# 2024 | CANCER RESEARCH

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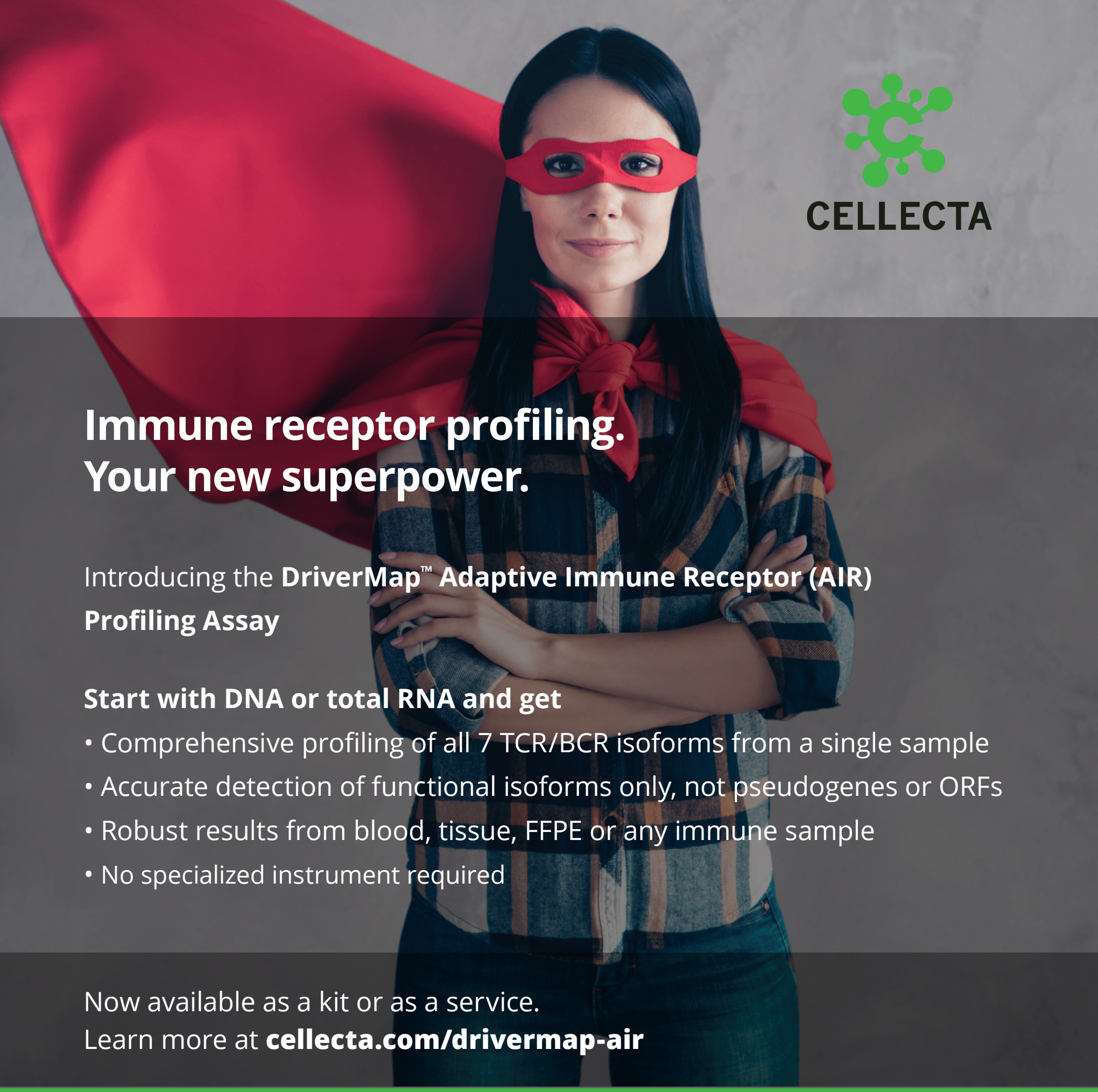
# contents





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# Chemotherapy's Hidden Origin

*From chemical warfare trenches, scientists glimpsed an opportunity to transform deadly mustard gas into a revolutionary cancer treatment.*

BY YUNING WANG, PHD

On the night of December 2, 1943, German bombers attacked the southern Italian port of Bari. Serving as a vital hub for the Allied troops during World War II, the harbor was crowded with ships loaded with ammunition and supplies. As explosions lit up the sky, seventeen ships sank, including the United States Liberty ship, John Harvey. The ship's cargo of bombs blew up, spilling oil into the water and dispersing smoke into the air.

Hundreds of sailors covered in oil escaped, but after a few hours, the sailors began exhibiting unusual symptoms. Burns and blisters appeared on their skins, and eye irritation and labored breathing set in. Within a day, their health rapidly deteriorated, and some sailors died. The doctors could not determine the cause of their deaths or recommend a treatment.

The United States Army dispatched Lieutenant Colonel Stewart Francis Alexander, a young medical doctor from New Jersey, to investigate the sailors' mysterious deaths. "When he arrived at Bari, he appreciated a garlic-like smell of mustard gas, so he immediately suspected," said Michael Nevins, a retired New Jersey cardiologist and internist who was Alexander's friend and colleague. "The credit goes to his nose."

The John Harvey had been carrying a secret load of 2,000 mustard gas bombs. Mustard gas gained its worldwide infamy as a chemical warfare agent during World War I. By the time World War I ended in 1918, mustard gas had intoxicated an estimated 1.2 million soldiers and contributed to 80% of the chemical casualties, making it the deadliest war gas of its time (1). The outbreak of World War II in 1939 intensified nations' efforts to develop, manufacture, and stockpile chemical weapons. Not long after, mustard gas made its reappearance in Bari.

Alexander's subsequent investigation marked an unexpected turn in the story of mustard gas. Scientists in various laboratories and medical institutions around the world started to recognize the potential for harnessing mustard gas' toxic properties and transformed it into a weapon to combat a different enemy: cancer. Their efforts eventually converted this deadly chemical agent into chemotherapy. Alexander's subsequent investigation marked an unexpected turn in the story of mustard gas. Scientists in various laboratories and medical institutions around the world started to recognize the potential for harnessing mustard gas' toxic properties and transformed it into a weapon to combat a different enemy: cancer. Their efforts eventually converted this deadly chemical agent into chemotherapy.

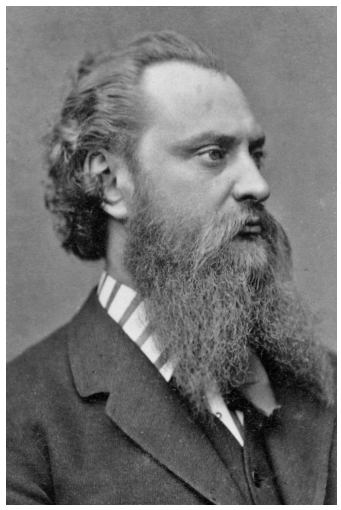
## From skin to blood

Working with mustard gas had never been safe. In 1860, when Frederick Guthrie from the University of Edinburgh first synthesized mustard gas by reacting ethylene and sulfur dichloride, he experienced irritation on his own skin (2). That same year, Albert Niemann at the University of Göttingen repeated the reaction and suffered skin reddening and blistering (3). Later, Viktor Meyer at the University of Göttingen achieved the first reliable synthesis of pure mustard gas by modifying the formula, resulting in a more toxic product (4). In 1913, Hans Thacher Clarke at the University of Berlin further developed Meyer's formulation and introduced a more efficient method for synthesizing mustard gas (5). Due to accidental exposure, Clarke was hospitalized, but ultimately, his method was employed for large-scale mustard gas production during warfare.

Dangerous chemicals never intimidated Alexander. "Back in high school, he had a chemistry lab in the basement of the house," said Nevins. In 1941, two years before the tragic incident in Bari, Alexander had just begun his medical practice in New Jersey when he received a summons from the United States Army. The United States Chemical Warfare Service (CWS), which was established during World War I for producing poison gas and defensive equipment, sought out medical experts across the country to bolster the army's preparedness for potential chemical gas attacks.

The CWS brought Alexander into the Medical Research Laboratory at the Edgewood Arsenal facility in Maryland to conduct research on chemical warfare agents and develop prevention and treatment methods. At Edgewood, Alexander consulted specialists, evaluated the toxicity of different agents on animals, and learned to identify different toxins, including chlorine, phosgene, and mustard gas by their odors. Soon, he became a chemical weapons expert.

In early 1942, the CWS assigned Alexander to investigate a new chemical agent called nitrogen mustard, a volatile liquid with a chemical structure similar to mustard gas that had little to no odor. Using rabbits, Alexander set up experiments on various organs and systems, including the skin, eyes, respiratory tract, and blood. Like mustard gas, nitrogen mustard caused burns on the skin and different organs.



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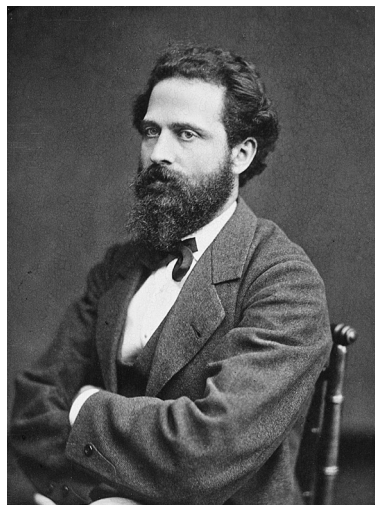

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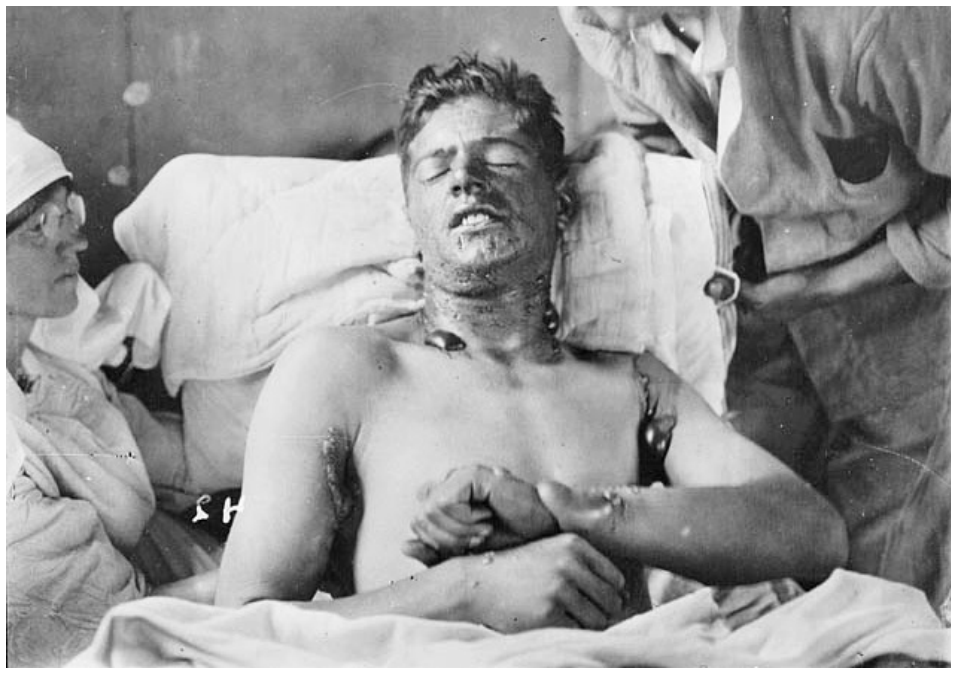
***Chemists Albert Niemann (left) and Viktor Meyer (right) pioneered the synthesis and large-scale production of mustard gas during the 1800s.***



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***A Canadian soldier suffered burns caused by mustard gas during World War I.***


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***A poster during World War II taught soldiers to identify mustard gas by its smell.***

"Chemotherapy created an entire new specialty, practice, and field of medicine. At this point, there will absolutely be leaps, breakthroughs, and huge paradigm changes."

