20 years of *Nature Biotechnology* biomedical research

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Authors of some of the most highly cited *Nature Biotechnology* biomedical papers from the past 20 years discuss their work and challenges for their fields.

On our 20th anniversary, *Nature Biotechnology* looks back at some of our greatest hits. In this Feature, we focus on a limited selection of papers with applications in biomedicine; readers are referred to other Features in this issue on research tools (p. 256) and applications outside of biomedicine (p. 267).

This being our third such effort—we profiled similar collections in our tenth and fifteenth anniversary issues—we chose to highlight only papers not previously profiled. A short summary of other papers with biomedical applications from those previous Features can be found in **Box 1**; the complete articles can be found here[1,2]. We acknowledge that the papers below provide just a limited snapshot of all the research appearing in our pages, and we thank the entire research community for its contributions to the journal over all these years.

Unlocking the CAR

Chimeric antigen receptor (CAR) technology is taking the world of cancer immunotherapy by storm. In certain adults and children suffering from blood cancers, CAR therapy—in which T cells are engineered with a tumor antigen–specific single-chain antibody domain hooked up to a T-cell receptor (TCR) signaling domain and various co-stimulatory molecules—can result in remission, even among those who haven't responded to conventional therapies or have relapsed following a bone marrow transplant. Yet the application of CAR-T cells to treat cancer is limited by the paucity of cell surface tumor antigen targets that are not also commonly found on normal cells. "There are not that many cell surface antigens known to this day that you could safely unleash these powerful T cells onto," says Michel Sadelain, director of the Center for Cell Engineering at the Memorial Sloan Kettering Cancer Center in New York.

In their 2002 paper, Sadelain and co-workers reported a second-generation of CARs—one that integrated co-stimulatory and activation signaling molecules in a single T cell. This work was the first to show that a synthetic receptor can redirect the function of a T cell and enable it to expand upon antigen exposure, according to Sadelain[3]. "This early study establishing principles of T-cell engineering was central to the CAR field," he says.

Michel Sadelain has pioneered and refined the application of CAR-T cell therapy in cancer.

More than a decade later, Sadelain's laboratory further improved on nature and engineered a T cell that requires recognition of two different antigens for activation[4]. "We are now going even further away from nature because, physiologically, each T cell recognizes one and only one antigen." The approach is designed to address the lack of known tumor-specific antigens: by making T-cell activation dependent on recognition of two antigens, neither has to be absolutely tumor-specific, as long as normal tissues do not express both antigens. The novel technique promises to widen the range of tumor types that could be treated with CAR-T cells. "This increases the options of antigen targets quite a bit," says Sadelain.

The two-antigen T cell expresses a CAR that alone results in only partial immune cell activation and a second chimeric co-stimulatory receptor that recognizes a different antigen, such that the signal is spread between the two receptors. "It's only when the two receptors are engaged on a tumor cell that has both antigens that the T cell becomes fully active," says Sadelain.

Although this new approach to engineering T-cells has yet to be tested in the clinic, Sadelain's group is focusing on developing these for cancers for which unique antigens are missing. The laboratory checks candidate tumor antigens for expression on normal tissues to make sure the pair of chosen antigens is not expressed together on any one tissue. Surprisingly, there appears to be a lack of information on expression of cell surface markers on wild-type tissue, rather than on tumors. Sadelain says, "What we're finding, though, is that there is often more information on what is expressed on tumors than what is known about normal tissues."

Toward liquid biopsies

Tumors shed into the bloodstream information in the form of cells, lipids, proteins and DNA molecules and particle packets known as exosomes. In recent years, the notion of exploiting these lipid-encased messengers to noninvasively detect and monitor malignancies has moved from big idea to clinical possibility. A critical step is having the right lure to fish out circulating exosome material that has originated from cancer cells rather than normal cells. Two years ago, Hakho Lee and his colleagues at Massachusetts General Hospital (Boston) described a novel assay capable of both detecting and profiling cancer exosomes, providing a new tool in the search for disease biomarkers and a foundation for the development of diagnostics.

Anna Azvolinsky, Charles Schmidt, Emily Waltz and Sarah Webb are freelance writers.

