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SURVIVING THE STOMACH IS KEY

Over the past two decades, biologic drugs — drugs composed of proteins produced by living cells — have become the safest, most effective top sellers within the pharmaceutical industry.

Approved to treat a variety of diseases including rheumatoid arthritis, diabetes, multiple sclerosis, Crohn’s disease, and a whole range of cancers, these drugs include monoclonal antibody therapeutics, hormones, and immune system signaling molecules. Their safety and efficacy depend largely on the complex, three-dimensional structure of the protein product itself — which is incredibly delicate and time-consuming to develop on a large scale. The majority of these are administered via injection directly into the bloodstream for maximum potency.

So, why can’t we just swallow a biologic pill? The simple answer: biologics would not survive the acidic pH and digestive enzymes of the gut. Even if survival were possible, the next issue to contend with is absorption; if the protein is not broken down properly, it will not be absorbed into the bloodstream. Companies seeking to develop oral delivery of biologics must overcome both hurdles.

Injectable delivery is cumbersome at best. It has a significant impact on a patient’s quality of life, which in turn affects compliance to drug therapy regimes. In many cases, patients must make regular trips to an infusion center to receive required treatments. Thus oral administration is the holy grail in terms of delivering biologics for maximum potency.

This may sound like the stuff of science fiction — and indeed, the device is still in preclinical trials — but the robotic pill has attracted big name pharma partners, including AstraZeneca (London, UK) and Novartis (Basel, Switzerland), as well as investment from Google Ventures (Mountain View, CA) among other venture funds. If the device ultimately succeeds, it will revolutionize the delivery of biologic drugs.

ENGENE’S GUT CELL FACTORIES

enGene (Vancouver, Canada) is bypassing the delivery obstacle by attempting to turn the cells of the gut into drug-producing factories. The trick is to deliver a gene — the instructions for a specific therapeutic protein — to those cells. enGene is using tiny carbohydrate-based nanoparticles to encase the gene. The carbohydrate coating protects the gene as it passes through the stomach, yet allows it to be absorbed into the intestines. The protein is then made in the intestines. This approach is especially appealing for diseases affecting the colon and small intestine, such as inflammatory bowel syndrome or Crohn’s disease.

enGene is currently using this platform in the preclinical development of the anti-inflammatory protein IL-10, for the treatment of inflammatory bowel disease.

APPLIED MOLECULAR TRANSPORT

Applied Molecular Transport South San Francisco, CA) is using a protein scaffold adapted from pathogenic microbes such as salmonella, which colonize the gut by secreting immune-crippling proteins into our body. Proteins from these “gut bugs” work by tricking the intestines into absorbing toxic proteins in the same way...
that they absorb nutrients from food. Applied Molecular Transport scientists have tweaked these microbial proteins to carry a therapeutic payload, rather than the toxins. Development efforts are currently aimed at delivering anti-inflammatory proteins to the intestines. Swallowing biologic drugs is no longer a pipe dream. Sensing the opportunity to make a significant impact on the biopharma industry, innovative companies are approaching the drug delivery problem with a range of strategies. It is likely that no single strategy will work for all types of biologics, but any success with a handful of products would represent a major breakthrough for the industry.
HOW DO ALLERGIES DEVELOP?
Watching a game at the ballpark and digging into a bag of peanuts is a source of entertainment for many Americans. For the 15 million who suffer from peanut allergies, the idea of being taken out to the ballgame elicits concern — or even anxiety.

Food allergies — think tree nuts, milk, eggs, wheat, soy, fish, and shellfish — are on the rise. The mere dust particle of a freshly cracked peanut can be responsible for an unpredictable cascade of reactions, including death brought about by anaphylaxis.

This WEEKLY takes a swing at explaining how allergies develop, the current treatments, and what new products might change the way allergen desensitization therapy is delivered.

SOMETHING TO SNEEZE AT
The host of symptoms dubbed “allergies” are the end result of the immune system’s response to a normally harmless substance, as if that harmless substance were a threat. An initial allergen exposure results in the production of a class of antibodies called Immunoglobulin E (IgE). A second exposure to the allergen results in an “allergen-IgE antibody complex” These newly produced complexes bind to and activate mast cells — a type of immune cell. As the image below shows, activated mast cells send out chemical alarms in the form of histamine.

TERM OF THE WEEK: HISTAMINES
Histamines are small molecules produced by mast cells. Once released, histamines bind to receptors on the surface of blood vessels, increasing their permeability — the ease with which fluid moves out of the blood vessels. Histamines also bind to receptors on certain types of nerve cells, resulting in muscle contraction. A host of symptoms can be triggered, ranging from annoying to deadly. Typical signs of histamine release include:

- Increased blood vessel permeability resulting in a runny nose and watery eyes.
- Increased muscle contraction leading to throat constriction and difficulty breathing.
- Extreme fluid release from tissues causing a sudden drop in blood pressure, potentially bringing on a heart attack.
- Difficulty breathing and swallowing, swelling, heart palpitations, and unconsciousness — sometimes causing death.

Mild allergy symptoms such as runny nose and watery eyes can often be successfully controlled by the use of an over-the-counter antihistamine, a drug that works by blocking the interaction of histamines with receptors on nerve and muscle cells. However, once anaphylaxis — a severe allergic reaction that includes difficulty breathing and heart palpitations — has occurred, it is too late for antihistamines to be effective. These symptoms can only be treated with an injection of the hormone epinephrine — and the sooner, the better in cases where a life is on the line.

Epinephrine helps to reverse histamine’s effects by decreasing blood vessel permeability, relaxing muscle cells, and stimulating the heart. People at risk for anaphylaxis need access to an epinephrine auto-injector — a spring-loaded syringe that makes the lifesaving shot readily available. This type of product is referred to as a “combination product” because it combines a device (the auto-injector) with a medicine (epinephrine). Mylan (Canonsburg, PA) EpiPen is an epinephrine auto-injector, as is Amedra’s (Horsham, PA) Adrenaclick.
THE HYGIENE HYPOTHESIS
Epidemiologists have noticed an interesting trend as countries rise from developing to developed status: an improvement in sanitation and access to antibiotics means less pathogenic exposure and lower infection rate. As the infection rate drops, the incidence of allergies shoots up.

Many scientists think early exposure to infection helps shift the immune response towards fighting pathogens while minimizing the production of IgE antibodies. Exposure to potential allergens (while the immune system is still developing) helps to desensitize the allergic response.

THE STRATEGY BEHIND DESENSITIZATION THERAPY
The idea of allergy desensitization through controlled exposure has been around for decades. Desensitization is the principle behind allergy shots shown to be effective against pet dander, dust mites, and pollen. Desensitization therapy was once considered to be too risky for food allergies, but a number of new studies support the idea that gradual exposure to food allergens may be beneficial.

In January 2017, the National Institutes of Health (NIH) released new guidelines recommending the early introduction of peanuts into children’s diets, including those considered to be at high risk for developing peanut allergies because they already have severe eczema or egg allergies. These new guidelines are based on the results of the NIH-funded Learning Early About Peanut Allergy (LEAP), a study which randomly assigned 600 high-risk infants to either peanut-avoidance or the regular inclusion of small amounts of peanut products in their diet for the first five years of life. At age five, peanut allergy was assessed – and an 81 percent reduction in allergy was found in the children who regularly consumed peanuts when compared to those who avoided them.