*– Dieter Lindskog, Yale School of Medicine*

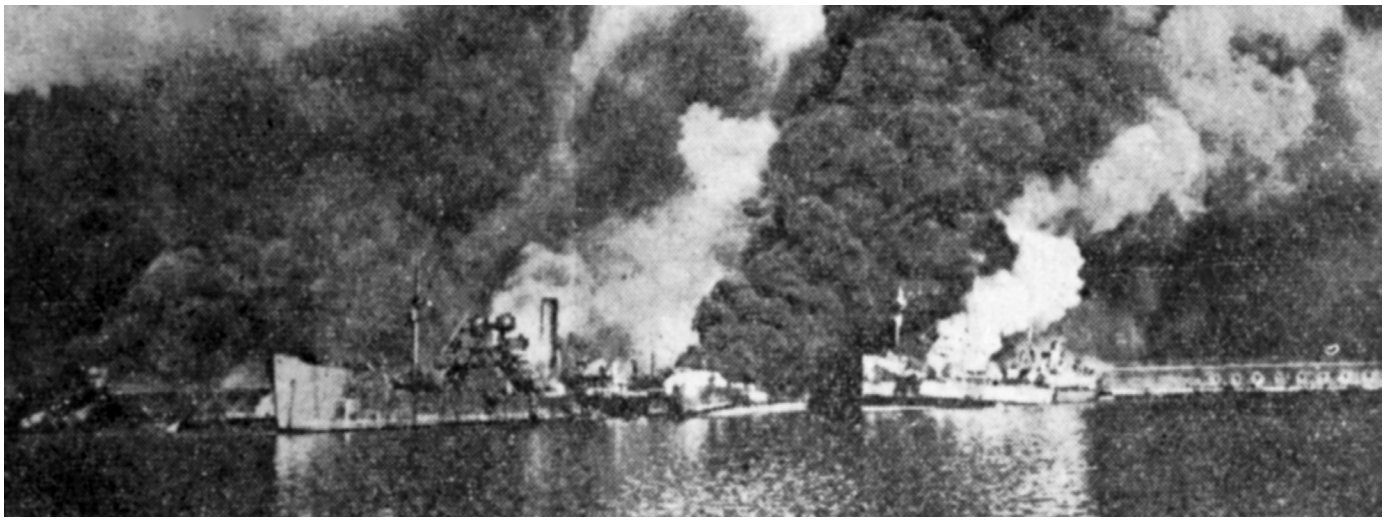
However, the agent's effects on the blood astonished Alexander.

For a typical burn injury, the immune system usually ramps up white blood cell production to fight off infections. In Alexander's nitrogen mustard experiments, the opposite happened. Within several days after exposure to nitrogen mustard, the rabbits' white blood cell numbers drastically dropped until they completely disappeared. The rabbits' lymph nodes melted away, and their bone marrow became depleted of blood cells (6).

After obtaining the same results from other laboratory animals such as rats and mice, Alexander believed that nitrogen mustard disrupted the body's blood production by attacking white blood cells. He began to wonder if nitrogen mustard would have the same effects on humans. If it did, it might be developed to treat leukemia, a condition marked by uncontrolled growth of white blood cells. Alexander documented his findings and hypothesis in a report published internally within the CWS in June 1942, but his report went unappreciated (6).

A year later, in the aftermath of the Bari attack, Alexander examined the sailors and confirmed his suspicions of mustard gas exposure. As he went through the doctors' medical case sheets and pathology reports, one recurring observation stood out: the white blood cell counts of severely injured patients took a sharp downward turn on the third or fourth day after the incident. These results agreed with Alexander's animal studies at Edgewood, supporting Alexander's interest in turning a poison into a drug to treat leukemia and lymphoma.

In December 1943, after conducting extensive blood and bone marrow tests, Alexander submitted a report on his investigation of the Bari disaster (6). His report was immediately classified by the government to avoid triggering a chemical war. But the United States military medical advisers, including Cornelius Rhoads, chief of the CWS's Medical Division, carefully appraised Alexander's work, finally taking note of nitrogen mustard's therapeutic potential. "Rhoads was very excited about the possibilities of how this could be turned into something useful in peacetime," said Nevins.



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*On December 2, 1943, German bombers launched an air attack on the Allied port in Bari, Italy, causing ammunition loaded on the ships to explode.*

## The substance x project

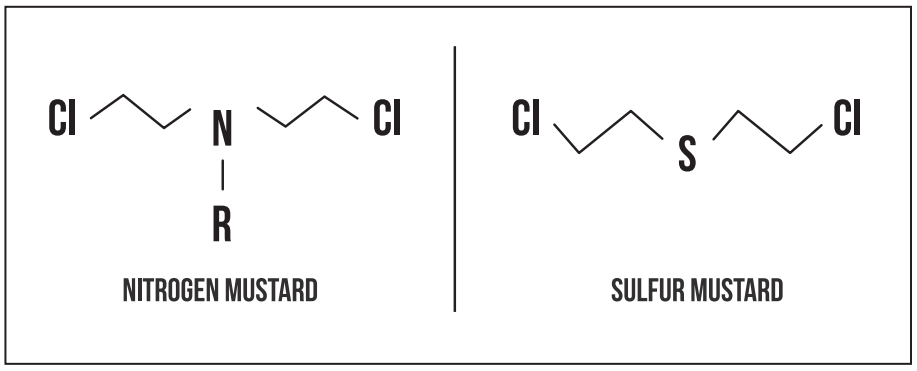
While Alexander served in the CWS investigating chemical weapons at Edgewood, another research program on war gas was secretly occurring 200 miles away. In 1942 at Yale School of Medicine, the school's Dean, Milton Winternitz, signed a government contract with the Office of Research and Development.

Winternitz had previously studied war gas poisonings during World War I and recognized the urgent need to investigate new chemical warfare agents and develop antidotes. He assigned two young pharmacologists, Alfred Gilman and Louis Goodman, to study the toxicity of the new chemical agent nitrogen mustard, which was coded as substance X.

Gilman and Goodman began their testing by exposing rabbits to substance X. Like Alexander, they observed the rapid disappearance of lymphocytes and granulocytes in the rabbits. "The systemic effects of the nitrogen mustards were far more fascinating than the blisters they produced on the skin," Gilman recalled in an article published in The American Journal of Surgery in 1963 (7). They wondered if this agent could destroy fast growing cancer cells before it attacked the host.

The pair consulted Thomas Dougherty, an anatomist at Yale School of Medicine, who provided them with a mouse transplanted with advanced lymphosarcoma. When Gilman and Goodman treated this mouse with nitrogen mustard, its tumor softened and shrunk after just two administrations of the chemical. With more doses, the tumor disappeared. Much to everyone's surprise, the mouse survived a remarkably long time (7).

Gilman and Goodman eagerly wanted to move forward with a human trial and presented their animal experimentation data to Gustaf Lindskog, the chair of surgery at Yale School of Medicine. Intrigued by the encouraging results, Lindskog agreed to supervise the trial. The team soon found a potential candidate, a 47-year-old patient with cancer at Yale New Haven Hospital.




*Mustard gas and nitrogen mustard share similar chemical structures and toxicities.*

The patient had terminal lymphosarcoma and had undergone all possible treatment options without any success. Since all hope for recovery seemed lost, Lindskog approached the patient, known to history as JD, with a bold experiment that might save his life.

"They had a detailed conversation with him about what they were undertaking, and that this was an experimental option, and that nobody knew what was going to happen," said Dieter Lindskog, grandson of Gustaf Lindskog and orthopedic surgeon at Yale School of Medicine.

JD decided to take the risk. "They had no idea, clue, or anything resembling guidance on dosing," said Lindskog. Their best hint was their toxicity studies on rabbits, which suggested a dosage of 0.1mg per kilogram of nitrogen mustard. A few days later, JD became the first recipient of intravenous chemotherapy for cancer.

"The patient's tumor had a marvelous response," said Lindskog. Within 48 hours, the tumor began to soften, and the patient felt better. By the tenth day, when the series of injections ended, his tumor was no longer palpable, and all cancer symptoms had disappeared.

By day 49, the patient's tumor made a vigorous return and became resistant to nitrogen mustard. Despite receiving two additional courses of treatment, his condition failed to improve. He eventually passed away on day 96 (8). Due to the confidentiality surrounding chemical warfare agents, the medical records of this trial were censored and were later lost to time, leaving many details undisclosed

## Modern chemotherapy

In June 1943, the nitrogen mustard research group at Yale University dispersed, but the clinical trials testing nitrogen mustard's therapeutic potential continued. Goodman collaborated with researchers in several institutions around the country to treat additional patients.

Gilman, Goodman, and their collaborators released details about their studies in 1946 after World War II had ended and the secrecy associated with the war gas program had been lifted. In this landmark report, they documented 67 nitrogen mustard clinical trials, including JD's case, for treating Hodgkin's disease, lymphosarcoma, and various types of leukemia. The report demonstrated significant tumor regression in most patients, with clinical remissions lasting from weeks to months (8).

Rhoads, who previously appreciated Alexander's Bari report, was further encouraged by Gilman and Goodman's clinical trials. He started vigorously seeking funding for developing chemotherapy after the war. In 1948, Rhoads became the director of the Memorial Sloan-Kettering Cancer Center, which spearheaded the development of several important chemotherapy drugs, including mustard derivatives and other antitumor compounds.

In 1949, the United States Food and Drug Administration approved mechlorethamine, a nitrogen mustard compound, as the first chemotherapy drug for hematologic malignancies (9). Around that time, scientists started to understand how the agent works in the body. Two chemists, Philip Lawley and Peter Brookes at the Royal Cancer Hospital, unraveled the molecular mechanisms behind the agent's cancer-killing properties.

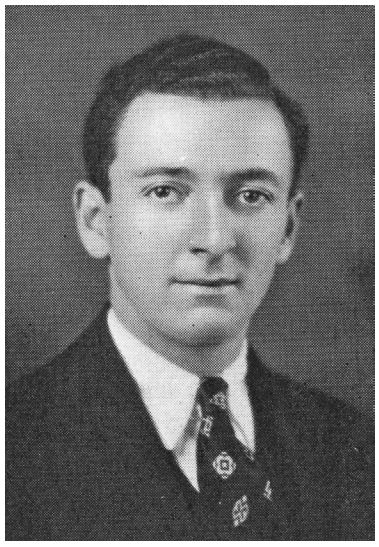
Mustard gas and nitrogen mustard both belong to a class of chemicals known as alkylating agents. In the cell, these agents undergo a series of reactions to form a highly reactive intermediate, which covalently modifies DNA in a reaction referred to as DNA alkylation. DNA alkylation disrupts cell replication and causes cellular damage, which makes alkylating agents particularly effective at destroying rapidly dividing cells such as white blood cells and cancer cells (10,11).

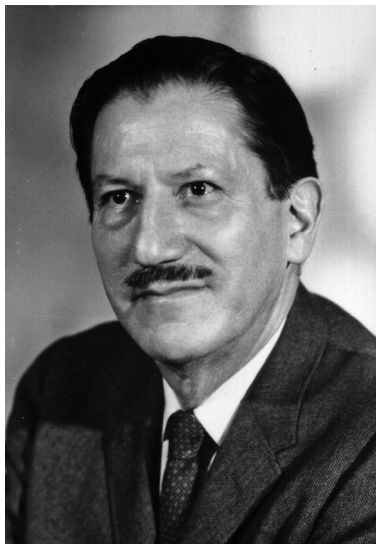
This information stimulated researchers to synthesize and test more alkylating compounds to fight cancer. Several new chemotherapy drugs emerged during the 1950s, such as chlorambucil and busulfan, alkylating agents that treat leukemia by stopping white blood cells from growing and spreading (12). Over the following decades, scientists introduced more than 100 different chemotherapy drugs into clinical practice to treat numerous cancer types, including leukemia, lymphoma, myeloma, sarcoma, and breast, lung, and ovarian cancers.

