Moving the needle on monitoring skin cancer

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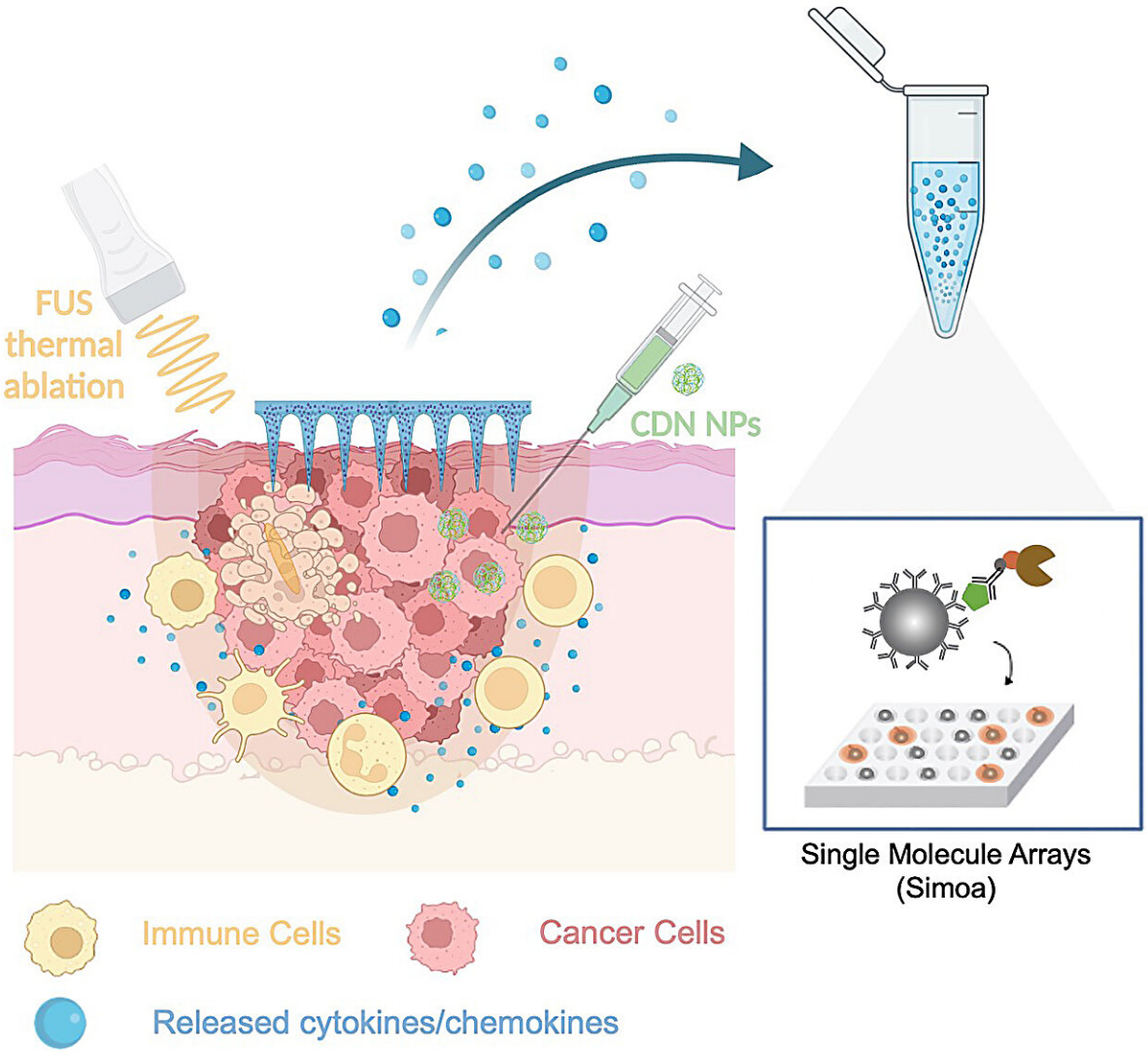