ALLERGENS BY THE DOSE
The current allergen immunotherapy market includes allergy shots (which require monitoring by a physician) and drops or tablets dissolved under the tongue (which can sometimes be taken at home). Aimmune Therapeutics’ (Brisbane, CA) AR 101 is a dissolvable tablet made of pharmaceutical grade peanut protein that can be mixed with food as a means of delivery. AR 101 Phase II studies report patients becoming desensitized to doses at least twenty times greater than the original allergy-inducing dose — and in some cases, more than 100-fold greater. The goal is to reach a tolerance level that offers protection against accidental eating of peanuts. The product was awarded Fast Track status by the FDA and is now in Phase III. Aimmune has plans for clinical studies on immune system training for egg and milk allergies to begin later this year.

DBV Technologies (Bagneux, France) is also in the allergy fight. They are currently conducting clinical trials on their Viaskin skin patch which delivers low doses of either peanut, milk, or dust mite allergens. By delivering an allergen through the skin instead of the blood, the body will react less severely, reducing the risk of anaphylaxis as a side effect. The Viaskin peanut patch, currently in Phase III clinical testing, has been awarded Fast Track designation by the FDA.

As allergen desensitization treatments continue to make their way through the drug pipeline, allergy sufferers remain hopeful for better and easier treatment options, and maybe even one day — a cure.
New Hope For Spinal Muscular Atrophy

FIRST THERAPY APPROVED FOR SMA

Squeaking by on December 23rd as the last new drug approval of 2016, Biogen's (Cambridge, MA) Spinraza now provides hope for the thousands of families affected by a debilitating neuromuscular disorder known as spinal muscular atrophy (SMA). SMA robs people of their ability to walk, eat, and ultimately, breathe.

In addition to Spinraza, there are 13 other new therapies making their way through the clinic, according to the patient advocacy group Cure SMA. The increase is largely due to a better understanding of the disease and a surge in funding for basic and clinical research. SMA affects about 1 in 10,000 babies born in the United States.

In this weekly, we'll decipher the science behind SMA, explain the novel mechanism of action used by Biogen's new drug, and find out how other drugs in development are zeroing in on this genetic disease.

SMA PRIMER

Our nervous system consists of the brain, spinal cord, and a vast network of nerves that feed into every tissue of the body. Motor neurons are a type of nerve cell that sends messages from the spinal cord to muscles, enabling movement.

In order for the motor neurons to do their job, a functional protein called the survival motor neural (SMN) protein is necessary. The survival motor neuron 1 (SMN1) gene is responsible for producing most of the SMN protein used by the body. A second, closely related gene is the survival motor neuron 2 (SMN2) gene, which produces a much smaller amount of SMN protein and is seen as a sort of “back-up” version to SMN1.

SMA is caused by a variety of mutations in the SMN1 gene. Without functional SMN protein, the neurons do not work correctly and eventually die. How soon they die depends on the extent of the SMN deficiency, which correlates with the severity of the disease: the less SMN produced, the more severe the disease.

The back-up gene, SMN2, produces a small amount of functional SMN protein. However, differences in the way SMN2 functions means most (but not all) of the protein is non-functional and degrades shortly after being produced. Patients with less severe forms of the disease usually have extra SMN2 copies because ultimately, even tiny amounts of SMN protein provides some motor nerve function.

The four generally accepted classifications of SMA are:

- **Type 1**: The most severe and the most common. Babies do not move, but lay perfectly still in their cribs. As the disease progresses, toddlers have trouble with swallowing and respiratory function. SMA Type 1 is usually fatal by age two.
- **Type 2**: Symptoms manifest between six and eighteen months. These children can typically sit but not stand or walk. Respiratory function is often compromised, however with the help of machines many of these patients live into adulthood.
- **Type 3**: Symptoms occur after age one. These kids are usually able to walk, but may lose that ability as the disease progresses. Respiratory function is less impaired, and life expectancy is often near normal.
- **Type 4**: This is the adult-onset form, typically developing at age 30 or later. Muscles gradually weaken, and the patient often needs to use a wheelchair later in life. Life expectancy is not affected.

SMA Type 1 is the most common and most severe, making up 60% of cases. As a result, many companies are looking to tackle this segment of the disease population. Below find a few treatment approaches for SMA Type 1.

ON THE MARKET: ANTISENSE THERAPY

Developed by Biogen in partnership with Ionis Pharmaceuticals (Carlsbad, CA), Spinraza is one of a small but growing class of drugs: antisense therapeutics. It is a synthetic mRNA molecule that binds to the naturally occurring SMN2 mRNA in such a way that more of the mRNA is used to make the protein. The result is greater amounts of full-length, functional SMN protein. Recall from high school biology that mRNA provides the instructions to make proteins. If the mRNA is working properly, the correct, functional protein is made.
IN DEVELOPMENT: SMALL MOLECULE ENHANCERS

PTC Therapeutics (South Plainfield, NJ) — in partnership with Roche (Basel, Switzerland) — has begun Phase II clinical studies on its proprietary small molecule drug, RG67800. RG67800 is similar to Spinraza in that it changes the way nerve cells process the SMN2 mRNA, resulting in increased production of functional SMN protein. However, a notable difference is the delivery mechanism—Spinraza is an injectable while RG67800 is a pill. Novartis (Basel, Switzerland) is preparing for Phase I clinical testing of a small molecule modulator of SMN2 mRNA.

IN DEVELOPMENT: A GENE THERAPY CURE?

As a single gene disorder, SMA is an ideal candidate for a gene therapy approach. Scientists deliver the corrected version of the mutated gene by a viral vector — a virus that has been stripped of its disease-causing ability, and modified to carry a correct version of the mutated gene. The viral vector can also be engineered to drop its genetic cargo into specific cells. In the case of SMA Type 1, the AAV9 vector crosses the blood-brain barrier and delivers corrected copies of the SMN1 gene into motor neuron cells in the brain.

AveXis (Bannockburn, IL) has a gene therapy candidate, AVXS-101, in Phase I/II clinical studies. The company reported promising results in mid-2016, noting that the drug appears to be safe and effective. Babies who received this gene therapy showed marked increases in SMN production and in movement. Also in the gene therapy mix is Voyager Therapeutics (Cambridge, MA) with their treatment currently in preclinical development.

With additional approvals hopefully to follow in the wake of Spinraza, SMA patients and their family members are hoping to kiss this devastating illness goodbye.
Coming soon to a supermarket near you: apples that have been genetically modified to resist browning. Dubbed Arctic Apples, the plants were approved two years ago by the USDA and will begin appearing on select retailers’ shelves in Midwestern U.S. states this month.

Developed by Okanagan Specialty Fruits (Summerland, B.C., Canada), the Arctic Apple also represents a new type of genetically altered food — one that has been engineered to directly appeal to and benefit the consumer rather than the farmer. Their debut may also help ease lingering safety concerns voiced by GMO critics due to the innovative technology used to create Arctic Apples. Let’s take a look at what causes apple browning, how science is able to produce a remedy, and preview what’s on the horizon for consumer-oriented genetically modified food.

Oxidation Causation

The browning observed in conventional apples is the end result of an oxidation reaction — a chemical reaction between oxygen and another substance. Typically, oxidation reactions result in a color change (think rusting car — the oxidation of metal). In apples, browning is the oxidation of “phenolic compound” by an enzyme called polyphenol oxidase (PPO).