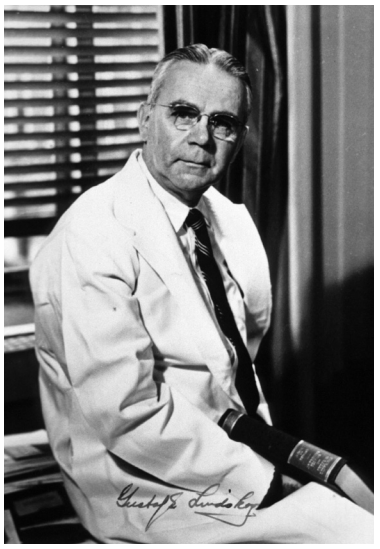
Chemotherapy has led to remarkable remission rates and prolonged survival for numerous patients. Its success in killing fast-dividing cancer cells laid the foundation for the development of more effective therapeutic approaches, such as targeted therapies and immunotherapies. "Chemotherapy created an entire new specialty, practice, and field of medicine," said Lindskog. "At this point, there will absolutely be leaps, breakthroughs, and huge paradigm changes."

## Setting the record straight

As researchers continue to make leaps and bounds in chemotherapy development, others are still uncovering hidden details about its







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***Alfred Gilman (left), Louis Goodman (middle), and Gustaf Lindskog (right) conducted the first human trial of nitrogen mustard to treat lymphosarcoma.***

CREDIT: ROBERT UDELSMAN

***In 2011, Robert Udelsman (left) and John Fenn (right) uncovered the medical records of the first intravenous use of chemotherapy in humans.***

***Today, chemotherapy drugs remain a mainstay of cancer treatment while often working in conjunction with innovative approaches like immunotherapy.***

early days. Although JD's case was published in Gilman's report in 1946, the details of the case were minimal, and JD's medical records disappeared.

In 2010, John Fenn, a surgeon and Lindskog's colleague at Yale School of Medicine, learned about the saga of the first chemotherapy trial Gilman, Goodman, and Lindskog conducted during World War II. Fascinated by it, Fenn teamed up with his colleague, Robert Udelsmam, a former clinical professor of surgery at Yale School of Medicine and current surgeon at the Miami Cancer Institute, to unearth JD's medical records.

"We contacted a senior colleague in pathology," Udelsmam recalled. "The pathology department had an independent database for the archive of every patient ever seen at Yale based on their diagnosis, the patient's name, their medical record, and the year."

Lacking key information such as name, date of birth, and medical record number, Fenn and Udelsmam had only the patient's initials, which matched with numerous pathology reports from the early 1940s. After months of searching, they identified a promising pathology chart of a patient with lymphosarcoma and a medical record number, but that did not lead them to JD's medical records. Suspecting errors in the record number, they began rearranging the number sequences and inserting additional numbers. After several failed searches, they eventually tracked down JD's complete medical records. "It was exciting at the time," said Udelsman. "I remember when John put the chart down on my hands. I had the original chart right in front of me. I couldn't believe we found it!"

The uncovered records shed new light on JD's personal background and clinical course. As a Polish immigrant, JD moved to the United States at age 18 and lost his family during the war. "He didn't speak English and worked in a ball bearing factory. He had no spouse, no children," said Udelsman. "It sounded like a very lonely, isolated life."

The lost files also added context to JD's previous treatments, offering insights into why he might have chosen to participate in the experimental trial. He was diagnosed with lymphosarcoma in 1940 and underwent multiple radiation treatments at Yale New Haven Hospital in 1941. Unfortunately, his tumor became progressively unresponsive, causing respiratory distress, dysphagia, and weight loss. Physicians noted in JD's medical records that his outlook was "utterly hopeless" (13).

"They were watching his tumor grow on a daily basis," noted Udelsman. "In difficult situations, doctors are more inclined to use potentially toxic agents and toxic doses of an agent to try to have a therapeutic benefit."

Although the treatment did not ultimately save JD's life, Udelsman was not surprised. "It is common in chemotherapy that patients develop clones of lymphocytes that are refractory to treatment. A few cells remain that are surviving, and they become more and more refractory to the drug," he explained. "Nowadays, we use combination chemotherapeutic treatments for this very reason."

In 2011, Fenn and Udelsmam published their investigation of JD's case in the *Journal of the American College of Surgeons*, finally setting JD's record straight six decades after the case and bringing light to the complicated history of chemotherapy treatment (13). "If this is really the first form of intravenous chemotherapy with an antineoplastic agent in the world, it's giant!" said Udelsman. "It should be in every textbook." ■

> "If this is really the first form of intravenous chemotherapy with an antineoplastic agent in the world, it's giant! It should be in every textbook."
>
> *– Robert Udelsmam, Miami Cancer Institute*

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# Turning Brain Tumors Against Themselves

*Using tiny chambers that release signals from a patient's own tumor, scientists trigger immune cells to mount a personalized attack against the cancer.*

BY STEPHANIE DEMARCO, PHD

Whether they're in the brain, on the skin, or in the liver, solid tumors are difficult to treat. Because they arise from healthy cells that go haywire, every person's tumor is different. This tumor heterogeneity means that there is often no "one size fits all" treatment, even for the same types of cancer.

Now, a team of immunologists and neurosurgeons at the biotechnology company Imvax plan to change that.

"We have an immunotherapy that's based on using the patient's own tumor cells as a source of antigen," said Mark Exley, an immunologist and chief scientific officer at Imvax. While the idea to use a cancer patient's tumor cells to fight against the tumor is not novel on its own, Exley added, "There are ways in which this is quite radically different, and that explains the substantially better looking clinical data that we've got as well as all the preclinical data we have that looks very positive."

For example, scientists have isolated patient dendritic cells, exposed them to antigens from the tumor, and reinfused the dendritic cells back into the patient. The challenge with this approach is, "you need to have a lot of [these cells] to reinfuse back into the body because eventually they die," said Corinne Ying Xuan Chua, a nanomedicine and cancer researcher at Houston Methodist Research Institute who is not associated with Imvax. That approach only leads to minor activation of the immune system against the cancer.

Instead, by triggering a patient's own tumor cells to release both immunostimulatory molecules and tumor-specific antigens within a biodiffusion chamber temporarily inserted in the patient's body, the researchers at Imvax turn the uniqueness of the tumor against itself. With positive results in a phase Ib clinical trial in brain cancer, this new immunotherapy may lead to personalized treatments for difficult-to-treat solid cancers.

For Imvax's cofounder and chief medical officer, David Andrews, the road to this personalized immunotherapy began with glioblastoma, an aggressive type of brain cancer. As an academic and practicing neurosurgeon, Andrews regularly treats patients with glioblastoma and has witnessed the need for better treatments firsthand.

The standard of care for glioblastoma is to remove as much of the brain tumor as possible and then treat the patient with radiation and chemotherapy, but Andrews said, this "is where we've been since 2005." Even with this treatment regimen, people with glioblastoma only have a 15-month survival prognosis.

Glioblastoma is difficult to treat for several reasons. In addition to being heterogeneous and located behind the blood-brain barrier, glioblastomas actively release factors to suppress the immune system (1).

Andrews and his colleagues at Thomas Jefferson University and later at Imvax reasoned that if they could give the immune system a chance to fight glioblastoma cells that remain after surgery, patients may have better outcomes.

They focused their attention on decades of research on the insulin-like growth factor I receptor (IGF-IR), which cancer cells overexpress to protect themselves from apoptosis (2). Andrews' and Craig Hooper's team at Thomas Jefferson University designed an antisense IGF-IR RNA molecule that inhibits glioblastoma tumor's IGF-IR and triggers tumor cell apoptosis in multiple rodent models (3,4). When they encapsulated both the rat glioblastoma tumor cells and the antisense IGF-IR RNA in a biodiffusion chamber and placed the chamber in the rat's abdomen for 24 hours, the rats that received the treatment lived three times longer than those that received no treatment, and their tumors never came back (5).


CREDIT: IMVAX

***Imvax's biodiffusion chambers contain a cancer treatment that uses a person's own tumor cells to stimulate the immune system to fight cancer.***

The biodiffusion chamber "has 100 nanometer pores, so it allows out peptides, proteins, exosomes, antigens, and debris in various forms, but not the viable tumor cell," said Exley. "It also allows out the antigenic stimulus and other components that stimulate the immune response — innate immune stimuli as well as the adaptive immune antigens — at the same time as keeping in some of the immunosuppressive components."

In recent efforts to understand the mechanisms driving these immune responses to the filled biodiffusion chambers, the Imvax researchers studied them in mouse models. They observed that the tumor cells with the IGF-IR antisense molecule triggered an increased T cell response, a greater interferon gamma response, and a shifting away from immunosuppressive cytokines in the tumor environment. "As well as the antigen specific response, we're also getting a general change in the immune milieu," said Exley.

Based on the positive preclinical data and a small but promising phase Ia trial, Andrews initiated a phase Ib clinical trial in patients with newly diagnosed glioblastoma. (The Imvax team assumed control of the clinical trial near its conclusion.) When people with glioblastoma arrived at the hospital for their tumor removal surgeries, Andrews and his neurosurgeon colleagues made additional incisions on either side of their abdomens to form small pockets where surgeons would place the filled biodiffusion chambers the next day.

"That's the advantage of being able to be done while the patient is in hospital anyway, so then they're one and done," said Exley.

Once surgeons removed the tumor cells from the patient, they sent them to Imvax's manufacturing facility where Imvax scientists treated the tumor cells with the antisense IGF-IR molecule and placed both in multiple biodiffusion chambers. They then irradiated the chambers with a low dose of radiation, which pushes the tumor cells toward immunogenic cell death. In less than 24 hours, the Imvax team then shipped the biodiffusion chambers carrying the patient's personalized treatment back to the hospital.

Depending on which treatment group the patient had been assigned to, surgeons either placed 10 or 20 biodiffusion chambers into the patient's abdomen for either 24 or 48 hours. A typical hospital stay for someone with glioblastoma after tumor removal surgery is six days, so even with receiving the Imvax team's immunotherapy, the patients didn't have to stay in the hospital any longer than usual.

When Andrews and his colleagues at Thomas Jefferson University assessed these patients six weeks later, they found that patients who had received 20 biodiffusion chambers for 48 hours not only had a strong immune response to the therapy, but they also had longer rates of progression-free survival than the patients in the other treatment groups.

"We went back to the Institutional Review Board and said we want to stop the randomization. It seemed ethical, since there's no difference in safety, that we're just going to finish this with the highest [dose] cohort," said Andrews. Six weeks after having the biodiffusion chambers removed, the patients received standard-of-care radiation and chemotherapy.

The results of the trial, published in 2021, were incredibly positive (6). "We're now up to 38 months overall survival, and we're maintaining progression-free survival above 17 months. So, that was unexpected," said Andrews. "As a neurosurgeon in practice for 33 years, I'd never seen [that] before."

Chua agreed that this approach looks very promising. "You have a local place where you put your processed tumor cells from the patient, and then you expose them directly within that niche to drugs that can perhaps teach the immune system to say, 'Look, these are the cancer cells. Go out and attack these cancer cells wherever you find them in the body.' So, this can be helpful not just for glioblastoma; it could be helpful for any other solid tumors."