The team engineered a strategy for monitoring the immunological responses to a newly devised, locally applied immunotherapy against melanoma. The therapy acts locally on tumor lesions by combining non-invasive focused ultrasound

(FUS) with the delivery of a previously developed nanoparticle-bound activator of an inflammation-inducing protein known as stimulator of interferon genes (STING). To monitor biomarkers informing about the responsiveness of immune cells to the therapy, first, interstitial skin fluid is sampled with microneedles from the lesions, and then biomarkers contained in it quantified with ultra-sensitive Simoa assays. Credit: Wyss Institute at Harvard University

Patients with melanoma, the most concerning form of skin cancer in which pigment-producing cells start to grow out of control, can benefit from existing immunotherapies, but by far not all of them do. More than 50% of patients do not respond to current immunotherapy drugs and among those that initially respond, many become resistant to the drugs' effects.

Thus, besides developing more effective immunotherapies, doctors need to be able to determine which patients respond well at the start of treatments and, which ones keep or stop responding in order to make the best treatment decisions.

Because cancerous skin lesions of melanoma patients are easily accessible, an effective way to eradicate them could be to apply immunotherapies locally, instead of systemically infusing them into the blood circulation. Also, monitoring the immune system's reaction to the therapy right at the tumor site, by sensitively and continuously measuring different biomarkers that signal the intended immune cell activation and a desirable inflammatory response, could enable better and more personalized patient care.

Now, a research team at the Wyss Institute at Harvard University, MIT, and Brigham and Women's Hospital in Boston has developed a new approach that integrates a minimally invasive, painless microneedle

platform capable of absorbing the cell-surrounding, biomarker-containing fluid from deeper layers of the skin with an ultra-sensitive, single-molecule detection method (Simoa) that detects often rare, yet relevant biomarkers with higher sensitivity than conventional methods.

The researchers provided proof-of-concept for their approach in a mouse melanoma model in which they treated cancerous lesions with a novel therapy. The therapy acts locally on tumor lesions in that it combines non-invasive focused ultrasound (FUS), which generates heat at the tumor site to instantly kill tumor cells, with the delivery of a previously developed nanoparticle-bound activator of an inflammation-inducing protein known as stimulator of interferon genes (STING). The findings are reported in *Advanced Functional Materials*.

"Rapid readout of the responses to melanoma therapy using microneedles may enable effective drug screening and patient stratification to maximize therapeutic benefits," said Wyss Associate Faculty member Natalie Artzi, Ph.D., who led the study. Artzi is also an Associate Professor of Medicine at Harvard Medical School (HMS) and a Principal Research Scientist at the Institute for Medical Engineering and Science at MIT.

Immunotherapy made local

Artzi and her group first developed a locally applied immunotherapy for melanoma that leveraged some of their previously pioneered methods and expertise. In a recent publication, which built on the known fact that activation of the inflammation-inducing STING protein contributes to tumor cell killing, they reported a significantly more effective way to activate the protein in immune cells. Natural activators (agonists) of STING are not sufficiently stable in the body and need to be given in high doses that also can produce side effects.

The group's solution was to deliver multiple copies of a synthetic STING agonist, called a synthetic cyclic dinucleotide (CDN), via nanoparticles (NP) that easily traverse the plasma membrane and, with the help of an engineered enzymatic reaction, release their cargo inside cells. This CDN-NP therapeutic can be directly injected in or close to cancerous skin lesions to additionally increase the drug concentration in tumors.

"Here, we chose to boost the processing of antigens from dying tumor cells following focused ultrasound together with STING-agonist delivery in the tumor microenvironment to coordinate a broader immune response," said first-author Daniel Dahis, Ph.D. Dahis, is a Research Scientist at the BioDevek startup, and performed his graduate work on the study with Artzi while being co-supervised by co-author Haim Azhari, Ph.D., a Professor at Technion Israel Institute of Technology and an expert in medical imaging.

The team first showed that the focused ultrasound (FUS) treatment, which transiently and in small areas increases the temperature up to 60°C, potentiated the effects of CDN-NP treatment in co-cultures of immune and cancer cells in a dish, and in melanoma tumors in mice, that they treated with the combination.