In order for oxidation to occur, PPO has to come into contact with the phenolic compounds. Within the apple cells, phenolic compounds are typically sequestered inside of membrane-bound compartments. If the cell is disrupted, by slicing or dropping the apple, the seal of these compartments is broken and the phenolic compounds exposed. PPO is then able to act, oxidizing the compounds to create browning.

Silencing the Gene

Scientists at Okanagan Specialty Fruits chose to tackle apple browning by blocking the production of the PPO enzyme. No enzyme, no chemical reaction, no browning. They used a gene-silencing technique known as RNA interference (RNAi) to block the production of PPO.

Recall that RNA serves as the intermediary between DNA and proteins. The information in DNA is converted to RNA, which is then translated into a protein by organelles called ribosomes. One way to stop PPO production is to get rid of the PPO RNA before it enters the ribosome. Enter RNAi.

RNAi is a short, complementary RNA sequence that attaches to a target RNA, in this case the target is PPO RNA. This creates a double-stranded RNA, which is universally recognized as viral RNA and is destroyed by another enzyme known as RNA-induced silencing complex (RISC). In the case of the Arctic Apple, RNAi attaches to the PPO RNA and is destroyed by RISC. No PPO RNA ever makes it to the ribosome, so no PPO is ever produced by the apple. No enzyme, no chemical reaction, no browning.

Why Less Brown?

Why go to all this trouble to make apples less brown? This modification is expected to benefit growers, packers, shippers, and retailers by limiting the amount of bruising and other visual imperfections in their apples — meaning less waste and more product to sell. Food processors, likewise, will be able to produce more consistent juices, sauces, and sliced apple products, without relying on antioxidant treatments currently in use.

And since Arctic Apples contain no foreign DNA, they are likely to be more palatable to GMO-wary consumers. The apples have undergone 10 years of field testing, and do not differ in any significant respect from non-modified apples, apart from the lack of PPO enzyme. Based on field studies, blocking the production of PPO does not make apple trees more susceptible to pests, and so their adoption should not result in any increased pesticide use.

On the Market

The first two varieties of modified Arctic Apples appearing on the market are Golden Delicious and Granny Smith. If successful, they could pave the way for other consumer-oriented products such as non-browning cherries and pears, also in development at Okanagan Specialty Fruits.
A similar product — the Innate Potato — was approved in November 2014 by the USDA. Developed by J.R. Simplot (Boise, Idaho), Innate also uses RNAi technology to decrease production of the PPO protein, again with the goal of reduced browning. Innate potatoes also use RNAi to knock out a second protein, asparagine synthetase-1.

When potatoes are cooked at high temperatures, asparagine synthetase-1 reacts with the potato sugars to produce a chemical called acrylamide — which has been linked to cancer in rodents. The Innate potatoes are already on the market in the U.S. at select retailers.
INDUCING STEM CELLS TO HEAL
Headlines touting stem cells often claim the therapies heal everything from hair loss to hearing loss. While many of these treatments are not FDA approved, there are some promising innovations winding through preclinical and clinical development. Here at WEEKLY headquarters, we like to tease out the science behind the scene, so let’s review regenerative medicine basics and survey the companies attempting to repair damaged tissue with these high potential cells.

STEM CELL PRIMER
Stem cells are unspecialized cells that have the ability to develop (differentiate) into 1 of 200 cell types in the body. There are two general classifications:

- **Embryonic stem cells**, found only in developing embryos, can become any cell type within the adult body.
- **Adult stem cells**, found within organs of a fully developed body, can become only certain cell types. Typically these cell types come from the organ in which they are found.

Embryonic stem cells have the most therapeutic potential. A main focus of research is sussing out which combo of hormones cause a stem cell to commit to becoming a specific tissue; for example, spinal cord tissue vs. brain tissue. The billion dollar market: grow and transplant stem cells into patients who have lost tissue due to acute trauma.

Since embryonic stem cells may come from unrelated donors, there is the likelihood of tissue rejection. Thus there is considerable interest in developing therapies using a patient’s own adult stem cells.

In certain tissues such as bone marrow, muscle, liver, and skin, damaged or worn out areas are naturally and regularly replaced by activated adult stem cells. In other tissues such as heart and brain/spinal cord, adult stem cells exist in such small numbers, our body cannot readily activate them to replace tissue. Enter stem cell therapy.

Let’s take a look at companies trying to boost the regenerative capacity of these tissues less adept at self-repair.

PUSHING THE PROGENITOR
Progenitor Therapeutics’ (Stevenage, UK) name hints to the company’s main technology. A progenitor cell is one that has taken the first steps to becoming a specialized cell, such as a cardiac cell. Once these steps are taken, the stem cell is “committed” to becoming that specialized cell type.

Progenitor Therapeutics is focused on discovering small molecule drugs that will nudge adult stem cells down the differentiation pathway. They do this by testing hundreds of thousands of drugs on stem cells to see which bring out progenitor cell types. Although still in preclinical development, their work offers a tantalizing future of popping a pill to regenerate damaged tissue.

IGNITING NEURAL STEM CELLS
Neuronascent (Clarksville, MD) and Neuralstem (Germantown, MD) are leading the charge in developing small-molecule activators of neurogenesis, or the generation of neurons (brain cells) from stem cells. By screening large chemical libraries, scientists at these companies have identified compounds that activate neurogenesis from adult neural stem cells both in the lab and in mouse models of various neurodegenerative disorders.

Neuralstem’s lead neurogenesis candidate, NSI-189, increased the size of the hippocampal region of mice brains by 20%. Neuralstem is beginning Phase II of NSI-189 for major depressive disorder. The drug is already in preclinical development for Alzheimer’s disease.

Neuronascent’s first focus is the development of small molecule compounds that promote neurogenesis for the treatment of Alzheimer’s disease. In an Alzheimer’s mouse model, the lead compound NNI-362 promoted the growth of new hippocampal neurons that not only migrated to the correct functional location, but also differentiated and survived long enough to reverse previously observed cognitive declines. Neuronascent is preparing for Phase I of NNI-362.
ON THE FAST TRACK WITH ANGIONETICS

Last month, Angionetics (San Diego, CA) won fast track designation for its gene therapy candidate, Generx, which is currently in Phase III clinical studies.

Generx is a viral vector — a virus that has been modified to deliver a specific gene to a target cell type — that delivers a gene for fibroblast growth factor 4 (FGF4) to cardiac tissue damaged due to lack of oxygen during a heart attack. The damaged tissue then produces the FGF4 protein, which promotes the formation of new blood vessels in damaged regions, increasing the heart’s ability to deliver oxygen-rich blood. The FGF4 gene delivered by Generx does not incorporate into the cardiac cell’s genome. This means the growth factor is only produced for a few weeks — just long enough to jump start the growth of new blood vessels.

NAKED DNA

Juventas (Cleveland, OH) is also developing a gene therapy for heart disease. However, instead of using modified virus, Juventas relies on plasmids — small, circular pieces of DNA engineered to contain the therapeutic gene. In this case, the plasmids are injected directly into damaged cardiac tissue. Advantages to this “naked DNA” approach include reduced patient immune response and lower manufacturing cost.

Dubbed JVS-100, the Juventas plasmid contains a gene for stromal-cell derived factor-1 (SDF-1), which recruits cardiac stem cells and promotes new blood vessel growth. Like Generx, JVS-100 does not integrate into the recipient’s own DNA, making the production of SDF-1 temporary. JVS-100 has completed Phase I clinical studies.