Since completing the phase Ib clinical trial, the Imvax researchers have explored exactly that. Working in mice, the team has reported positive results using the personalized biodiffusion chambers to target endometrial, liver, bladder, and ovarian cancers. They have also just begun a phase IIb clinical trial for glioblastoma to assess the effectiveness of their treatment platform. For the phase IIb trial, the Imvax team has optimized the phase Ib trial procedure, and they recently dosed their first patient.

Andrews, Exley, and the entire Imvax team hope that this treatment will lead to a more effective therapy for patients with glioblastoma and other hard-to-treat solid cancers.

Recalling the phase Ib trial results, Andrew said, "What was really thrilling was not only was it safe, but getting these responses was probably the most rewarding experience I've had in neurosurgery."

Exley agreed: "As an immunologist, to apply immunology and make it work for patients, it's the peak of my career as well." ■

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# milestone
# The Rise of Herceptin

BY DANIELLE GERHARD, PHD

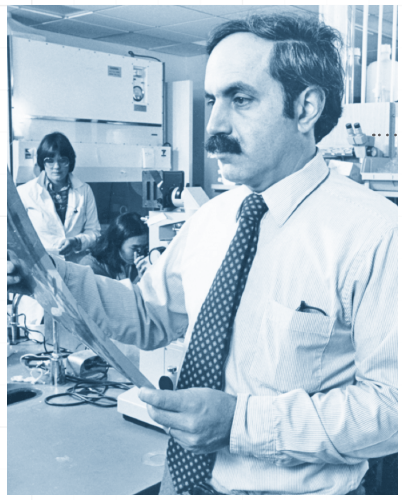
The monoclonal antibody drug Herceptin dramatically changed the lives of those diagnosed with human epidermal growth factor receptor two (HER2) overexpressing cancers. The drug also changed the landscape of cancer treatment as one of the first targeted therapies.

## 1981–1982 Transforming cells

In the early 1980s, cancer biologist Robert Weinberg and his research team at the Massachusetts Institute of Technology were hunting genes responsible for transforming healthy cells into cancer cells. To do this, they treated pregnant rats with chemical carcinogens, which reliably produced offspring with brain tumors. When the researchers transfected healthy cells with DNA from these tumors, the cells adopted an oncogenic phenotype, providing valuable evidence that the tumor DNA contained the instructions to drive this cancerous transformation (1). Weinberg's group investigated the mechanisms driving this transformation and discovered a heavy phosphoprotein known as p185 (due to its 185 kilodalton mass) in the sera of mice that were injected with the transformed cells (2).

At that time, it was difficult to say which gene was responsible for transforming the cells and whether the same gene might also produce p185. Cloning technology was still in its infancy, and DNA sequencing took months. The researchers ruled out the rat sarcoma virus (Ras) oncogenes since they were known to encode a 21 kilodalton protein but had few other clues to the identity of these transforming genes. "This raised the possibility that there were other groups of oncogenes from other gene families," said Weinberg.

These questions led Weinberg to team up with Mark Greene at Harvard University. Now an immunobiologist at the University of Pennsylvania, Greene was similarly interested in whether the same factors that induce cancer growth also induce antigens on the surface of cancer cells. Weinberg's research team had been trying to produce a monoclonal antibody targeting p185. Jeffrey Drebin, a surgical oncologist at Memorial Sloan Kettering, was an MD/PhD student in Greene's lab when he was tasked with producing this antibody.



CREDIT: ROBERT WEINBERG

***Robert Weinberg used autoradiography to show that the human HER2/neu gene mapped to human chromosome 17 (15).***

## 1984 A brand *neu* oncogene

The two teams sampled DNA from four separate brain tumor cell lines and found that they all produced the same p185 protein and exhibited comparable DNA cleavage in the presence of restriction enzymes, suggesting that specific DNA sequences were present across the cell lines. This further supported Weinberg's hunch that another family of genes was at play. Given their origins in neuroblastoma, the researchers dubbed this group of apparently related genes *neu*.

In 1984, Weinberg and Greene published the first results of their fruitful collaboration. They reported that the *neu* oncogene shared homology with the epidermal growth factor related gene erythroblastic oncogene *B* (*erbB*), which produced a similarly weighted protein product, the epidermal growth factor (EGF) receptor (3). Although the protein products were similar in size, the monoclonal antibodies that Drebin used to target p185 and the EGF receptor bound to different proteins (4). Together, these findings provided additional evidence that *neu* was a distinct gene but also strongly suggested that p185 was a *neu* oncogene product.

## 1985 Targeting p185

With a monoclonal antibody targeting p185 in hand, Drebin wondered what effect it would have on cancer cells. When he added the monoclonal antibody to cells expressing the *neu* gene, he observed a reduction in p185 levels and a reversion back to the pretransformed cell state (5). Furthermore, the treated cancer cells were unable to grow in soft agar, a phenotype that most recapitulates cancerous growth in animals. These studies provided the first evidence that monoclonal antibodies targeting p185 could inhibit the growth of cells expressing *neu*.

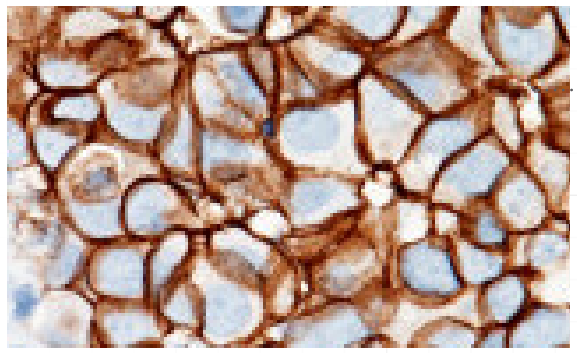
Around this time, the researchers established another important characteristic of p185: the necessity of its expression for the malignant behavior of cancer cells. While other monoclonal antibodies had been developed with the goal of treating cancer cells, it was unknown whether the expression of their targeted antigens was critical for cancer growth. "In other words, many other tumor-associated antigens could be shut down without the cancer cell needing to pay a price in terms of a loss of proliferation," said Weinberg. This was not the case for p185.

Weinberg's and Greene's teams did not suspect the impact these findings would eventually have on cancer treatment. "At the time I wrote my thesis, I don't think we were even sure there was much relevance to human cancer," said Drebin.

## 1985–1987 HER2/*neu* in human cancer

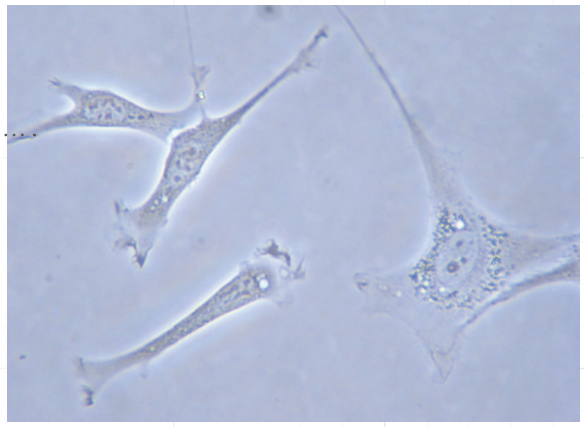
Two important papers published in *Science* in 1985 opened a new chapter in the story of the *neu* oncogene. First, Stuart Aaronson, a biologist at the National Cancer Institute at the time, reported on an *erbB*-related gene that was frequently amplified in human breast tumor cells (6). Only one issue later, Aaronson published a paper with two Genentech scientists, Axel Ullrich and Lisa Coussens, confirming that this *erbB*-related gene was in fact the *neu* oncogene (7).

Up to this point, the relationship between oncogenes and the induction or maintenance of tumors in humans was merely circumstantial. Dennis Slamon, an oncologist at the University of California, Los Angeles, was aware of Ullrich and his colleagues' findings and wondered what role oncogenes played in human cancer. Ullrich supplied Slamon with DNA probes targeted to the *neu* gene, which he used to examine expression patterns in nearly 200 primary human tumor samples. What Slamon observed was unprecedented; more than a quarter of the tumors expressed the *neu* oncogene in levels that exceeded those found in noncancerous cells, and this expression pattern associated with a more aggressive form of breast cancer (8).



CREDIT: WIKIMEDIA COMMONS

***Nearly 30 percent of breast cancer tumors overexpress HER2/neu, which now serves as an important biomarker and target for therapies.***



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***When Robert Weinberg and his team transformed NIH 3T3 cells (pictured) with rat neuroblastoma oncogenes, the cells produced the phosphoprotein p185, which they subsequently found was a product of the newly identified oncogene neu.***

CREDIT: MEMORIAL SLOAN KETTERING CANCER CENTER

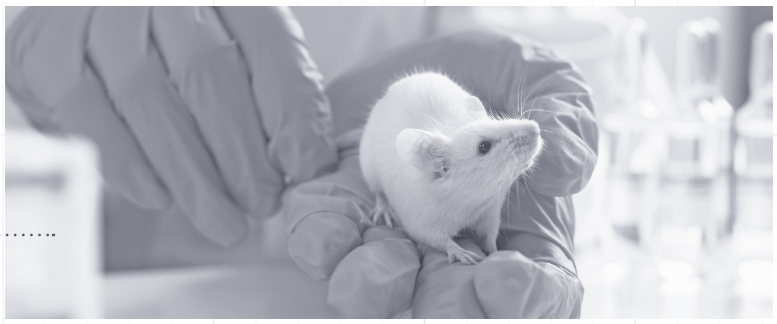
***Jeffrey Drebin, a surgical oncologist at Memorial Sloan Kettering, designed early mouse monoclonal antibodies targeted to the neu gene product p185.***

# 1989–1990 Pursuing a new antibody

Over the next few years, HER2, the human homolog of the *neu* gene first discovered in rats, became the dominant nomenclature for the gene, HER2-positive breast cancer was the grim diagnosis given to patients with HER2 overexpressing tumors, and Genentech scientists got to work making HER2-targeted antibodies.

Ullrich and his colleagues generated a panel of mouse antibodies that bound to the HER2 protein p185HER2. In two key studies, the group demonstrated that these antibodies inhibited the growth of p185HER2 overexpressing tumor cells in soft agar and sensitized the cells to the antitumor activity of tumor necrosis factor, a key player in the human immune system (9,10).

These findings provided further evidence of the clinical potential of these antibodies for treating HER2 overexpressing cancers, but adapting these drugs for the clinic posed another great hurdle.



***The first antibodies targeted to HER2 were created in mice. However, for clinical use in humans, researchers needed to humanize the antibodies.***

# 1998 FDA approval

Despite these incredible results, Genentech, then only a small biotech company, had reservations about moving forward with the antiHER2 antibody (12). However, in 1992, they reversed their decision and funded the first clinical trial on their antiHER2 antibody called Herceptin (trastuzumab). With the need for an effective treatment option and the desperation felt by many patients, Genentech became inundated with requests to join the trial before any conclusive safety and efficacy data had been collected.

After four years, the results were clear. Compared to chemotherapy alone, Herceptin in combination with chemotherapy slowed disease progression, extended survival, and reduced risk of death by 20 percent (13). By 1998, the Food and Drug Administration approved Herceptin plus chemotherapy for people with HER2-positive metastatic breast cancer.

***Herceptin has converting the cancer treatment landscape for many, c a deadly form of cancer into a curable cancer.***

CREDIT: GENENTECH

***Paul Carter collaborated with others at Genentech to humanize the antiHER2 mouse monoclonal antibody.***

# 1990s Humanized mouse antibodies

Paul Carter, an antibody engineer at Genentech, recalled when the new head of research, Arthur Levinson, toured Genentech's research departments to showcase company-wide research programs. The HER2 program piqued Carter's interests. He saw clear parallels with his own research expertise. In the 1980s, Carter was a graduate student under the tutelage of the Nobel Prize-winning molecular biologist Gregory Winter at the MRC Laboratory of Molecular Biology, where he worked on monoclonal antibody development.