Previously, Lee and his colleagues had used nuclear magnetic resonance to detect exosomes, but realized that this technique was impractical for high-throughput screening. So they looked to another analytical platform, surface plasmon resonance, and designed a chip, called a nanoplasmonic exosome (nPLEX) sensor, which is an array of evenly spaced nanoholes on a metal film. They attached ligands specific for tumor-surface proteins to the sensor, the depth of which is perfectly suited to detect objects the size of these vesicles (50–100 nm in diameter), says Lee. The refractive properties of light passing through the chip are altered where exosomes are bound, providing a sensitive system for detecting the presence of exosomes and the abundance of proteins within each one.

Using ovarian cancer markers EpCAM and CD24 on the particle surface, they could distinguish cancer exosomes from those from normal cells. The assay is 100 times more sensitive than an enzyme-linked immunosorbent assay (ELISA) and able to detect as few as 3,000 exosomes[5]. Although it is only capable of capturing exosomes for which validated cancer markers have been identified, their multiplex system allows the measurement of several different markers at the same time.

Unlike tests for circulating tumor cells and cell-free DNA, exosome sensing can detect proteins, Lee emphasizes. That means that the technique can be used on its own or in conjunction with another noninvasive monitoring strategy to provide more accurate clinical results. "Initially, this paper attracted considerable attention because it established a sensitive approach to isolate and analyze exosomes, which requires rather small amounts of blood," says Klaus Pantel, a tumor biologist at the University Medical Center Hamburg-Eppendorf in Germany. Such a diagnostic tool could serve as a 'liquid biopsy' for cancer patients, he adds.

Since their publication, Lee's team has collaborated with several clinical teams to look at exosome markers in brain, pancreatic and lung cancers. Commercial companies in Boston are interested in the development of a diagnostic, Lee says. In the meantime, he and his colleagues are working on ways to make exosome profiling more useful—techniques that would avoid the need for membrane filtering, and more sensitive methods that would allow researchers to profile the contents of single exosomes.

Hakho Lee's sensor is 100-fold more sensitive than ELISA in detecting cancer cell–derived exosomes in blood.

Pinpointing somatic variants in cancer

It isn't often that an algorithm attracts more attention than the scientific discoveries it helps to generate. But that's what happened when a team led by Gad Getz, from the Broad Institute (Cambridge, MA, USA) introduced an algorithm called MuTect in 2013 (ref. 6). Designed to detect, or 'call', somatic point mutations in cancer, MuTect is a freely available tool for academics that has since become indispensable for scientists working to understand how tumors differ from normal tissues.

Getz and his group began developing MuTect in 2008, prompted by shortcomings in the available tools for analyzing next-generation sequencing data in cancer. He says that somatic mutation callers at the time were based on tools used for finding germline mutations in DNA. But scientists were becoming increasingly aware that these tools were not sensitive enough to detect somatic mutations, which can develop at any time during a person's life. Getz was especially interested in detecting mutations with low allelic fractions, which exist in subclones of cancer cells in a given tumor. Malignant tumors contain a mixture of normal cells and various genetic subclones of cancer cells, each with potentially varying aggressiveness and resistance to treatment. Some mutations in those subclones are either drivers that accelerate tumor growth or passengers that come along for the ride. "But before we could distinguish drivers from passengers, we first had to identify all the mutations," Getz says.

To do that, Getz needed a robust analytical tool that wasn't prone to false-positive results—no easy task because somatic mutations are rare, with a few thousand mutations in the 3-billion-base human genome. Finding low allele fractions is made harder because their signal is weak, diluted by normal cells or tumor cells lacking the mutations. Up to 95% of the cells in a pancreas tumor, Getz points out, are normal.

So Getz teamed with Kristian Cibulskis, a software engineer at the Broad, on a multiyear endeavor to create what became MuTect. Their paper described a novel approach. MuTect analyzes genomic data from both tumor and normal tissue samples, and, after removing low-quality reads, confirms that a somatic mutation is not just a random sequencing error. It does that by running the data through a statistical test that determines whether each position is more likely to be a somatic mutation than a sequencing error, and then through a set of filters designed to further remove false positives. Finally, it screens the output against a panel of normal tissues obtained from a variety of people. According to Getz, that latter step helps to characterize noise in the data, and it allows researchers to more easily remove germ-line mutations and other sequencing artifacts.

The paper also describes a benchmarking approach for comparing MuTect's accuracy with that of its competitors. Results showed MuTect was superior in terms of both its sensitivity, or ability to pick up hard-to-detect mutations, and specificity, or avoiding false positives, that is, calling true mutations accurately.