HEAR, HEAR

Good hearing depends on thousands of tiny hair cells within the inner ear that mechanically amplify low-level sound. As these hair cells are damaged or lost through exposure to excessive noise, toxic drugs, and normal aging, hearing loss occurs and we are not able to generate new hair cells.

That may change. The leaders behind Cambridge-based startup Frequency Therapeutics reported the discovery of a small-molecule activator of hair cell precursors — LGR5+ cells — which are a type of adult stem cell. Once activated, LGR5+ cells differentiate into hair cells. The work was done on animal models in the lab using cochlea — the portion of the inner ear which contains the hair cells. The findings suggest Frequency may be on the path to developing a drug to restore hearing loss.

WANTING THE WNT PATHWAY

Just last month, a new regenerative medicine company, Surrozen, was launched in South San Francisco. The company founders include scientists who have spent decades deciphering a cell signaling pathway known as the Wnt pathway, which plays a central role in stem cell maintenance and tissue regeneration. Surrozen’s focus is on developing drugs that activate this pathway, potentially leading to treatments for degenerative diseases.

If any of these companies are successful a shot of stem cells — not major surgery — may repair previously unfixable tissue damage. We’ll keep a close eye on these exciting developments and be sure to report them back to you.
BASICS & INNOVATIONS

Monoclonal antibody (mAb) therapeutics burst onto the healthcare scene 20 years ago, and today they remain one of the most versatile and effective therapeutics available. The tried and true mAbs are still in high demand, and we suspect this first wave of derivative products clamoring their way through the pipeline will be equally as successful. In this WEEKLY, we’ll review the basics of monoclonal antibodies and highlight some of the recent innovations within this therapeutic modality.

MAB PRIMER

Antibodies are proteins produced by B-cells, a type of white blood cell, in the immune system. Each antibody has a distinct shape that recognizes a unique target, often that unique target is a protein on the surface of a pathogen. These foreign proteins are known as immunogens. When an antibody recognizes its specific immunogen, it physically binds to it. This binding action alerts the immune system to attack and eliminate the pathogen.

The specific protein that triggers antibody recognition is called an immunogen, and the exact part of the immunogen that is recognized by the antibody is called an epitope.

This ability of antibodies to recognize and bind to a specific target is what makes them effective therapeutics. And researchers have expanded on this concept — for example, scientists may identify an antibody that binds to a protein on the surface of a cancer cell. When injected into a patient’s body, this binding triggers the patient’s immune system to attack the cancer.

EASILY CONFUSED: MONOCLONAL VS. POLYCLONAL ANTIBODIES

Commercially available antibodies are divided into two categories: Monoclonal and polyclonal. Monoclonal antibodies are antibodies that are all produced by the same B-cell, or by the clones (descendants) of that B-cell. This means they all recognize the same epitope on a target immunogen. Therapeutic antibodies are always monoclonal — this is required to ensure a consistent therapeutic effect.

Polyclonal antibodies are produced by a collection of different B-cells, and recognize multiple epitopes on the same immunogen. Polyclonal antibodies are used in diagnostic and research applications, where detection of a specific immunogen is the only requirement, and the specific epitope recognized doesn’t matter.

A BISPECIFIC CONNECTION

Antibodies are Y-shaped proteins. In antibodies produced by our own immune system, as well as most therapeutic antibodies, the two “arms” of the Y are identical, meaning that each antibody recognizes only one target. Bispecific antibodies, in contrast, have been genetically engineered by splitting and fusing the genes for two different monoclonal antibodies together to make a new Y. This way, the bispecific antibody is able to recognize two different targets and bring them in contact with one another.

For example, one arm might recognize a cancer cell, while the other arm might recognize and bind to a killer T-cell — a type of white blood cell that has the ability to inject toxins directly into the cancer cell. By bringing a cancer cell into direct contact with a killer T-cell, the T-cell is activated to kill that cancer target. This is the mechanism of action used by Blincyto (Amgen; Thousand Oaks, CA), the first and so far only bispecific antibody approved by the FDA.
THE ABCS OF ADCS

Antibody-drug conjugates (ADCs) are highly potent, targeted therapeutics that work by combining the targeting capabilities of monoclonal antibodies with the cancer-killing ability of toxic drugs, enabling the killing of cancer cells with less impact on healthy cells. ADCs have three key components:

- A *monoclonal antibody* that is highly specific for a tumor-associated immunogen that has little to no expression on healthy cells.
- A *small molecule drug* that is highly toxic and designed to kill the cancer cell once internalized.
- A *chemical linker* that connects the small molecule drug to the antibody. The linker is stable in blood circulation but releases the toxin once inside the tumor.

How does it work?

- The antibody binds to its target immunogen on the surface of the cancer cell.
- The antibody-drug conjugate is then taken up or internalized by the cancer cell.
- Once inside the cancer cell, the linker is degraded and the active drug released.

The ability to target only cancer cells allows drug designers to use drugs that are much more toxic than traditional chemotherapy. The ADC has a higher specificity and only attacks cancer cells, avoiding nearby healthy tissue which is often destroyed by chemotherapy.

Currently, there are two ADCs on the market: Seattle Genetics’ (Seattle, WA) Adcetris for the treatment of Hodgkin’s lymphoma, and Roche’s (Basel, Switzerland) Kadcyla for the treatment of HER2-positive breast cancer. There are dozens more in clinical development by companies including AstraZeneca (Cambridge, UK), Roche, Immunogen (Waltham, MA), Novartis (Basel, Switzerland), and Fortis Therapeutics (San Diego, CA).

PHOTOIMMUNOTHERAPY DELIVERS A PUNCH

Photoimmunotherapy delivers the latest twist on antibodies. Developed by the National Institutes of Health, this technology uses light to deliver a punch.

- A light sensitive, non-toxic drug is attached to the end of an antibody. This drug becomes toxic when exposed to infrared light.
- Infrared light is shone, activating the drug and damaging the cancer cell membrane. This damaged membrane allows water to enter, causing the cancer cell to burst and die.
- Upon bursting, the cancer cell releases tumor immunogens that serve to activate the immune system to further recognize and target more tumor cells.

One caveat to using photoimmunotherapy is the tumor must be accessible to an infrared laser in order to activate the photosensitive drug. Aspyrian Therapeutics (San Diego, CA) is in Phase II clinical studies of photoimmunotherapy antibodies that target head and neck cancer.

Over the years, monoclonal antibodies have proven to be safe and effective therapeutics in a number of different indications, most notably various cancers and autoimmune disorders. Innovations in antibody technology ensure monoclonal antibodies will remain one of the most powerful tools in the drug development arsenal.
The Mechanics Of Melanoma

SPECTRUM OF THERAPIES
Melanoma accounts for less than 1% of skin cancer cases yet causes the vast majority of skin cancer deaths. If detected early enough, melanoma is almost always curable. If not, its ability to metastasize makes it difficult to treat.

Melanoma is more common in young adults than many other types of cancer, with 25% of new cases occurring in people under age 45. Its prevalence is growing — the number of new cases per year relative to the total population has doubled since 1973. In 2017, there will be an estimated 87,110 new cases of melanoma in the U.S. and 9,730 melanoma-related deaths, according to the Aim at Melanoma Foundation. Let’s review the basics and find out the latest treatments in the battle against melanoma.

MELANOMA’S METHOD
Melanoma is the uncontrolled growth of melanocytes, the pigment-producing cells located in the bottom layer of the skin’s outermost layer (the epidermis). In skin cancer, a tumor initially grows and spreads within the epidermis due to DNA damage, which is usually caused by ultraviolet (UV) radiation.