Genentech was pursuing antiHER2 mouse monoclonal antibodies in clinical trials, but Carter was acutely aware of the limitations of these drugs. "In the 1970s, there was great hope for antibodies to be a very important class of drugs, but those products got largely dashed in the late 70s and early 80s," said Carter. Mouse monoclonal antibodies taken into the clinic consistently failed. This was primarily due to the human immune system identifying mouse antibodies as foreign and rejecting them. "This was kind of a low time in the field," said Carter.

Years prior, Winter pioneered a technique to humanize mouse monoclonal antibodies or disguise the mouse antibody under the cloak of a human antibody. Carter thought this technique was worth pursuing, so he forged an alliance with Genentech scientists Michael Shepard and Len Presta to innovate this technology for Genentech's products.

Using one of Ullrich's antibodies, Carter and his colleagues reserved key sections that bound to HER2 and skillfully grafted these onto a human antibody. By the end of 1990, the group developed eight humanized variants (11). Compared to the original mouse antibody, one humanized variant in particular exhibited excellent binding affinity to p185HER2 and comparable inhibitory activity for cancer cells. As an added bonus, their humanized design facilitated the killing of HER2 overexpressing cells by attracting other immune cells to the tumor cells to initiate cell death. Much to their surprise, it was a great success.

# 2012 Two antibodies are better than one

After the groundbreaking trial in the 1990s, Genentech continued searching for better treatments. In another clinical trial, clinicians gave a combination of chemotherapy, Herceptin, and Perjeta (pertuzumab), another antiHER2 antibody (14). By giving two antibodies instead of one, patients lived nearly 16 months longer and had a median survival of almost five years, which was previously unheard of for patients with such aggressive cancer.

"The development of the two-antibody therapy took HER2-positive cancer from the worst kind of breast cancer — the most likely to recur, the most rapidly growing, the most lethal — to the best kind of breast cancer — the most likely to be cured," said Drebin.

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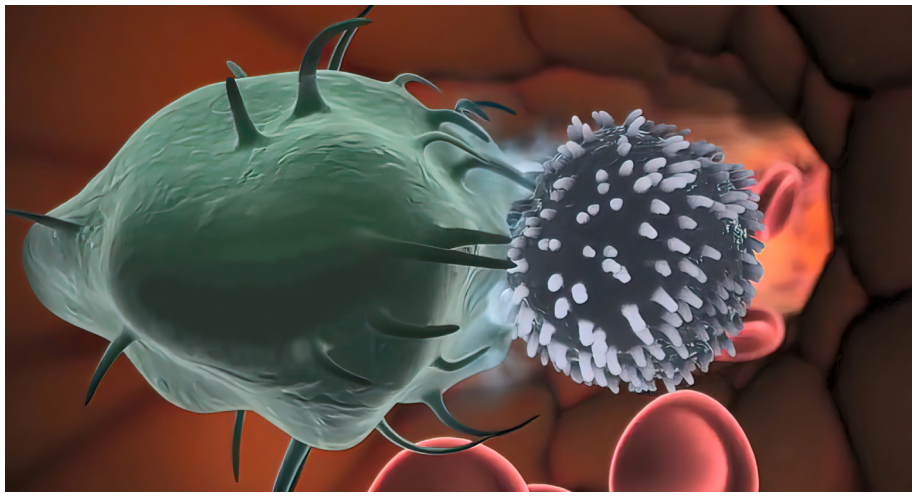
# Modifying Macrophages to Attack Solid Tumors

*A team of industry and academic researchers developed a new way to engineer the immune system, leading to the first clinical trial using genetically modified macrophages.*

BY DANIELLE GERHARD, PHD

OVER THE LAST TWO decades, advancements in genetic engineering have led to new cancer immunotherapies. Genetically modified T cells express customizable chimeric antigen receptors (CAR) that efficiency hunt down specific proteins on cancer cells and initiate T cell attack (1). The now six U.S. Food and Drug Administration (FDA)-approved CAR T cell therapies are a game changer for treating certain lymphomas and leukemias. However, their efficacy in treating solid tumors is limited. While researchers are looking into modifying CAR T cells for solid tumors, others are focusing their efforts on another big player in the immune system: macrophages.



"The natural armamentarium of the nonengineered macrophage was too weak to combat the cancer."

—Michael Klichinsky

### A new CAR on the lot

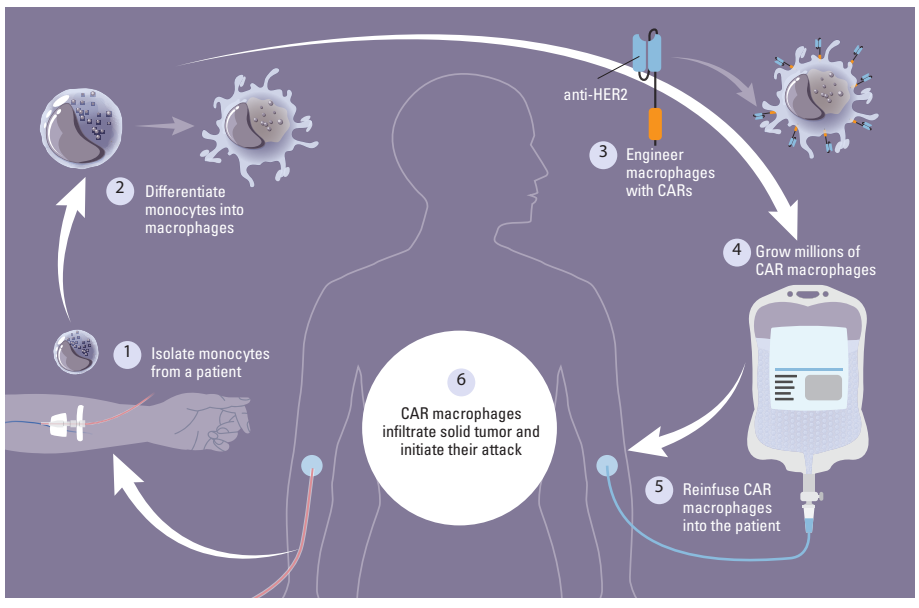
Solid tumors work hard to limit T cell trafficking and create unwelcoming, immunosuppressive conditions. Unlike B cell malignancies, that share common available targets for CAR T cell therapies, it is very difficult to find a universal antigen expressed on all solid tumor cells. Therapies that target a single antigen ignore antigen negative cells within the tumor, causing selective pressure and eventual resistance to those therapies.

This is where macrophages come into play. As the most abundant immune cell in the tumor microenvironment, macrophages readily traffic into solid tumors. In fact, solid tumors welcome macrophages because they are plastic. Tumor cells can easily convert macrophages from an anti-inflammatory phenotype to an immunosuppressive phenotype to help tumors grow. Outside of the tumor environment, macrophages are central regulators of the innate immune system and direct an antitumor immune response. These qualities led to early attempts by scientists to harvest monocytes from patients, grow them into macrophages in the lab, and reintroduce them into patients in high volumes to attack tumor cells (2). While these studies failed to show antitumor effects, they provided valuable information on the safety and feasibility of using macrophages in the clinic.

"The natural armamentarium of the nonengineered macrophage was too weak to combat the cancer," said Michael Klichinsky, a pharmacologist at Carisma Therapeutics. This led Klichinksy and a team of researchers at the University of Pennsylvania, which has a rich history of CAR T cell therapy innovations, to search for new ways to equip macrophages with CARs. They published their findings in *Nature Biotechnology* in 2020 (3).

The team of researchers quickly learned that engineering macrophages was more difficult than engineering T cells. Traditionally researchers use retroviruses or lentiviruses to introduce CARs into cells, but these methods were ineffective, leading Klichinsky and his team to turn to adenoviruses. Macrophages express cluster of differentiation 46 (CD46), a protein that allows adenovirus 35 (Ad35) to attach to the cell and release its genetic cargo. Klichinsky and his team modified Ad35 to transport CARs, creating a new Ad5f35 vector that exhibited excellent efficiency in delivering engineered CARs to macrophages. Adenoviral infections activate an immune response, so the researchers hypothesized that the vector would induce a proinflammatory macrophage state regardless of the cargo inside the Ad5f35 vector. However, further investigation into Ad5f35 revealed a secondary effect. To their surprise, the vector also locked macrophages into a permanent proinflammatory state, thus preventing tumors from turning macrophages to their advantage.

"When CAR macrophages get to the tumor, not only do they resist immunosuppression, but they drive inflammation. They help warm up the otherwise cold tumor microenvironment," said Klichinsky. Just like CAR T cells, CAR macrophages kill cells expressing the targeted antigen. However, because macrophages are professional antigen presenting cells, they also gobble up tumor cells, process other tumor-derived antigens, and use these to prime secondary T cell adaptive immune responses. "You are essentially therapeutically vaccinating the patient against their own tumor antigens," said Klichinsky. Ultimately, this leads to long term immune memory that protects from antigen negative relapse.




**The lifecycle of CAR macrophage therapy. (1) After patients receive a bone marrow stimulator to encourage white blood cell release from the bone marrow, clinicians isolate the monocytes using apheresis. (2) The monocytes arrive at the lab, where scientists differentiate them into mature macrophages. (3) Next, they transduce the macrophage with the anti-HER2 CAR CT-0508. (4) Finally, scientists produce millions of these engineered cells, cryopreserve them, and ship them back to the clinic. (5) Clinicians reintroduce the patient's genetically engineered macrophages, (6) which enter the solid tumor and kill HER2-expressing cells. In a secondary line of attack, macrophages identify other antigens expressed by the cancer cells and prime T cells with this information.**

### CAR macrophages in the fast lane

These promising results from Klichinsky and colleagues prompted the U.S. FDA to grant Fast Track designation to CT-0508, a CAR macrophage designed to target HER2-positive solid tumors, in September 2021. The research team is now enrolling patients with HER2 overexpressing solid tumors for which treatments are either unavailable or have failed, in a Phase I clinical trial for CT-0508 (4).

Currently, both CAR T cell and CAR macrophage therapies are autologous cell therapies, meaning they use a patient's existing cells. For this clinical trial, patients receive a bone marrow stimulator to trigger the release of monocytes. Once extracted, researchers differentiate the monocytes into macrophages in the lab, transduce them with the Ad5f35 vector carrying the anti-HER2 CAR, and cryopreserve the engineered cells for reinfusion.

To assess the safety and tolerability of CT-0508, the clinical team administered genetically modified macrophages across three separate infusions into the first seven patients enrolled in the trial. The researchers also investigated a number of secondary measures, including clinical efficacy, cell kinetics, and T cell characteristics.

### Paving the way to a bolstered immune landscape

In June 2022, researcwdata at the American Society of Clinical Oncology conference (5). They demonstrated a favorable safety profile with no major toxicities for CT-0508. Importantly, none of the patients exhibited neurotoxicity or major cytokine release syndrome, both of which are potential serious side effects for approved CAR T cell therapies. The authors found an initial cytokine surge in the bloodstream that quickly dissipated and corresponded with increased levels of CT-0508 in the tumor microenvironment. This aligned with the fact that mature macrophages do not linger in the bloodstream. "They're there for a minute, and then they go park in the tissue," said Kim Reiss, a medical oncologist at the University of Pennsylvania and principal investigator on the trial.