Maria Nemchuk, BIC

Gad Getz tackled the problem of identifying somatic mutations in heterogeneous tumor cell populations.

Getz says the main challenge in developing MuTect was understanding that, depending on the "purity" of the tumor, or the degree to which it is contaminated with normal cells, and subclonality of the mutations, a somatic mutation could be present in any allelic fraction. "That was also a conceptual advance that wasn't fully appreciated in somatic mutation callers before this paper was published," he says.

Brad Chapman, a biostatistician at the Harvard T.H. Chan School of Public Health, in Boston, says MuTect remains well-regarded worldwide, though it's limited to single-nucleotide polymorphisms. The algorithm cannot call small insertions or deletions, he says. According to Getz, that and other issues are now being addressed in MuTect's second version, which is under development.

Catherine Wu, an associate professor at Harvard Medical School, says MuTect remains the best somatic mutation caller available today. She uses it in genomic studies of chronic lymphocytic leukemia, and claims that it has led directly to our modern day understanding of key genetic events affecting the disease, its progression and its evolution over time. "MuTect has opened the door to the highly sensitive detection of somatic mutations from massively parallel sequencing data from cancer samples," she says. "And in doing so, it's provided the path for the

discovery of critical insights into the genetic events and networks that drive cancer."

A recipe for pancreatic beta-like cells

It is 16 years since researchers at the University of Alberta in Canada first published their work showing that islet cells isolated from donor pancreases and transplanted into the liver allowed patients with severe type 1 diabetes to forego insulin therapy[7]. But this procedure, known as the Edmonton protocol, requires scarce donor organs, limiting the number of patients who can be treated to just a few hundred in North America each year. Thus, the Holy Grail in diabetes cell therapy has been to develop an unlimited source of insulin-producing pancreatic beta cells by controlled differentiation of pluripotent stem cells. A 2014 paper[8] from a team led by University of British Columbia professor Timothy Kieffer and Alireza Rezania at BetaLogics (a division of Janssen, New Brunswick, NJ, USA) reported important progress toward this goal.

The study built on previous work from the regenerative medicine company ViaCyte (San Diego) showing that pancreatic precursors produced *in vitro* (in a four -step differentiation protocol) from human embryonic stem cells differentiate toward beta cells and reverse diabetes in mouse models. This type of cell is now undergoing phase 1/2 testing. But transplanting a precursor that can take three or four months to mature might not be the best therapeutic strategy, according to Kieffer. "Optimally, it would be preferable to make a bona fide beta cell in the lab." In addition, precursors could differentiate into other pancreatic cell types that secrete counteracting hormones, such as glucagon, which boosts glucose concentrations in the bloodstream.

Kieffer, Rezania and their colleagues spent nearly a decade tweaking pancreatic differentiation protocols for human pluripotent cells and comparing the resulting cells with stage-four precursors and beta cells from the pancreas[9]. Using high-throughput screening, the BetaLogics researchers identified several critical differentiation factors, such as the thyroid hormone tri-iodo thyronine. Eventually, using a seven-stage protocol, the team produced "maturing beta cells" that express MAFA, a hallmark of mature pancreatic cells, and are nearly equivalent to beta cells from the pancreas[8]. When transplanted into mice, the cells reverse diabetes four times faster than stage-four precursors, with just one-quarter of the cellular dose, Kieffer says.

Timothy Kieffer's work on differentiation of human pluripotent stem cells may someday provide beta cells for reversing diabetes.

According to Neil Hanley at the University of Manchester (UK), the study "took the whole field forward by generating monohormonal cells secreting insulin and attracted a lot of comment at meetings for its thorough, detailed approach." But as Kieffer acknowledges, there is more work to be done. "Our cells are a little sluggish" in response to glucose, he says, and show differences in calcium signaling […] We've fallen short of the end goal, but we're on the five-yard line. We're getting close." Laboratory-derived beta cells could help with the treatment of type II diabetes as well, particularly by allowing researchers to study patient-derived cells and understand the underlying processes that limit their response to insulin.

Thwarting the foreign body response

Interest is increasing in implantable devices for use in biomedical research and the clinic, but these devices must overcome the human body's defenses—the foreign-body response—that coat them in proteins that attract macrophages and other immune cells. Eventually collagen encapsulates these structures, often rendering medical devices or other implants useless. Materials, such as polyethylene glycol (PEG) and sulfobetaines, can slow the biofouling process, but ultimately they can't prevent it.