If melanoma is detected during the epidermal level growth stage, it can often be surgically removed. Penetrating the deeper layers of the skin as it grows, it will eventually come into contact with lymph and blood vessels — which act as a cancer highway. When melanoma spreads to other parts of the body it is known as metastatic melanoma.

Lighter-skinned people are at higher risk for melanoma because the increased skin pigmentation found in darker skin tones helps to block UV rays from penetrating and damaging skin cell DNA. However, darker-skinned people can and do get skin cancer. Another general risk factor for melanoma is atypical moles, or moles with irregular shape, color, or size. Moles are clusters of melanocytes and so sudden changes in them may be an early warning sign of melanoma.

GENETIC FACTORS: P53 & BRAF
Although most cases of skin cancer are traceable to excessive UV radiation from sun exposure, about 10% are likely due to genetic factors. The gene most commonly mutated in familial melanoma is p53. p53 is a “tumor suppressor” — it detects DNA damage in cells and triggers either DNA repair pathways or activates cell death if the damage cannot be repaired. Another gene, known as the BRAF gene, regulates cell growth and is mutated in inherited forms of melanoma. About half of all genetically-based melanomas have the BRAF mutation.

Let’s take a closer look at BRAF. BRAF codes for a protein required for the transmission of a growth signal from a cell surface receptor to the cell nucleus (growth signal transduction). Growth signaling is initiated by a growth factor binding to its receptor. This binding transmits a signal through the membrane, causing the internal portion of the receptor to interact with and activate a protein inside of the cell. This activation is then transferred to the next protein in the pathway, and so on until the signal reaches the last protein in the pathway. When this protein is activated, it enters the nucleus, where it turns on specific genes that make proteins which initiate cell division. BRAF is one of the proteins in this pathway. In BRAF-associated melanoma, the mutated BRAF is always turned on even when no growth factor is present.

The small molecule drugs Zelboraf (Genentech; South San Francisco, CA) and Tafinlar (Novartis; Basel, Switzerland) inhibit overactive BRAF and are approved for the treatment of late-stage melanoma.
IMMUNOTHERAPIES IN THE FIGHT

A few different checkpoint inhibitor therapies are on the market for metastatic melanoma. These therapies enable killer T-cells – immune system cells that recognize and kill threats such as cancer cells – to become fully activated and able to kill tumor cells. Keytruda (Merck, Kenilworth, NJ) and Opdivo (Bristol-Myers Squibb, New York, NY) both target PD-1, an inhibitory protein on the surface of T-cells; Yervoy (Bristol-Myers Squibb) targets a second inhibitory protein, CTLA-4. Both PD-1 and CTLA4 essentially act as “off switches” for killer T-cells. By inhibiting these off switches, the killer T-cells become fully activated, and able to kill melanoma cells.

A second type of immunotherapy approved for melanoma is Amgen’s (Thousand Oaks, CA) oncolytic virus therapy, talimogene laherparepvec (T-VEC). An oncolytic virus is a virus that infects and kills cancer cells. The cancer cells are killed through cell lysis: as the virus multiplies inside of the cells, it causes them to burst open. This in turn releases new infectious particles that can target remaining tumor cells. In addition to killing cancer via lysis, the presence of an actively replicating virus triggers the immune response to target the tumor. T-VEC was designed to infect and kill melanoma cells, and is so far the only FDA-approved oncolytic virus.

INCYTE’S INSIGHT

Incyte (Alapocas, Delaware) has a new type of small molecule immunotherapy in Phase III called Epacadostat – it inhibits the enzyme IDO1. IDO1 helps regulatory T-cells to develop and become activated. Regulatory T-cells suppress the immune response, and therefore help cancer cells to escape immune surveillance. Inhibiting IDO1 should suppress the development of regulatory T-cells, bolstering the immune response against melanoma.

THE IMPLICATIONS OF MICRORNA

Researchers at Tel Aviv University examined how melanoma metastasizes in an article last summer: the melanoma cells release tiny vesicles containing microRNA, a type of regulatory RNA produced by all cells. The microRNA-filled vesicles induce changes in the layer of skin just below the epidermis, where melanoma originates. That lower layer of skin, known as the dermis, contains blood vessels which cancer cells are able to access. The Tel Aviv team is identifying drug candidates that may interfere with this process, preventing the metastasis that makes melanoma so deadly.

Increased understanding of the molecular pathways that contribute to melanoma’s development and spread will provide additional tools to fight those cases of metastatic melanoma that inevitably will continue to arise.
THE VERSATILITY OF HIFs
Quite a few headlines touting a term called “HIF compound” or “hypoxia-inducible factor compound” have intrigued us here at WEEKLY headquarters. GlaxoSmithKline (London, England), Akebia Therapeutics (Cambridge, MA) and more have HIF-inducing drugs in the pipeline which may prove to be attractive alternatives to Amgen’s (Thousand Oaks, CA) injectable Epogen currently on the market. In earlier stages of research, HIFs are being studied for their connection to tumor growth. How can one compound be versatile enough to affect both anemia and cancer? Without further ado, let’s take a look at the story behind hypoxia-inducible factors.

TERM OF THE WEEK: HYPOXIA-INDUCIBLE FACTOR
Hypoxia occurs when the amount of oxygen reaching a person’s cells and tissues is inadequate. Hypoxia may be triggered by lower oxygen concentrations at higher altitudes or by disease processes seen in pulmonary disorders, anemia, or circulatory deficiencies.

The low-oxygen environment in hypoxia causes cells to make a protein called hypoxia-inducible factor (HIF). HIF is a transcription factor — a protein that binds to cellular DNA in a defined location and “turns on” specific genes (which then make their intended proteins).

HIF activates genes involved in the production of oxygen-carrying red blood cells and the formation of blood vessels (angiogenesis). Both of these processes assist in increasing oxygen delivery to hypoxic (oxygen-deprived) tissues.

ACTIVATING HIF FOR ANEMIA
Anemia is the decrease in the total amount of red blood cells in the body, resulting in a lowered ability of the blood to transport oxygen. In healthy people, when the number of red blood cells in circulation drops, the kidney releases a hormone called erythropoietin, which stimulates the bone marrow to produce more red blood cells. In chronic kidney disease (CKD), the kidneys don’t produce adequate amounts of erythropoietin in response to reduced circulating red blood cells, leading to anemia.

Erythropoietin production can be enhanced by increasing the amount of HIF present.

Under normal oxygen conditions, small amounts of HIF are produced, but are quickly degraded though the action of an enzyme called HIF prolyl-hydroxylase (HIF PHD). HIF PHD is inhibited in low-oxygen conditions, enabling HIF levels to increase. Several companies are developing small molecule, orally available drugs to inhibit HIF PHD, facilitating the activation of HIF under normal oxygen levels. HIF PHD inhibitors in clinical development include:

- Akebia’s vadadustat, Phase III clinical development for CKD anemia
- FibroGen’s (San Francisco, CA) roxadustat, Phase III clinical development for CKD anemia
- GlaxoSmithKline’s daprodustat, Phase III clinical development for CKD anemia

DISRUPTING ANGIOGENESIS IN CANCER
HIF is also thought to contribute to the process of angiogenesis — the growth of blood vessels into tumors. Most solid tumors have a hypoxic environment, due to their high cell density and lack of supporting vascular networks. This hypoxic environment causes cancer cells to produce HIF, which in turn activates the secretion of vascular endothelial cell growth factor (VEGF). VEGF triggers angiogenesis, which provides a way for tumors to get oxygen and nutrients, enabling the tumor to continue growing. Angiogenesis also provides a possible route for individual tumor cells to exit the tumor and spread to other parts of the body.