With respect to the clinical profile, at eight weeks post-infusion, four of the seven

**Michael Klichinsky (left) helped design the CAR macrophage used in the clinical trial (Carisma Therapeutics). Kim Reiss (middle) is a principal investigator on the clinical trial (University of Pennsylvania). Michel Sadelain (right) is a pioneer in CAR T cell therapies (Memorial Sloan Kettering).**

patients had stable disease, meaning minimal tumor shrinkage or growth, while the other three patients exhibited progressive disease, defined as at least a 20% increase in tumor growth.

The researchers ran T cell receptor sequencing on a subset of the patients to monitor changes in T cell repertoire following treatment. Reflective of an active immune response, they observed T cell expansion in the tumor periphery and microenvironment. These findings suggest that CT-0508 initiates an immune response and may also drive antitumor immunity.

To dig deeper, the authors used single cell RNA sequencing to assess remodeling of the tumor microenvironment following CAR macrophage treatment. After four weeks, the tumor microenvironment shifted towards an inflammatory state, evidenced by elevated proinflammatory macrophages as well as activated CD8 and CD4 T cells. "These findings suggest that these new T cells were not just randomly coming in, they were in fact, tumor-reactive," said Klichinsky.

> "When CAR macrophages get to the tumor, not only do they resist immunosuppression, but they drive inflammation. They help warm up the otherwise cold tumor microenvironment."
>
> —Michael Klichinsky

It is important to note the small sample size of this study. However, the authors are optimistic and are currently enrolling patients for group two of the Phase I trial. Group two patients will receive a single infusion instead of three spaced infusions. "We're looking to see if [fewer infusions] change the safety profile, but it is not expected to," said Reiss. Additionally, Carisma Therapeutics is opening a combination study using CT-0508 alongside pembrolizumab, an anti programed cell death protein 1 (anti-PD1) antibody and T cell checkpoint inhibitor. The researchers hope this will combat T cell exhaustion, a common problem in late stage cancer, and thus work synergistically with the CAR macrophage to bolster immune system attack.

"Nowadays, if you can quickly retarget, repurpose, or reprogram an immune cell, that will hopefully be beneficial to patients with a range of cancers and other diseases," said Michel Sadelain, an immunologist at Memorial Sloan Kettering and pioneer of CAR T cell therapy who was not involved in the recent studies. The preliminary results from the CAR macrophage trial are promising for the treatment of not only solid tumors but autoimmune disorders and other immune-related disorders as well. "There's a big exploration now, let's see what comes out of it all. It's research, not everything will work," said Sadelain. "But there are so many opportunities and possibilities that certainly many of us believe and hope that there will be many more cell therapies in the years to come." ■

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explained

# How Do Cells Migrate?

*Cells possess a remarkable ability to move. Understanding the mechanisms underlying their choreographed movements unveils the secrets of many biological processes.*

BY YUNING WANG, PHD · ILLUSTRATED BY EMILY LAVINSKAS

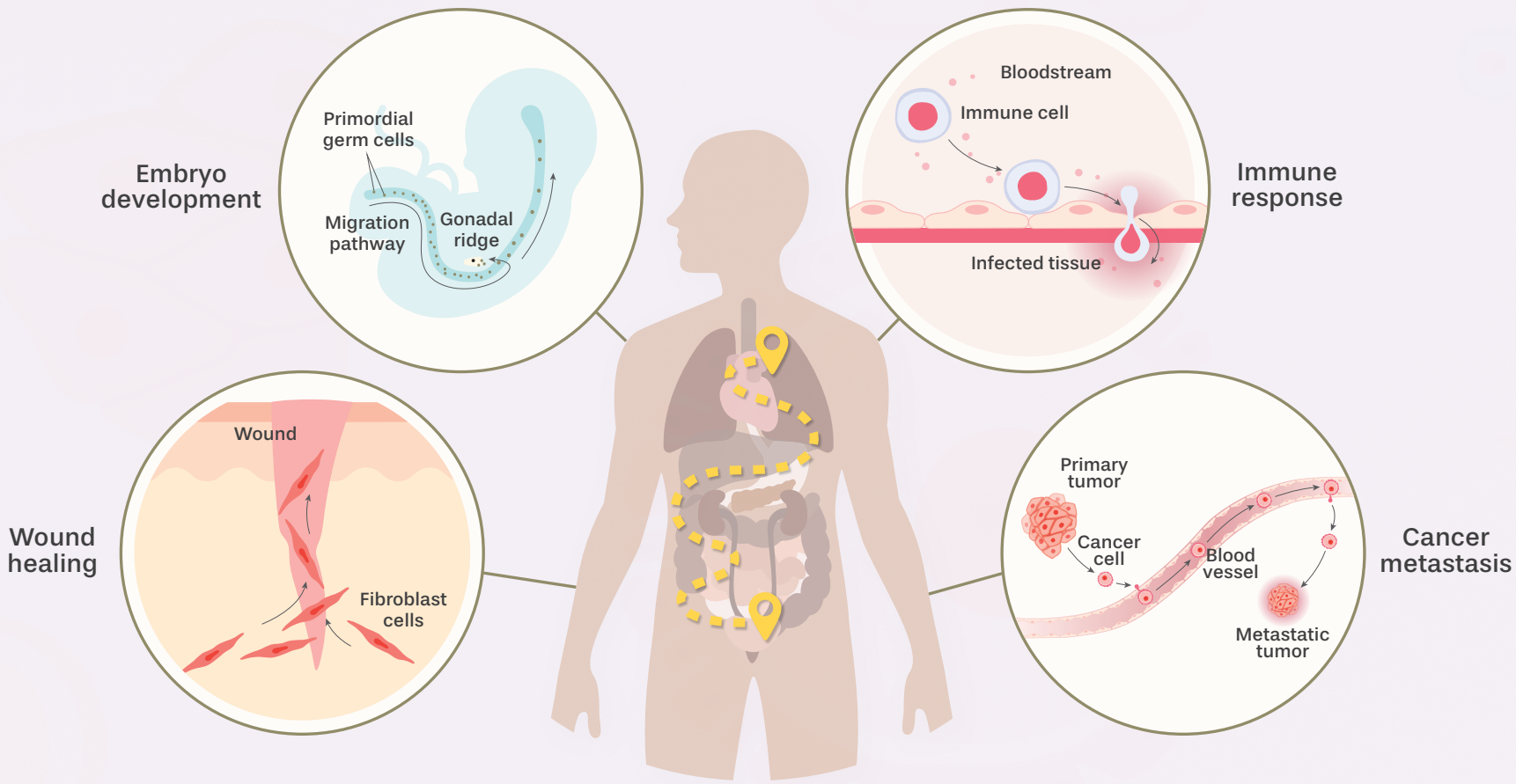
When microbiologist Antonie van Leeuwenhoek first peered at bacterial cells through his microscope, he described these motile entities as little animalcules. Today, scientists know that cell movement is a fundamental process in all living organisms. Cells frequently move through crowded microenvironments and travel long distances to reach remote tissues, enabling diverse biological phenomena in health and disease.

## Why do cells migrate?

Cell migration begins from the earliest stage of an animal's life. As the embryo develops, it transforms from a continuous epithelial sheet of cells into a complex, multidimensional structure that gives rise to diverse tissue shapes. The pivotal force of this transformation is the migration of embryonic epithelial cells, which move inward to form three primary germ layers. Cells in these layers continue to move, reaching their designated locations within the embryo, where they specialize into distinct cell populations (1). These early migration events determine the precise shape and position of cells during organogenesis, crafting the ultimate architectures of tissues and organs in the adult body.

In adult organisms, cell migration remains prominent. During wound healing and tissue regeneration, fibroblasts migrate from underlying tissue layers to replace old or damaged cells, maintaining tissue integrity and homeostasis. Immune cells constantly patrol the body, exiting the bloodstream and entering the tissue during immune responses (2). Such motility allows them to effectively defend the body against pathogens and foreign invaders.

To ensure proper functioning, the body precisely regulates cell migration in space and time. When cells fail to migrate correctly, severe pathological conditions can occur, including birth defects, chronic wounds, and immune deficiencies. Similarly, undesirable migratory events can lead to detrimental consequences like metastatic cancer, where tumor cells break free from their origins to invade normal tissues (2).

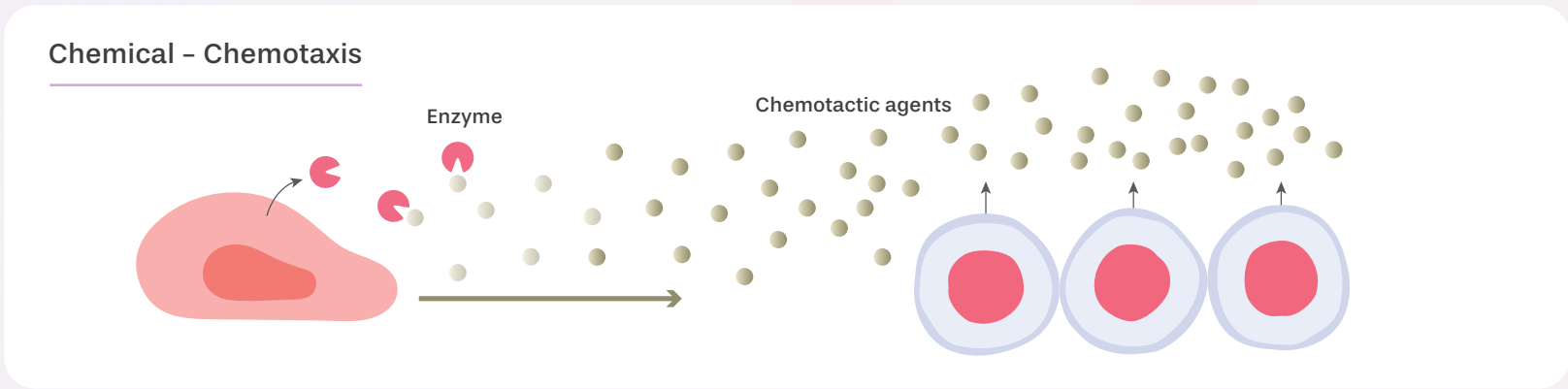


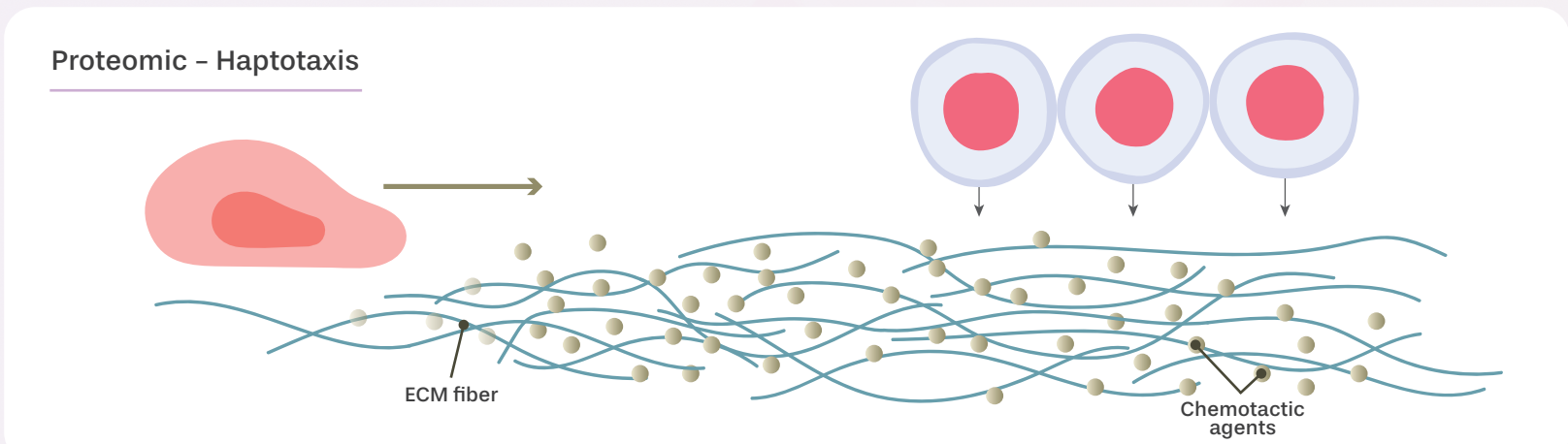
# What determines the direction of cell movement?