Shaoyi Jiang and his colleagues at the University of Washington (Seattle) spent nearly ten years trying to solve this problem before they developed zwitterionic hydrogels from pure polymers of carboxybetaines[10]. Such structures exist in nature as protein stabilizers, but in the hydrogels they form regular structures that evenly alternate positive and negative charge across the hydrogel surface. The surface then strongly attracts water molecules, holding them tighter than hydrogen bonds do. This wettened surface prevents protein and cell binding, stopping the cascade that leads to collagen capsule formation, Jiang says. He reported that poly-carboxybetaine methacrylate (PCBMA) hydrogels implanted subcutaneously in mice could be in place for three months without capsules being formed. In addition, the PCBMA hydrogel attracted greater blood vessel formation than commonly used hydrogels.

Over the last 25 years, several companies have tried using zwitterionic materials against the foreign body response. The University of Utah's David Grainger cites examples such as coatings on the surfaces of contact lenses and catheters. Those materials "didn't make that much of a hit," he says. But this new material has "some remarkable features," he says, such as low coagulation tendencies in flowing blood *in vitro*, adding that it looks promising for short-term *in vivo* use. However, Jiang's study, like many in this field, tests wound healing around an implant using a rodent model, an imperfect one for mimicking the physiology and immune cell response in human wound healing. Grainger says the field awaits data from the gold standard large-animal model, the pig.

Jiang agrees that the hydrogels should be tested in larger animal models. Since publication of his study, he and his colleagues have also tested carboxybetaines as an alternative to PEG-tagging for some types of protein-based drugs. In addition, they've shown that the hydrogels can serve as a protective matrix for mesenchymal stem cells, keeping them alive and preventing their differentiation. Jiang and his team are already testing these new hydrogels for hematological and immunological applications in the clinic.

Shaoyi Jiang has tried to sidestep the foreign body response with zwitterionic hydrogels.

ZFNs move closer to clinic

In 2008, zinc finger nucleases (ZFNs) had become a promising research tool for genome editing but proof-of-concept studies in a therapeutic context remained elusive. That's when the University of Pennsylvania's (Philadelphia) Carl June and his team described the use of ZFNs to edit the genome in human T-cells to confer HIV resistance[11].

The gene they targeted encodes a cell surface receptor, chemokine CC-motif receptor 5 (CCR5), which is HIV's gateway into T cells. People with a delta32 CCR5 mutation express a truncated version of the protein, shutting the

Box 1 Previously, in our hall of fame

Previous incarnations of this Feature have highlighted other advances in biomedicine published in our pages. Below, we provide brief summaries of these studies and their advance. For further details, readers are referred to the originals[1,2].

1997. Didier Trono created the first lentiviral vector from multi-attenuated HIV for gene delivery[18].

2000. Benjamin Reubinoff and Martin Pera showed that human embryonic stem cells can be differentiated *in vitro*[19].

2002. John Rossi repressed the expression of HIV by four orders of magnitude using RNAi[20].

2004. Shuming Nie showed how quantum dots could be used to image cancer in live animals[21].

2005. David Lockhart and colleagues at Ambit Biotech (San Diego) published an interaction map of small molecules and protein kinases[22].

2006. Weida Tong and Leming Shi led an FDA consortium reporting the results of a quality control exercise for microarrays involving 137 participants from 51organizations[23].

2006. Ed Baetge's group at Novocell (San Deigo) converted human embryonic stem cells into hormone-expressing endocrine cells[24].

2007. John Frangioni and Moungi Bawendi determined how small a quantum dot had to be to be cleared out of the body by the kidney[25].

2007. Napoleon Ferrara's team at Genentech (S. San Francisco, CA, USA) showed that suppression of myeloid cells reduces resistance in Avastin-refractory tumors[26].

2008. Tomer Shlomi described a program for modeling metabolism in human tissues[27].

2008. Shinya Yamanaka's group made Myc-free induced pluripotent stem cells from both mouse and human cells[28].

door on HIV. The discovery of the gene's function in the mid-1990s gave rise to a whole class of anti-retroviral drugs. But attempts at mimicking the mutation's effects with various gene therapy tricks fell short.