Because of this relationship between HIF, angiogenesis, and cancer, there is considerable interest in developing HIF inhibitors in an attempt to block angiogenesis. The drugs Torisel (Pfizer; New York, NY) and Zortress (Novartis; Basel, Switzerland) stop mTOR, a protein that activates HIF. It is thought that the anti-angiogenesis effects of these mTOR inhibitors are a result of suppressing HIF. Torisel is FDA-approved for the treatment of renal cell cancer; Zortress is FDA-approved for advanced kidney cancer, metastatic pancreatic neuroendocrine tumors, hormone-positive, and HER2-negative breast cancer.
COCKTAIL FODDER: THE HIF ADVANTAGE

You may have heard of “altitude training” — the practice of training at high altitudes in order to increase performance, especially in endurance events such as long distance running or cycling. Altitude training works because at elevations higher than about 5,000 feet there are fewer oxygen molecules per volume of air due to reduced atmospheric pressure. Every breath taken at higher elevations delivers less oxygen than it would at lower elevations, creating a slightly hypoxic environment inside the athlete’s cells. This hypoxia increases the levels of HIF, leading to more erythropoietin and subsequent red blood cell production. The enhanced oxygen-carrying capacity lasts for about ten to twenty days after returning to lower elevations, so an athlete who trains at a higher altitude and then competes at sea level will have an advantage over those who complete all of their training at sea level.
The PARP Race Is On

PARP1 INHIBITOR LINEUP

PARP1 inhibitors are making a strong statement! Tesaro’s (Waltham, MA) just-approved Zejula has garnered predictions of blockbuster status. AstraZeneca’s (Cambridge, UK) Lynparza was the first PARP1 inhibitor to make it to market back in 2014, and their recent clinical trial results showed significant survival benefit in ovarian cancer. Clovis Oncology (Boulder, CO) achieved the second FDA approval of a PARP1 inhibitor with Rubraca in December 2016. AbbVie (North Chicago, IL) and Medivation (San Francisco, CA) both have PARP1 inhibitors in late-stage development.

The race is in full swing, so let’s pick up the science of PARP1 inhibition.

DNA DAMAGE RUNS DEEP

Simply put, PARP1 inhibitors work by exploiting the cellular pathways found in DNA damage repair. So, how exactly does DNA get damaged?

DNA incurs approximately 10,000 to 1,000,000 “molecular lesions” per day from breaks or “nicks” to the double helix, or chemical modification to the A, C, G, or T bases. This may sound high — but remember, our DNA contains 6 billion bases (3 billion base pairs), so this is equivalent to .001% to .1% of the total DNA in each cell. This damage occurs as a result of normal DNA replication errors and environmental exposures, such as ultraviolet radiation, X-rays, and chemicals.

The good news is our cells have mechanisms to fight against this damage before it causes harm. DNA repair proteins find and fix different types of DNA damage. If DNA damage exceeds a threshold amount (beyond which repair is possible) a protein called p53 triggers cell death — also known as apoptosis. DNA repair proteins prevent errant cells from turning into cancerous cells, a likely outcome if the damage accumulates in genes important for regulating cell growth and division.

BEHIND BRCA

Arguably the most famous DNA repair proteins, BRCA1 and BRCA2, are found in breast and ovarian cells. If these repair proteins themselves are non-functional, the cells in which they would normally do their job are prone to sustaining DNA damage at a much higher rate than normal. This higher rate of DNA damage increases the chances of cancer developing in those cells. BRCA1/BRCA2 positive cancer is cancer that is associated with mutations in the BRCA1/BRCA2 genes. The mutations are most strongly associated with breast and ovarian cancer, but are also associated with increased risk of developing stomach, pancreatic, prostate, melanoma, leukemia, lymphoma, and colon cancer.

THE POINT OF PARP

Poly ADP ribose polymerase 1 (PARP1) is a DNA repair protein. By stopping the PARP1 repair pathway in cells already deficient in BRCA1/BRCA2-mediated repair, cancer cells become extremely vulnerable to DNA damage. Because of this, DNA damage accumulates and triggers apoptosis. A PARP1 inhibitor is usually administered in combination with chemotherapy or radiation therapy, which increases the incidence of apoptosis-triggering DNA damage. Healthy cells, which still have BRCA repair pathways intact, are less sensitive to additional DNA damage.

What cancers are PARP1 inhibitors aiming to fight? AstraZeneca’s Lynparza and Clovis Oncology’s Rubraca are both approved for ovarian cancer. Zejula (Tesaro) targets ovarian, fallopian tube, and primary peritoneal cancer. The Phase III clinical pipeline includes:

- Lynparza: Prostate, gastric, breast, and pancreatic
- Rubraca: Prostate
- Talazoparib (Medivation): Breast
- Veliparib (AbbVie): Breast, lung, and ovarian
- Zejula: Breast
BEYOND CANCER

Preclinical research suggests that PARP1 inhibitors may also be relevant to other disease areas, such as autoimmune and inflammatory disorders. PARP1 has been shown to play a role in activating proteins that drive inflammation. Preclinical models demonstrate that in cases without the PARP1 gene, subjects were less vulnerable to rheumatoid arthritis than with the gene. Inhibiting PARP1 resulted in reduced signs of inflammation in models of multiple sclerosis, irritable bowel disease, and allergic airway inflammation.

EASILY CONFUSED: DNA DAMAGE VS. DNA MUTATION

BRCA1, BRCA2, PARP1, and other DNA-repair proteins correct DNA damage, but they don’t fix mutations. What’s the difference?

DNA damage refers to alterations in the chemical structure of DNA. This may mean a break in the DNA strand, a substitution to one of the bases that make up DNA (A, C, G, or T), or even a missing base. These changes are detected and corrected by DNA repair enzymes.

A DNA mutation is a change to the actual base sequence (A, C, G, or T). Mutations can arise if DNA damage is not corrected. Recall that in undamaged DNA, an “A” base always pairs with a “T” base, and a “C” base always pairs with a “G” base. These base-pairing rules are what enable DNA to replicate faithfully from one generation of cells to the next. However, uncorrected DNA damage may cause that “A” base to mistakenly pair with a “G” during replication; or a “C” to pair with a “T.” This results in a sequence change – a mutation - in the replicated DNA. The gene now provides incorrect genetic information to the cell.
CRISPR/CAS9 UPDATE

CRISPR/Cas9 can’t seem to stay out of the news — from first in human to patent disputes, we here at the WEEKLY want to update you on this hot technology.

A group of scientists from the State Key Laboratory of Proteomics (Beijing, China) and the National Center for Protein Sciences (Beijing, China) recently reported the first ever edit using CRISPR/Cas9 in healthy human embryos — just two years after researchers from the University of Guangzhou (Guangzhou, China) used the genome-editing technique on abnormal embryos. This news comes just weeks after a report released by an international committee convened by the U.S. National Academy of Sciences weighed in on the topic, which stated the technique may be permissible in embryos if the goal is to cure or prevent serious disease.