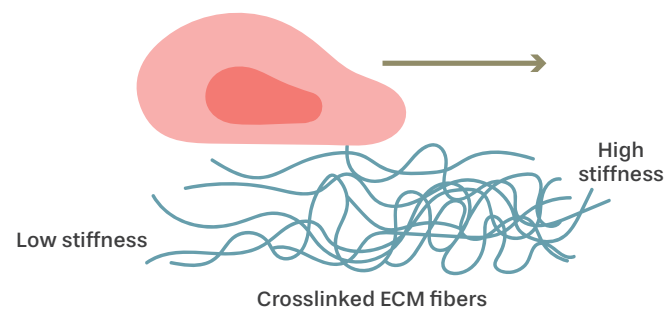
More than a century ago, microbiologist Theodor Wilhelm Engelmann from the University of Utrecht and botanist Wilhelm Pfeffer from the University of Tübingen discovered that bacteria migrated toward nutrient sources and away from noxious acids (3). Since then, scientists have found that cells within multicellular organisms also react to diverse chemical stimuli in their environments, including small peptides, metabolites, growth factors, and chemokines. When these agents form a gradient, cells orient their movement along the gradient in a phenomenon known as chemotaxis (4). Such gradients are transient, typically directing cells to migrate toward or away from the gradient sources over short ranges. Interestingly, migrating cells can generate the gradients themselves. They achieve this by secreting enzymes that degrade the initially distributed chemotactic agents in the environment, giving rise to a steep local gradient. As a result, the gradient constantly moves with the cells, allowing them to traverse long distances (4).

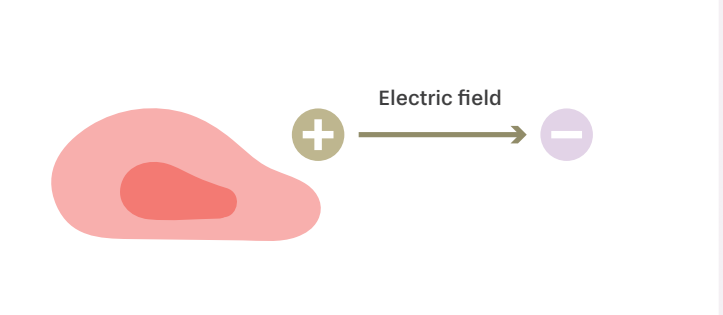
The extracellular matrix (ECM) contains a multitude of fibrous proteins such as fibronectin, laminin, collagen, and elastin that provide adhesion sites for cells. Variations in concentrations of these ECM proteins generate adhesion gradients. Similar to chemotaxis, cells migrate along these gradients in a process called haptotaxis (4). ECM proteins bind to chemotactic agents secreted by cells, creating immobilized gradients that serve as migration cues. During haptotaxis, migrating cells may deposit or break down ECM proteins. This remodels the ECM and modifies the haptotactic gradient, enabling cells to navigate toward specific locations.

In addition to chemical cues, mechanical and electrical stimuli also dictate the direction of cell movement. In a behavior known as durotaxis, cells sense extracellular rigidity and tend to migrate toward stiffer parts of the ECM. ECM stiffness arises from the crosslinking of ECM components such as collagen and fibronectin. While moving, migrating and surrounding cells can actively modulate ECM stiffness by depositing or degrading ECM components (5). Galvanotaxis, on the other hand, is directed cell motion guided by electric fields. For example, when an injury occurs, ions seep from damaged cells, producing abnormal electrical currents. The detection of these electric fields by nearby cells triggers their migration toward the wound to facilitate healing (5).



# How do cells move?

When moving on a 2D substrate, cells go through repetitive cycles of protrusion, adhesion, and contraction (6). In this cycle, the cell first protrudes lamellipodia, sheet-like projections composed of branched actin filaments, at its leading edge. Next, the cell establishes temporary adhesions to the substrate via integrins that bind to the matrix. These adhesions connect to the contractile actomyosin fibers within the cell, which pull from the front and squeeze from the rear, driving the cell body forward. After the cell body advances, the rear detaches from the substrate. The cycle then repeats with new cell protrusions at the leading edge.

In contrast to *in vitro* 2D substrates, cell movement in 3D living tissues faces more challenges as cells must navigate through a complex network of ECM barriers and surrounding cells. To accomplish this, cells employ different migratory modes, including mesenchymal, amoeboid, and lobopodial.

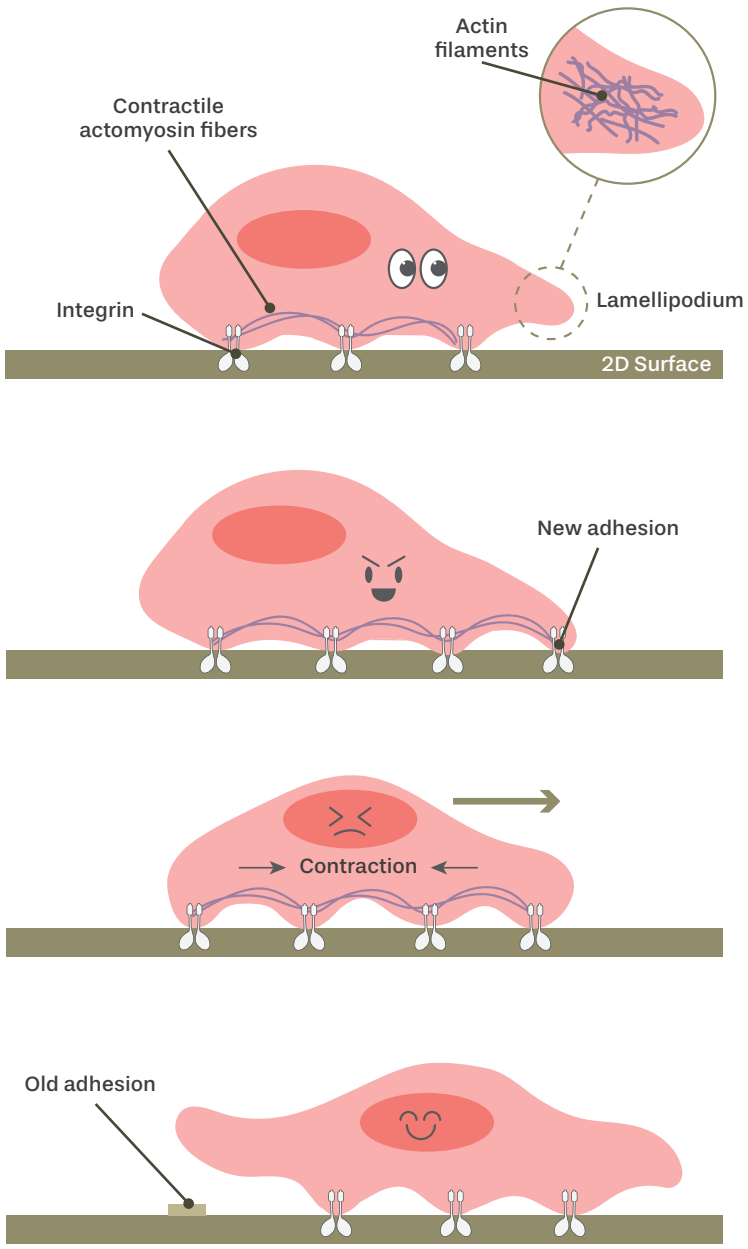
Mesenchymal cells, which are multilineage cells capable of self-renewal and differentiation into various cell types, orient themselves along the ECM fibers, while secreting proteases that digest a tunnel in the ECM. The microtubule-organizing center within mesenchymal cells is located ahead of the nucleus, facilitating the delivery of protease-loaded vesicles to the cell front for ECM remodeling (7).

In amoeboid migration, cells exhibit a rounded morphology and undergo extensive shape changes, resembling a moving amoeba. This migration mode requires few adhesions to the ECM. Instead, the cell forms actin protrusions or spherical membrane bulges called blebs and moves by extending these blebs through narrow pores in the ECM. The nucleus, which is typically located in the cell front, serves as a ruler that gauges the diameter of pores within the ECM, guiding the cell to choose the path with minimal resistance (7).

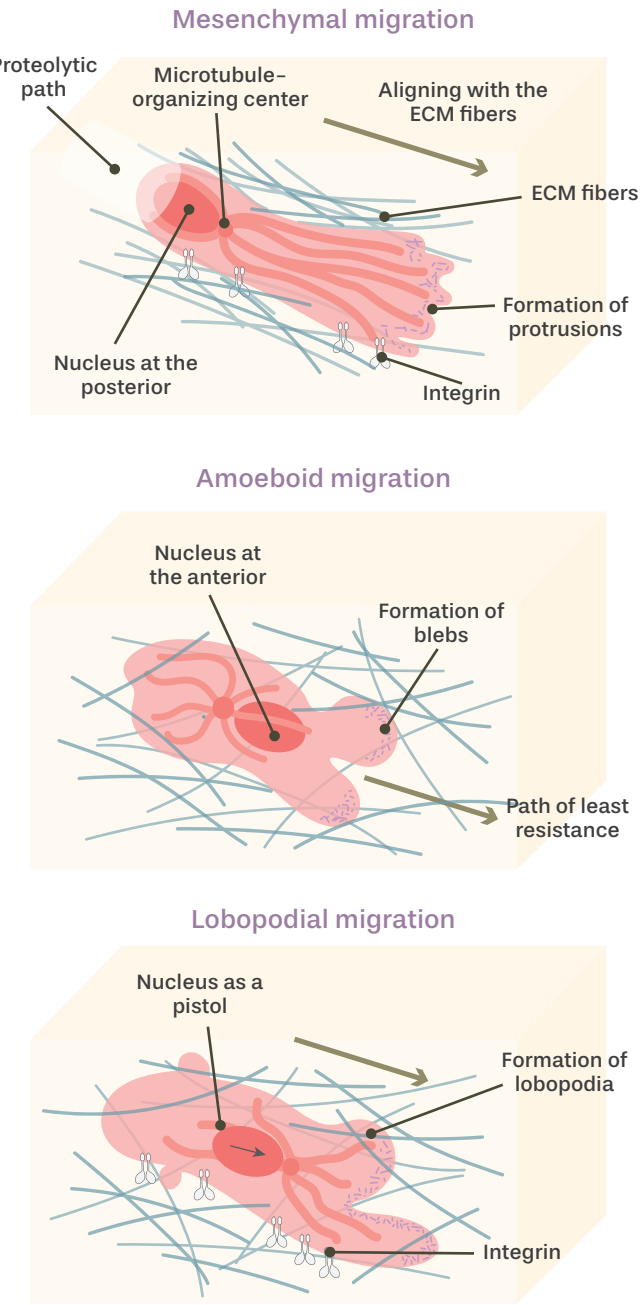
Often found in a highly crosslinked ECM, lobopodial migration exhibits features of both amoeboid and mesenchymal migration. The cell creates bleb-like protrusions called lobopodian at its leading edge. At the same time, the cell firmly attaches itself to the substrate, and the contractile actomyosin fibers pull the nucleus forward like a piston in a cylinder to generate pressure at the front, propelling the cell ahead (7).

