, the tumor suppressor p16 has been used for senescence detection (4), but it also detects cell cycle arrest, so a p16-positive cell is not necessarily a senescent cell.

Essentially, we've added a third powerful tool to the evaluation of tumor kinetics; before, we could assess proliferation via Ki-67 and apoptosis via the apoptotic index, but now we have access to a third metric: senescence via lipofuscin. And if we see a tumor or other pathological entity with a high proportion of senescent cells, it means we have the opportunity to examine it further. Is it the "bright side" of senescence – the side that stalls tumor growth? Or is it the dark side that encourages the disease to progress? The answer to these questions lies in double stainings to detect SASP factors. If the staining is positive for SASP mediators, then tumor-promoting senescence features prevail.

When can we get hold of it?

As lipofuscin and Sudan Black B teach us more about senescence, I expect that many of the old questions will be answered and a lot of new ones will emerge. We're already seeing the new marker's inclusion in clinical trials, even though we only published less than a month ago (5). It

seems the scientific community shares our enthusiasm! I've even had reviewers contact me to find out when our compound will be commercially available...

In answer to that question, I hope that the new method – and specifically, our highly pure GL13 – will become available to pathology departments in the next few months, because it's very important for the clinic. Until now, the only measure of a cancer patient's response to chemotherapy was the shrinkage of the tumor, which is caused by apoptosis. But what about the cancer cells that don't undergo that process, and instead enter senescence? The process makes them harmless but doesn't shrink the tumor. If we can include that parameter in our tumor kinetics, we can avoid giving patients chemotherapy they don't need. Better yet, we can now evaluate the effectiveness of novel therapeutic interventions that activate senescence and stall tumor growth. This is all-important: you can't kill something if you can't see it – a physician cannot choose an appropriate treatment unless he knows how the patient's disease behaves. Being able to measure senescent cells in tumors provides such an example by estimating how effective novel senescence-inducing therapies are. Moreover, this new method allows us to monitor the elimination of senescent cells in emerging rejuvenating therapies with senolytic drugs (6).

The future of senescence detection

Right now, we are on the verge of another major leap forward. So far, we've seen very positive results when testing the GL 13-mediated technique on samples of body fluids (for example, saliva and plasma), which is great because it will really boost the clinical applications. We can even combine the in situ tissue analysis with body fluid analysis for a more complete picture. And although this aspect is not yet fully developed, we believe that we will have it in the final stages as early as March!

As a final side note, I think the reason my colleagues and I were able to develop this new method is because we are also hybrids. I am a molecular pathologist – so I consider myself both a pathologist and a molecular biologist. There aren't that many of us in the world, but I think our ability to dive into both the basic and the clinical sides of research problems gives us added insight and helps lead us to advances like our new senescence test – and who knows how many others in the future?

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