CRISPR technology and its applications were discovered by two different research teams, one at University of California, Berkeley, and another at the Broad Institute (Cambridge, MA). Both have filed patents on various aspects of the CRISPR/Cas9 system. The Broad Institute had granted an exclusive license to Editas Medicine (Cambridge, MA), while Berkeley had granted licenses to Caribou Biosciences (Berkeley, CA), CRISPR Therapeutics (Basel, Switzerland and Cambridge, MA), Intellia Therapeutics (Cambridge, MA), and ERS Genomics (Dublin, Ireland). In February, the U.S. Patent Office ruled in favor of the Broad Institute and its licensee, while in March the European Patent Office ruled in favor of U.C. Berkeley patents. The legal battle is certainly far from over.

CRISPR/Cas9 entered its first human clinical trial at Sichuan University (Chengdu, China) last fall, and is widely expected to do so in the U.S. by the end of 2017. With all of these new developments making waves in the industry let’s review the basics.

CAS TO THE RESCUE

CRISPR was originally discovered as a key component of the bacterial immune response. Bacteria, like people, are plagued by viral infections, and have evolved clever ways to attack invading viruses. In the 1980s, scientists observed an interesting pattern in bacterial genomes: repeating, palindromic sequences, with unique sequences referred to as “spacers” between the repeats. They dubbed these regions a tongue twister of a name, “clustered regularly interspaced short palindromic repeats,” or CRISPR. Scientists also noticed CRISPR sequences were always located near a gene that coded for an enzyme that cut DNA. This enzyme became known as Cas, short for “CRISPR-associated”.

In the mid-2000’s, scientists realized the “spacer sequences” matched DNA sequences of invading viruses — the bacteria were storing away bits of invading viral DNA between its own bacterial CRISPR sequences! These bits of viral DNA create a “genetic memory” of the virus, enabling the bacteria to fight back if reinfected.

Reinfection triggers the following steps:

• Viral DNA present in the spacer sequences is copied into viral RNA.
• The DNA-cutting enzyme Cas is made, and attaches itself to the viral RNA produced from the spacer sequence.
• This newly minted viral RNA/Cas complex finds its “match” on the invading viral DNA.
• The Cas enzyme is now positioned to cut up viral DNA, destroying the invading virus.

USE IN HUMANS

In 2013, researchers adapted this bacterial defense for use in human cells. Human cells were engineered to contain both specially-designed RNA and Cas genes. When these human cells produce the RNA/Cas complex, the dynamic duo is ferried to its complementary DNA target. Once in position, Cas goes to work cutting the DNA. The particular Cas protein chosen for this work was one discovered in Streptococcus bacteria, Cas9 — hence the moniker CRISPR/Cas9.

The ability to cut human DNA in precise locations is an exciting innovation because of what the cell does next.

BREAKING & FIXING

Cas9 creates double-stranded breaks (DSB) in the specified DNA sequence. Double-stranded breaks cut both strands of the double-stranded DNA helix. Think of DNA as a two-lane bridge that, after experiencing...
an earthquake, has a section break off and fall into the water below.

DSBs activate two repair pathways to fix the break in the DNA:

- **Non-Homologous End-Joining (NHEJ)** closes the gap between the break by joining the two sections back together—visualize pushing the two sides of the bridge together, leaving the fallen section in the water. An unintended byproduct of NHEJ is the possibility of sequence error, much like the sections of the bridge not lining up properly. If the repair occurs in the middle of a gene, the minor error can be enough to disrupt gene function and halt the production of the corresponding protein.

- **Homology Directed Repair (HDR)** relies on a highly similar (homologous) DNA segment to repair the break—visualize the missing bridge section built elsewhere and helicoptered in to fill the break.

By engineering double-stranded breaks to occur at specific locations, scientists activate the NHEJ and HDR cell repair pathways. By activating the NHEJ pathway, scientists can disrupt a disease-associated gene, preventing the production of a protein that causes the disease. By activating the HDR pathway, a short sequence of DNA is delivered with CRISPR/Cas9 to correct the mutated sequence.

### CRISPR IN THE CLINIC

A clinical trial initiated last fall by Chinese researchers at Sichuan University uses CRISPR/Cas9 to disable the PD-1 gene in T-cells.

The PD-1 gene produces the PD-1 protein, which is located on the T-cell’s surface. When the PD-1 protein is activated, the T-cell doesn’t function. When the PD-1 protein is deactivated, the T-cell functions. Aggressive cancers take advantage of this on/off switch turning PD-1 on, effectively shutting down the T-cell. By turning PD-1 off, the T-cells can’t be suppressed—freeing them up to attack cancer cells.

### CRISPR COMING SOON

A U.S. clinical trial of CRISPR to disrupt PD-1 in T-cells is expected to begin before the end of 2017. This two-year study is funded by the Parker Institute (San Francisco, CA).

A number of private companies also have plans for CRISPR/Cas9 clinical trials that include both gene disruption and gene correction. The table below summarizes some key players in the genome-editing arena and their approaches to applying CRISPR. *In vivo* means the therapy will take place inside the human and *Ex vivo* means the treatment will be performed in cells taken from the body and then injected back into the patient.

<table>
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<tr>
<th>Company</th>
<th>Disease</th>
<th>Disrupt/Correct</th>
<th>In vivo/Ex vivo</th>
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<tr>
<td>CRISPR Therapeutics/Vertex</td>
<td>Beta-thalassemia</td>
<td>Disrupt</td>
<td>Ex vivo</td>
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<tr>
<td>CRISPR Therapeutics/Vertex</td>
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<td>Editas</td>
<td>Hereditary blindness</td>
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<td>Editas</td>
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<td>Editas/Juno</td>
<td>Engineer T-cells</td>
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<td>Intellia/Regeneron</td>
<td>Transhyretin amyloidosis</td>
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<tr>
<td>Intellia/Novartis</td>
<td>Engineer T-cells</td>
<td>Disrupt/insert</td>
<td>Ex vivo</td>
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As these and other potential treatments move through clinical trials, the world will be watching to see if this revolutionary technology will live up to the hype and change the way we prevent and treat disease.
PROMISING PEPTIDE THERAPIES

The front runners in the game of drug delivery include small molecule and large molecule drugs, but there is another class that lands right in between: peptides.

Several companies, including Rhythm Pharmaceuticals (Boston, MA), Kalos Therapeutics (San Diego, CA), Aileron Therapeutics (Cambridge, MA), and Bicycle Therapeutics (Cambridge, MA) have emerged as prominent players in the peptide arena.

Let’s review the differences between the drug classes and explain where peptides fit into the picture. Then we’ll take a spectator’s interest in the companies and products leading the charge in peptides therapeutics.

EASILY CONFUSED: SMALL MOLECULE VS. LARGE MOLECULE VS. PEPTIDE

Small molecule drugs are chemically synthesized — made by a series of chemical reactions in the lab. They are typically taken as a pill or capsule. The pill or capsule dissolves in the gastrointestinal tract and the active ingredient is easily absorbed into the bloodstream via the intestinal wall. The molecules that make up these drugs are so tiny they are able to penetrate cell membranes and get inside of cells.

In contrast, large molecule drugs — protein-based therapeutics known as biologics — are made by living cells. They must be administered via injection because they will be destroyed by digestive enzymes in the gastrointestinal tract if given orally. Their large size, anywhere from 50 to 1,000 times larger than a typical small molecule drug, makes it impossible for them to penetrate cells. On the flip side, large molecules are highly specific for their target — typically a cell-surface receptor on the outside of the cell.