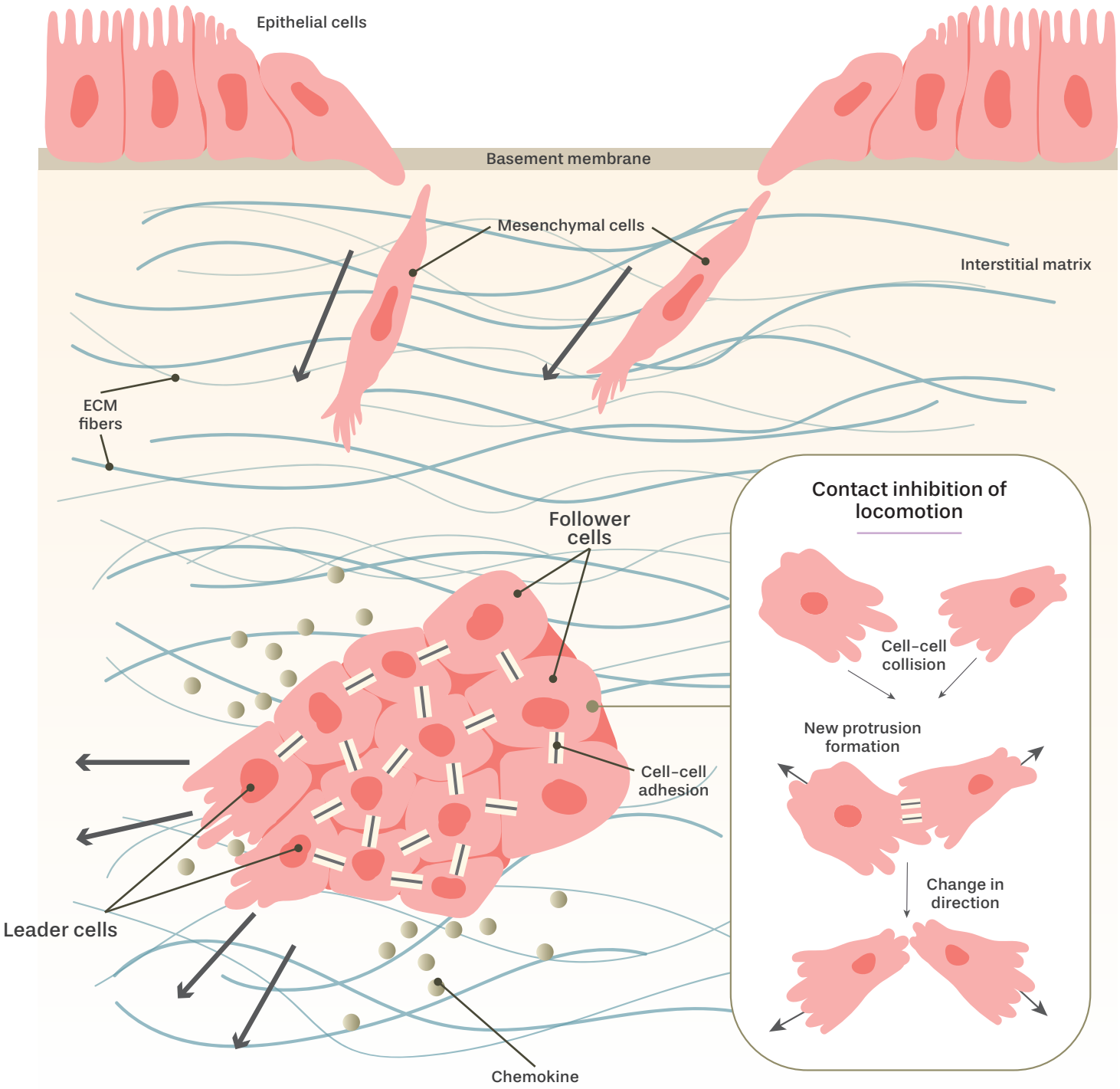
Various factors including cell-ECM adhesions, protrusion types, cellular contractility, and proteolytic capacity, influence cell migration. Cells adapt or switch between different migratory modes, opting for the most suitable approach to advance themselves.

## 2D cell migration




## 3D cell migration





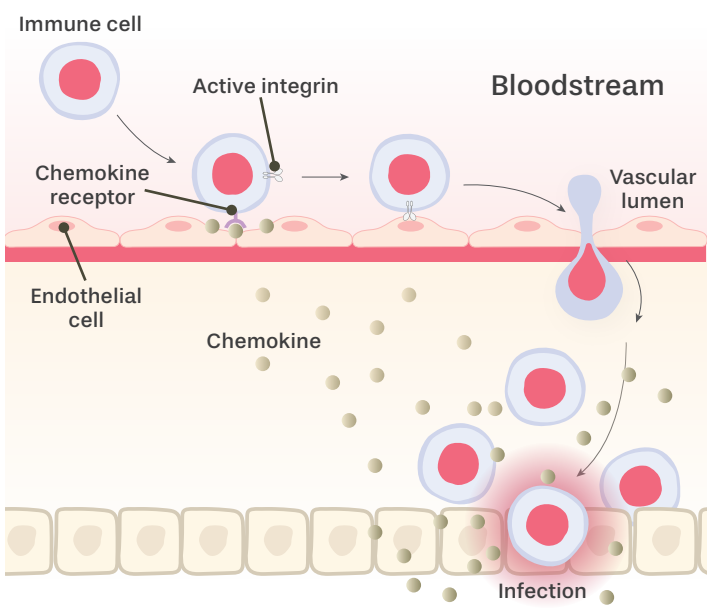

# How do groups of cells move together?

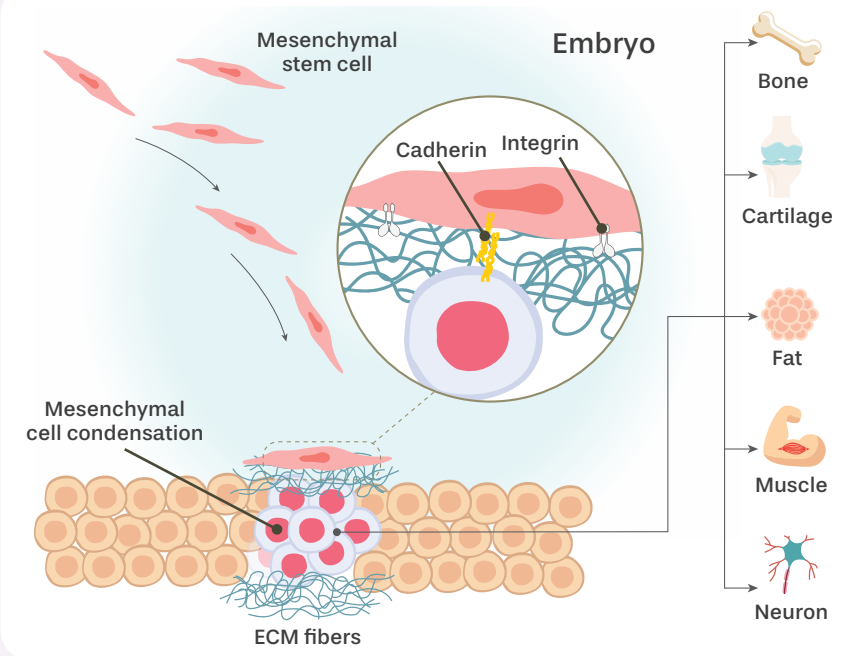
Some cells travel and find their ways individually. However, during the development of multicellular organisms, tissue regeneration, and tumor dissemination, cells often team up to form sheets, sprouts, strands, tubes, or clusters for more efficient migration. This collective movement typically begins with a process known as epithelial-to-mesenchymal transition (EMT), where static epithelial cells acquire migratory properties to transform into mesenchymal cells (7).

Once these motile mesenchymal cells form, they come together and join forces. Adjacent cells establish interactions between their surface receptors, which connect to the actin cytoskeletons within cells. This interconnectedness enables the cells to communicate and transmit forces among each other. Cells that lose contact with their adjacent partners halt their migration until they reconnect to the collective (7). By utilizing different adhesion receptors and cytoskeletal systems, cell-cell adhesions can be transient or stable, tight or loose, enabling diverse forms of collective cell migration (8).

During collective migration, leader cells pioneer the way. They sense the surrounding microenvironment and guide the direction and speed of follower cells. When exposed to environmental cues such as ECM fibers, chemokines, or growth factors, leader cells polarize themselves along the migration direction, elongate their shapes, and extend protrusions. To drag follower cells behind, leader cells transmit forces through their interconnected cytoskeletons as well as release chemotactic signals that prompt the follower cells to move. Additionally, while moving through the 3D environment, leader cells actively modify their migration paths by interacting with the ECM and secreting proteases that break down ECM fibers, facilitating follower cells' movements (8).

While leader cells guide the migration paths, follower cells help maintain the unity of the team. When two migrating cells come into contact, they undergo contact inhibition of locomotion (CIL). During CIL, both cells stop moving toward each other, retract their protrusions, and diverge in separate directions. This prevents follower cells from producing protrusions or stacking up on one another. As a result, only leader cells at the front edge maintain their protrusions in the direction of migration, ensuring a uniform cell polarity across the entire cell cluster (9).




# How do migratory cells stop moving and settle into their new environments?

Following an arduous journey, migrating cells settle at their intended destinations to execute their functions. Circulating immune cells stop moving once they encounter chemokines immobilized within the vascular lumen produced by endothelial or other immune cells near an infection. Binding these chemokines activates integrins on immune cells' surfaces, leading to their adherence to endothelial cells, which prompts them to exit the circulatory system and move toward an infection site (10). Metastatic cancer cells in blood vessels use a similar mechanism to leave the bloodstream and invade surrounding tissues (11).

To adapt to their new surroundings, migratory cells establish stable connections with local environments. In the case of embryonic development, once migratory mesenchymal stem cells (MSCs) reach their target regions, they express specific cell adhesion molecules such as cadherin, which tightly stick the cells together, forming a condensed cluster (12). As cell-cell interactions increase, the cells rearrange their actin cytoskeletons, changing their shapes from spread to round. During this condensation, MSCs also deposit new ECM components such as fibronectin, introducing additional contact sites to stabilize the already arrived cells and accommodate incoming ones (13). The high cell density and fibronectin-rich matrix make a conducive environment for MSCs to shift into the differentiation phase, leading to the development of a range of tissues such as cartilage, bone, fat, skeletal muscle, and neurons. Likewise, during wound healing, fibroblasts that have migrated to the wound secrete various ECM components, including collagen and fibronectin, which create a provisional scaffold that fosters epithelial cell proliferation and drives tissue regeneration (14).

## Tracking every cell step

Much like zoologists utilize radio telemetry and geolocation devices for tracking animal movement in the wild, scientists employ specialized tracking tools to study cell migration. Various microscopy techniques enable real-time visualization of cell movement within living organisms through fluorescent labeling or genetic modification. These methods are typically applied to laboratory animals like mice and zebrafish.

Human cell migration studies rely on *in vitro* methods, such as transwell chambers, which consist of a permeable membrane that separates two compartments in a culture dish. Cells in the upper chamber migrate toward a chemotactic agent in the lower chamber (15). By quantifying the number of cells that successfully cross over, scientists can gain insights into the cells' migratory abilities in response to different stimuli. Recent advances in microfluidics and organ-on-a-chip technologies have revolutionized human cell migration research. These innovations recreate cellular microenvironments, facilitating the precise manipulation of factors that affect cell migration. For example, 3D microfluidic devices with biochemical and biophysical cues mimic pathophysiological conditions associated with different tumor progression events, enabling scientists to closely follow each stage of cancer metastasis (16). These devices may also help uncover crucial factors in immune and neurological disorders and discover novel drugs that target specific steps of aberrant cell migration.

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