



## Anticancer autophagy inhibitors attract 'resurgent' interest

Despite industry's past setbacks with autophagy inhibitors, preclinical and clinical findings are starting to revitalize a once written-off strategy.

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The idea of targeting autophagy, the cell's recycling process, to treat cancer is experiencing a comeback. Years after most large pharmaceutical companies abandoned the therapeutic strategy for oncology applications, several academic groups and fledgling companies have begun zeroing in on cancer types and drug regimens in which autophagy inhibition may still hold promise.

For now, much of the academic community's interest remains focused on repositioning the antimalarial drug chloroquine and its derivatives, indirect inhibitors of autophagy that are cheap, safe and readily available for clinical trials. Case studies show that these agents, when given to the right patients in the right combinations, can yield dramatic responses, even in the face of drug resistance or when targeted inhibition of cancer drivers has failed.

Five years ago, for example, a team led by Jean Mulcahy Levy and Andrew Thorburn at the University of Colorado Denver [described in \*Cancer Discovery\*](#) the case of a teenage girl with *BRAF*-mutant brain cancer who had progressed while on anti-*BRAF* therapy but then experienced more than 2.5 years of disease regression on a combination of chloroquine plus a *BRAF*-targeted therapy. More recently,

in March, cancer biologist Martin McMahon and his colleagues at the University of Utah's Huntsman Cancer Institute [reported in \*Nature Medicine\*](#) on a 68-year-old man with *KRAS*-altered pancreatic cancer who had exhausted all approved treatment options but then responded to hydroxychloroquine plus a targeted inhibitor of MEK, part of the *KRAS*-mediated MAPK cascade.

"Autophagy inhibition may be a viable strategy for enhancing the responsiveness to — or diminishing resistance to — MAPK pathway-targeted therapies in a number of different disease areas," says McMahon.

Dozens of small trials continue to evaluate the benefits of adding hydroxychloroquine to other anticancer drugs.

Yet, hydroxychloroquine offers only limited autophagy modulation. "To call it an autophagy inhibitor is a rather loose definition," says Channing Der, of the University of North Carolina, who published [a companion paper in \*Nature Medicine\*](#) to McMahon's that used mouse and cellular models to explore why dual targeting of autophagy and MAPK signalling still offers clinical hope. "[Hydroxychloroquine] truly is a lysosomal inhibitor," says Der. It blocks the last step of autophagy, in which the autophagosome, the key organelle responsible for engulfing cellular debris, fuses with the lysosome, a waste-processing sac, to degrade unwanted cargo.

A handful of companies — including Vescor Therapeutics, Deciphera Pharmaceuticals and Sprint Bioscience — are as a result searching for more targeted inhibitors of the autophagy pathway.

"There's a revitalized interest clinically [in blocking autophagy]," says Alec Kimmelman, a radiation oncologist at New York University who co-founded Vescor last year to develop next-generation autophagy inhibitors. "But I don't think you're going to maximally know what can be done by inhibiting this pathway unless we get better chemical matter," he says.

### Recycling ideas

Victor Bedoya first showed that [chloroquine could inhibit tumour cell growth](#) in the lab in 1970. It would take another three decades for researchers to show mechanistically that the antimalarial drug actually [impacts autophagic flux](#) and that [autophagy-related proteins can regulate tumour development](#).

By the 2000s, the academic research community was hard at work elucidating the dichotomous — and context-dependent — roles of autophagy in cancer, showing that the metabolic process can both suppress the formation of new tumours and drive the growth of established ones. These insights led to dozens of academic-sponsored trials pairing chloroquine or hydroxychloroquine with radiotherapy, chemotherapeutic agents and targeted anticancer drugs in patients with refractory skin, brain, blood and other cancers.

The survival benefits from this first wave of trials were mostly modest, though — in part, says Thorburn, because of the spaghetti-at-the-wall nature of this early work. Looking forward, he says, "we should be a little bit more rational and mechanism-based than randomly throwing crap in combination."

Oncologists nevertheless remained excited, particularly around the opportunity for the use of autophagy inhibitors in *RAS*-driven cancers, which are thought to account for more than 30% of cancers. This was prompted in part by findings from Kimmelman and separately by Eileen White, at the Rutgers Cancer Institute of New Jersey, who [each showed](#) that autophagy suppression could limit the growth of these cancers.