Importantly, 60 days following the treatment, all animals that received only FUS therapy had died, while 75% of animals receiving only the CDN-NP therapy were still alive—the combination treatment allowed 100% of animals in their group to survive.

Tapping deep into skin biomarkers

To investigate if the survival benefits of the combination therapy are mirrored in the levels of biomarkers at the tumor site, which in the future could translate to the monitoring of responses in human patients treated with immunotherapy, Artzi's group had previously developed a

novel type of minimally invasive microneedles that are made of hyaluronic acid and, in principle, can be used to simultaneously deliver drugs and detect biomarkers.

These devices reach into the lower (dermal) layers of the skin where the polymer encounters the fluid surrounding skin cells, the so-called interstitial skin fluid (ISF), and, like a sponge, takes up tiny amounts of it.

"Merely a few microliters of ISF obtained with microneedles provide a wealth of biomarker information as normal skin cells, local immune cells, and cancer cells constantly secrete diverse signaling molecules and metabolites," said Dahis. "After the microneedles are retrieved, their tips can be simply dissolved to release the captured molecules into a test tube for us to start the biomarker analysis."

However, while the researchers saw FUS clearly added to the immune response evoked by CDN-NP in tumors, many biomarkers of interest, including genes that are switched on by the activated STING protein, were barely detectable or not detectable at all using conventional methods. To overcome this bottleneck, Artzi's team joined forces with that of Wyss Core Faculty member David Walt, Ph.D., who had previously developed the Simoa technology, which has ultrasensitive biomarker detection abilities.

Simoa essentially allows researchers to capture a biomarker protein of interest with a specific antibody molecule that is linked to a magnetic bead much larger in size than the antibody itself. The bound protein is then "sandwiched" with the help of a second detector antibody in individual wells of a multi-well plate that each can only fit one magnetic bead.

The detector antibody is labeled with an enzyme that generates a

fluorescent signal in the well. Thus, Simoa enables the digital counting of individual biomarker proteins at a single-molecule level, which far surpasses the sensitivity of commonly used detection assays.

Co-author Tal Gilboa, Ph.D. on Walt's team developed four Simoa assays to detect molecules whose expression is activated by STING: interferon-b (IFN-b), MCP-1 and KC, which both attract immune cells to tumors, as well as the general inflammation marker interleukin-6 (IL-6).

Indeed, this enabled the researchers to detect these biomarkers in microneedle-extracted ISF samples with 100 to 1000-fold increased sensitivities compared to commonly used assays, and the measurements mirrored parallel Simoa measurements of the same biomarkers in blood samples. "It was striking to see that animals with the most pronounced pro-inflammatory response were also those that survived the longest," said Dahis.

"The Artzi lab's remarkable microneedle technology containing engineered nanostructures, in principle, enables both, drug delivery and microsampling—a completely new concept for a theranostic, which provides an ideal, non-invasive and comprehensive solution to melanoma treatment," said Walt, who also is the Hansjörg Wyss Professor of Biologically Inspired Engineering at Harvard Medical School (HMS), Professor of Pathology at Brigham and Women's Hospital, and a Howard Hughes Medical Institute (HHMI) Professor.

Walt, together with co-author Rushdy Ahmad, Ph.D., leads the Wyss Diagnostic Accelerator, which aims to shorten the time frame for urgently needed diagnostics in multiple disease areas, including cancer.

"This work is a beautiful example of interdisciplinary collaboration and convergence of cutting-edge technologies that we strive for at the Wyss

Institute. This new advance has the potential to raise the quality of cancer immunotherapy to the next level by directly assessing therapeutic efficacy in individual patients," said Wyss Founding Director Donald Ingber, M.D., Ph.D., who is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital.

More information: Daniel Dahis et al, Monitoring Melanoma Responses to STING Agonism and Focused Ultrasound Thermal Ablation Using Microneedles and Ultrasensitive Single Molecule Arrays, *Advanced Functional Materials* (2023). DOI: 10.1002/adfm.202301659

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