When ZFNs came along, researchers had a more precise means for knocking out the CCR5 gene. But they needed to get it to work in human immune cells, not just in Petri dishes and cell lines. With help from Sangamo Biosciences (Richmond, CA, USA), which was developing ZFN technology, Elena Perez, a postdoc in June's laboratory, used an engineered ZFN to disrupt the CCR5 gene in human T-cells, and introduced the cells into mice. The team found that when challenged with HIV, the animals treated with the modified T cells had lower viral loads and higher $CD4^+$ cell counts than control animals with unmodified T cells.

In addition to demonstrating protection from HIV, these experiments showed that ZFNs had no signs of adverse effects in mice at efficacious doses. "It was the first demonstration of genome editing in primary human cells at levels that are clinically relevant," says Paula Cannon, of the University of Southern California (Los Angeles), who has a program with Sangamo in HIV as well. "It almost sounds trivial now because so many people can do this. But they were the group that took these new and exciting genome editing tools out of the toolbox and put them firmly in the clinical realm," she says.

The paper served as a blueprint for preclinical safety studies involving gene editing, says Cannon. "It shows you in a clear way the types of safety studies and analyses you might do" to prepare a preclinical package for the US Food and Drug Administration (FDA), she says. Researchers are developing ZFN gene editing for other therapeutic areas, including cancer, hemophilia and sickle cell disease. Cannon's group and others are targeting the CCR5 gene in hematopoietic stem cells[12].

Candace di Carlo

Carl June's work galvanized therapeutic development of ZFNs.

June's team, for their part, went on to conduct the first-in-human clinical trials of ZFN genome editing. In 2014, June and his colleagues reported the results from 12 people, whose T-cells were modified *ex vivo* and transfused into the participants[13]. After the treatment, the modified immune cells persisted in the body, and in one individual, there were tantalizing hints of HIV control.

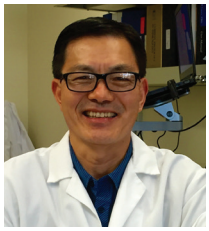
Like any seminal paper, a lot was riding on June's. If he had failed, things would have been harder for others hoping to use ZFNs in the clinic, he says. "If we would have had a disaster happen, it would have closed down the field," June says. "Fortunately that didn't happen."

Optimally arming antibodies

Antibody-drug conjugates (ADCs) burst onto the commercial scene in 2011 with the FDA's approval of Adcetris (brentuximab vedotin). But many kinks and wrinkles in the technology had to be worked out over the preceding decades to achieve this milestone. "ADCs are novel and exciting and much progress has been made in the past ten years," says Ben-Quan Shen, a senior scientist at Genentech (South San Francisco, CA, USA). After Adcentris, approval of other ADCs followed, including Genentech's Kadcycla (ado-trastuzumab emtansine) for metastatic HER2-positive breast cancer.

One area that has been improved since ADCs were first described in the 1980s has been the linkage between the drug and antibody. Early ADCs had unstable linkages, which resulted in the drug being released into the bloodstream, causing vascular leakage syndrome and limiting their clinical use. Attaching either a lysine or a cysteine on the antibody to a linker stabilized the molecule. However, the conjugation reaction generates a mixture of ADC molecules as a typical antibody has multiple sites where the linker can be attached. "The drug-to-antibody ratio ranges from about zero to eight, so you can get about a million different variants, [in a single reaction]," says Shen. Such a heterogeneous mix of conjugated molecules can result in multiple species with varying activities and pharmacological properties.

Shen's team at Genentech came up with a way to engineer a reactive cysteine residue that, unlike endogenous cysteines that form disulfide bonds with other cysteine residues, remains unpaired and does not form interchain disulfide bonds. The reactive amino acid can then be readily attached to a linker and several types of cytotoxic drugs, enabling researchers to choose a single conjugation site[14]. "This allowed us to conjugate an exact number of drug molecules to each antibody at a defined site, which has a lot of advantages. We showed that this improved the stability of the ADC, which in turn improved its safety," says Shen. The resulting ADCs are more homogeneous, with predictable properties, simplifying the analytics of the product. "When we have [a heterogeneous mix of conjugates] with different pharmacological properties, we need multiple assays to test them but with the site-specific conjugate, we know exactly what we will be dosing. There is less variability."



Ben-Quan Shen's group at Genentech found a way to reduce the complexity of antibody-drug conjugates.