Yet, industry scientists struggled to replicate the *RAS*-related findings. Despite working with several different tumour lines in cell culture and in xenograft mouse models, "we were never able to recapitulate those initial findings," says Pfizer's Christina Eng, a senior principal scientist in the company's oncology research unit. In her

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team's hands, knocking out autophagy did not impact the proliferation of *KRAS*-driven cell lines, nor did it sensitize cells to several anticancer agents.

Independently, researchers at Novartis had arrived at much the same conclusion in dozens of cell lines of their own.

Both companies presented their findings at a Keystone meeting on autophagy in 2014, and then jointly reported their negative results the following year in *Proceedings of the National Academy of Sciences (PNAS)*. Pfizer soon abandoned plans to search for small-molecule inhibitors of autophagy. Novartis, which had made a series of potent and selective inhibitors of VPS34, an enzyme that is involved in autophagosome formation, wound down its programme as well. (Other industry programmes suffered similar fates, including Sanofi's VPS34 inhibitor called SAR405, and Millennium Pharmaceuticals' small-molecule modulators of ATG7, an enzyme needed for autophagosome maturation).

The head of those drug discovery efforts at Novartis, Leon Murphy, has since moved on to other things. Last year, he helped launch Casma Therapeutics, a company focused on boosting autophagy activity for non-oncology indications. To this day, Murphy continues to doubt the future of anticancer autophagy inhibitors, at least as originally envisioned as modulators of the process inside cancer cells themselves. "I think it's going to be really hard to develop an autophagy inhibitor that's both safe and has a robust effect against tumours," he says.

New data — published earlier this year in the two aforementioned papers in *Nature Medicine* and in a third in *PNAS* — seem to refute that notion. When the three independent groups combined autophagy blockers with inhibitors of the MAPK cascade, which were not previously evaluated by the Novartis or Pfizer scientists, they were able to blunt the growth of various tumour lines.

"The autophagy dependence for proliferation and tumorigenesis only becomes apparent when a certain perturbation is applied," says McMahon, who led one of these studies.

### Backup system

The findings from Der's team, reported in the companion article, explain this phenomenon. Tumours compensate for MAPK inhibition — and the suppressed glycolysis and mitochondrial dysfunction this brings — by boosting autophagic activity. Autophagy can consequently be seen as a backup energy source for cancers, explains Der. And the addition of a drug like hydroxychloroquine puts the kibosh on this adaptive response, resulting in tumour cell death.

That was on Conan Kinsey's mind when the physician–scientist began treating his pancreatic cancer patient with hydroxychloroquine plus Novartis's MEK inhibitor trametinib on a compassionate-use basis at the Huntsman Cancer Institute. The man's tumour burden shrank by about 50%, and he lived another 7 months post-treatment, Kinsey and the rest of McMahon's group reported in *Nature Medicine*. "That doesn't sound like very much," Kinsey says. But considering the man likely had only about a month to live, "it was quite a significant increase." Plus, Kinsey notes, "he did have a very good quality of life," managing to hike again with his wife on his favourite trails near the Grand Canyon.

Notably, a growing body of evidence also suggests that autophagy inhibition restricts cancer growth through its effects on the tumour microenvironment and on host immunity. Last year, for example, Douglas Green and his colleagues at St. Jude Children's Research Hospital showed in *Cell* that myeloid cells found in the tumour microenvironment rely on autophagy to shut down the activity of anticancer T cells in the vicinity.

Thus, some autophagy experts now challenge the relevance of the negative findings from Novartis and Pfizer that relied on cell lines and immunodeficient mouse models of disease. "Studying cells growing in plastic is not the appropriate setting," says White, who is also a co-founder of Vescor. She stresses the need to examine autophagy in genetically engineered mouse models with intact immune systems that can impact metabolic health. "The core function of autophagy is related to metabolism," she says, "and one must therefore study it in physiologically relevant settings."

Eng stands by the finding that autophagic activity within malignant cells does not itself promote tumorigenesis in a cell-intrinsic manner. But she notes that Pfizer has unpublished data supporting a role for autophagy in host responses to tumour growth. "What's happening in the dish might

be very different from what's happening in the body," she says now.

But Novartis and Pfizer were not making such subtle arguments back in 2014, when a press release that accompanied their *PNAS* paper claimed that the two drug firms had united to "upend oncology dogma". In cancer circles at least, this study "kind of put the dampeners on autophagy inhibition", McMahon says. It didn't help that the publication came hot on the heels of another high-profile *Nature* paper that, according to McMahon, "completely misled the field" by purporting to show in mouse models of pancreatic cancer that autophagy inhibition could fuel tumour growth — a conclusion later challenged for focusing on tumour initiation, rather than maintenance, in a model that doesn't accurately reflect the human condition.

The pendulum is swinging, though. "We see a totally different interest than we had a couple years ago," says Jessica Martinsson, head of operations at Sprint Bioscience, which is developing VPS34 inhibitors. Vescor and Deciphera Pharmaceuticals have not yet disclosed their targets or frontrunner compounds.

### Renewed interest

Although Sprint initially explored the idea of positioning its autophagy blocker in the clinic in combination with targeted drug therapies like the kinase inhibitor sunitinib, the company has begun presenting the asset as something that can boost the efficacy of immunotherapies as well. "We now have [unpublished] data showing that autophagy is a key immune escape mechanism," Martinsson says. The company's VPS34 inhibitor seems to promote inflammation in the tumour microenvironment to enhance immune surveillance. "We're basically making cold tumours hot," she says, noting that the drug — which she hopes will enter first-in-human trials in 2020 — pairs well with checkpoint inhibitors in preclinical testing.

In addition to VPS34's critical role in autophagy, the enzyme also has functions in vesicular and endocytic trafficking, prompting many researchers to voice concerns about its safety as a drug target. Other scientists are therefore focused on

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finding inhibitors of a different enzyme: ULK1, a protein kinase that helps regulate autophagosome biogenesis. “It’s the most attractive target from a pharmacological point of view,” says Reuben Shaw, a cancer metabolism researcher at the Salk Institute for Biological Studies who has [discovered](#) an ULK1 inhibitor with colleagues.

“The autophagy pathway is highly druggable, and there are multiple druggable entry points to the pathway,” says the US National Cancer Institute’s Ji Luo, whose [recent PNAS](#) paper about co-targeting RAF and autophagy in RAS-mutant cancer cell lines is similarly renewing interest in this space.

Yet, counterintuitively, it may sometimes be preferable to activate rather than inhibit autophagy in certain oncology settings. Researchers have shown, for example, that autophagy in cancer cells can [increase the immunogenicity of tumours](#), enhancing the therapeutic potential of immunogenic anticancer drugs. And others [recently reported](#) that an autophagy-inducing drug can blunt the growth of HER2-positive breast tumours just as well as HER2-targeted therapy.

“Both a strong inhibition or a strong induction [of autophagy] can lead to cancer cell death,” says Héctor Pérez-Montoyo, director of biological research at Ability Pharma, to explain the paradox. “These are opposite strategies, but in the end they achieve the same goal.” Ability is developing



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a phase II drug candidate that’s thought to indirectly promote autophagy-mediated cancer cell death.

#### “There’s promise there”

For now, most clinical researchers continue to study various forms of chloroquine. Steve Jean, an autophagy researcher at the University of Sherbrooke, cautions that the drug is “so unspecific”. But at least a half dozen early-stage trials — informed by the newly discovered science surrounding the role of autophagy and MAPK inhibition — are nevertheless ongoing or planned to combine MEK inhibitors and hydroxychloroquine for patients with MAPK-mutant tumours of the brain, pancreas and skin.

For example, University of Pennsylvania oncologist Ravi Amaravadi is running a single-arm skin cancer study evaluating trametinib plus Novartis’s BRAF inhibitor dabrafenib with the addition of hydroxychloroquine for patients with BRAF-mutant melanoma. So far, nineteen of twenty-one patients have responded to the

front-line therapy with prolonged periods of disease regression compared with what would be expected.

“It’s not a home run, but it certainly shows that there’s promise there for blocking autophagy,” Amaravadi says of his unpublished results, some of which he [presented](#) at the 2018 annual meeting of the American Association for Cancer Research in Chicago. He is now planning a follow-up randomized phase II study to determine whether the triple combination is statistically superior to the standard combination.

Amaravadi is also working with Pinpoint Therapeutics, the company that he co-founded last year, to discover derivatives of chloroquine to advance the field further still. Earlier this year, he reported in [Cancer Discovery](#) with colleagues that one of these compounds, called DC661, blocks autophagy more potently and offers increased localization to the lysosome compared with hydroxychloroquine. “That is going to be very important for efficacy and to decrease off-target toxicity,” he says.

After years of being one of the only investigators evaluating autophagy inhibition in cancer patients, Amaravadi says the field is “resurgent”. And for those looking beyond hydroxychloroquine, companies no longer have to prove the pathway, he says. “That paves the way for focusing on the chemistry and the drug development. It’s an exciting time.”