Since then, Shen's group has taken the technology further by comparing the activity of antibodies with the linker-drug combination attached at three different sites[15]. With Kadcyla, the linker attachment site on the light chain rather than either of the two heavy chain sites resulted in the greatest activity, improving stability and efficacy in a mouse tumor model. "Selecting the right site for linker-drug conjugation is very important for developing an optimal ADC molecule. It is just like buying a house. It's all about the importance of location, location, location," Shen says.

"The technology described in this paper is both simple and robust," says Peter Senter, vice president of chemistry at Seattle Genetics, who was involved in the development of Adcetris. "[Researchers] are really interested in site specificity and this technology. This is one of several site-specific conjugation tools that will have a big impact on ADCs moving into clinical development."

Exosomes as nanomedicines

The brain is unique among organs in the body in that it has its own security system: a network of blood vessels that allows nutrients in and keeps other substances such as toxins and drugs out—the so-called blood-brain barrier (BBB). In 2011, researchers from the University of Oxford reported that they had successfully breached the BBB with a systemically delivered exosome 'nanoparticle'[16]. The nanoparticles were capable of delivering a small-interfering RNA (siRNA) capable of suppressing an Alzheimer's disease target, beta-secretase 1 (BACE1)[16].

Their paper made global headlines, yet the coverage in some ways missed the point, says corresponding author Matthew Wood. "It wasn't that we were on our way to curing Alzheimer's, but rather that we had engineered a natural nanoparticle, the exosome, for therapeutic purposes," he says. "That's the lasting legacy and it's not something that even I fully appreciated at the time."

Wood was investigating nucleic acid-based therapies for brain diseases in 2007 when he learned of a study showing that exosomes ferry RNA between cells[17]. That these tiny lipid-coated structures also transport proteins was already known. This new finding pointed to an opportunity. Wood envisioned exosomes loaded up with siRNAs traversing the BBB and hitting neurological targets. He speculated that if he could package siRNA in exosomes designed to shuttle through the BBB, they might survive long enough to do their job in target brain tissue.

So he and a pair of post-docs, Lydia Alvarez-Erviti, a Spanish neuroscientist, and Yiqi Seow, a molecular biologist from Singapore, came up with a strategy. They started by altering dendritic cells so that their exosomes would express a surface protein called lamp2. That protein, Wood explains, served as a scaffold to which the scientists affixed a peptide, RVG, isolated from the rabies virus. RVG binds to acetylcholine receptors enriched both on neurons and endothelial cells in the BBB (it's possible that RVG binding facilitates passage of rabies into the brain, but that's never been confirmed).

Though Wood didn't predict it, outfitting exosomes with RVG was the easy part of the study. The much harder part, he says, was packing these tiny structures with siRNA. Exosomes are tiny—measuring no greater than 100 nm across—and they also have charged outer membranes that siRNAs can easily stick to. The team needed a way to disrupt that membrane, which they finally achieved with electroporation; they applied an electric charge to exosomes and siRNA in solution, and "if the voltage and the duration were both right, some siRNA would become encapsulated within the vesicle," Wood says.

Wood's team worked with two kinds of siRNA, both packaged into exosomes that were injected into mouse tail veins. The first targeted a metabolic housekeeping gene called GADPH, which was knocked down in several brain regions after RVG exosome exposure. "That's when I started thinking that maybe this is more than just a clever idea," Wood says. The second targeted BACE1 and resulted in a roughly 60% reduction in the gene's activity—a slam-dunk covered by the news media.

Xandra Breakefield, a neuroscientist at Harvard University, in Boston, says Wood's paper provided a conceptual breakthrough. "It showed that extracellular vesicles could be used for therapeutic delivery of RNA with targeting to the brain and downregulation of an mRNA critical in Alzheimer's disease," she says. "And it really energized the field of extracellular vesicles as therapeutic delivery vehicles—which is a hot topic now with delivery of proteins, DNA, RNA and drugs." Before exosomes can be turned into a commercial payload delivery system, issues such as large-scale, reproducible manufacture remain to be addressed.

Matthew Wood enlisted exosomes to deliver a therapeutic cargo across the blood brain barrier.

Wood emphasizes, however, that exosomes show particular promise because many new experimental treatment modalities have high molecular weights that preclude cellular entry. "That makes them hard to deliver—cells don't ordinarily take up proteins or nucleic acids so you need a delivery method," he says. "Exosomes deliver them as part of their natural function so they're perfectly suited for this."

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