The FDA defines a peptide therapeutic as a chain of amino acids (the building blocks of proteins) containing 40 amino acids or less, and regulates them as small molecules. Peptide therapeutics are similar to small molecule drugs in that they can be synthesized in the lab using a peptide synthesis machine — a machine that links amino acids together in a specified order. Peptides also share key characteristics with large molecule drugs which include sensitivity to digestive enzymes, delivery by injection, and high specificity for their target.

Examples of peptide drugs on the market today include glucagon-like peptide-1 (GLP-1) receptor activators, such as Byetta (AstraZeneca; Cambridge, England), Victoza (Novo Nordisk; Bagsvaerd, Denmark), and Trulicity (Eli Lilly; Indianapolis, Indiana). These peptide drugs work by interacting with a receptor on the surface of pancreatic beta cells and stimulate the release of insulin for diabetes.

IN THE RHYTHM

Rhythm Pharmaceuticals is prepping to enter Phase III clinical studies of their anti-obesity peptide drug setmalnotide. Designated as a breakthrough therapy by the FDA, early clinical trial results in rare genetic forms of obesity were promising, helping to attract $41 million from key investors including Pfizer Venture Investments and Third Rock Ventures to fund the upcoming Phase III.

Setmalnotide works by activating the melanocortin-4 receptor (MC4R), a receptor present on the surface of cells in the hypothalamus of the brain, a region involved in regulating both appetite and satiety. Mutations in MC4R that result in reduced activation are the most common genetic cause of obesity, and account for approximately 6-8% of obesity cases.

KALOS FIGHTS CANCER

Kalos Therapeutics has a peptide drug in development based on a straightforward observation: despite constant activity, heart muscles don’t get bigger, and
cancers of the heart are extremely rare. At least part of the reason for this is a peptide known as atrial natriuretic peptide (ANP), which is produced in the heart. It helps to control cell growth and division, making sure that the heart doesn’t get too big for the chest. Since cancer is caused by out-of-control cell growth and division, a connection was made: perhaps these peptides could play a role in controlling tumor cell growth.

Kalos Therapeutics has identified a portion of ANP and is synthesizing and testing it as a potential anti-cancer agent. Dubbed KTH-22, the agent is cytostatic, meaning it halts the growth and division of cancer cells, but does not directly kill them as a cytotoxic (toxic to cells) agent would. KTH-22 is in preclinical research, with data supporting its use in the treatment of pancreatic and ovarian cancers.

**STAPLES & BICYCLES**

Most peptide therapeutics do not penetrate cell membranes. Designing peptides that could enter cells would truly endow them with the best characteristics of both large and small molecule therapies. **Aileron Therapeutics** and **Bicycle Therapeutics** are aiming to do just that.

**Aileron Therapeutics** is developing “stapled peptides.” These peptides are synthesized according to an optimized amino acid sequence. Next, a chemical linker is used to connect two amino acids within the chain, creating a folded or “stapled” version of the peptide. These stapled peptides still recognize their target protein, are more stable, and better able to penetrate cell membranes than the linear versions.

Aileron’s leading stapled peptide candidate, ALRN-6924, activates p53, a protein that triggers cell death in cancer cells but is inactivated in a range of malignancies. ALRN-6924 is in Phase II clinical studies for lymphoma. The company is pursuing the development of stapled peptides in a range of therapeutic areas, including inflammation and endocrine and metabolic diseases.

**Bicycle Therapeutics** also uses chemically linked peptides to increase stability, target interaction, and penetrate cells. Their peptides are formed — using a chemical linker — into the shape of a bicycle.

Bicycle’s lead candidate, BT1718, is a “bicycle drug conjugate” — a bicyclic peptide with a toxic drug attached. The peptide targets a protein called “membrane type 1 matrix metalloproteinase” (MT1-MMP) which is overexpressed in many tumors. BT1718 delivers its toxic payload to tumors overexpressing MT1-MMP. Preclinical trials have shown high efficacy against these tumors, and clinical trials are expected to start by the end of 2017.

Already capable of affecting a range of therapeutic targets with high specificity, continued innovations in peptide design and delivery should make this class of drug an important player.
NUCLEIC ACID THERAPEUTICS

Small molecule, peptide, and biologic drugs aren't the only players in the game of drug development. A fourth class of therapeutics differs from all three of these: nucleic acid-based drugs. These drugs are rising in prominence due to their potential to specifically target a wide range of diseases, including various types of cancer, autoimmune, and infectious diseases. Companies like Moderna (Cambridge, MA) are garnering unprecedented investor interest, while improvements in delivery methods have increased the efficacy of nucleic acid-based therapies.

In this WEEKLY — the first of a two part series — we'll dig up the different types of nucleic acids and unearth mRNA-based therapeutics in development.

TERM OF THE WEEK: NUCLEIC ACID

Nucleic acids are long chains of repeating units of nucleotides. Nucleotides are made up of a phosphate group, a sugar group, and a base.

[Diagram of DNA and RNA structure]

There are two types of nucleic acids: DNA and RNA. The nucleotides (or building blocks) of these two varieties of nucleic acids are quite similar, but there are marked differences.

DNA

• The deoxyribose is a more chemically stable sugar group because “deoxyribose” lacks the highly reactive oxygen atom.
• Two individual strands of linked nucleotides join together to make the double helix by forming complementary base pairs — “A” complements “T” and “C” complements “G.”
• Bases include Adenine (A), Cytosine (C), Guanine (G), Thymidine (T).

RNA

• The ribose sugar group is a less chemically stable sugar group because “ribose” has a highly reactive oxygen atom.
• RNA is typically single stranded.
• Bases include Adenine (A), Cytosine (C), Guanine (G), Uracil (U).

WHY MRNA?

You probably recognize DNA as the molecule of heredity, and may recall that it provides cells with the instructions for making proteins. Enter messenger RNA (mRNA) — the literal messenger that relays the DNA instruction to the ribosome where the protein-making process takes place.
So, why all this talk about mRNA? Well, protein therapeutics — the injectable protein-based drugs discussed last week — have revolutionized the treatment of a range of diseases, from diabetes to cancer to autoimmune disorders. However, they are time consuming and expensive to produce. Cells must be engineered to develop the desired protein, then grown in large (thousands of liters) tanks. Finally, the therapeutic protein must be painstakingly purified away from other proteins and cellular debris in the cell.

What if we could eliminate the huge biomanufacturing tanks and just have the patient make the therapeutic protein using their own cells? That is the idea behind mRNA therapeutics — figure out a way to provide the information contained in mRNA directly to the patient’s ribosomes and let the patient’s cells do the work. Not only would this be more efficient, it would also enable therapeutic proteins to be introduced directly inside cells or embed into the cell membrane. Recall that protein therapeutics injected into the bloodstream are too large to enter cells and are limited to interacting with proteins on the surface of cells or in the blood.

**THEORY VS. REALITY**

Like much in biotech, the concept of mRNA-based therapeutics is elegant in theory, yet rough in reality.

- **Reason 1:** The relative instability of the mRNA molecule itself; mRNA traveling through the bloodstream would typically be degraded by nuclease — enzymes that break down nucleic acids.

- **Reason 2:** “Foreign mRNA” coming from outside of the cell could trigger an immune response; our immune systems have evolved to recognize foreign mRNA as bad.

- **Reason 3:** Delivery of mRNA therapy is difficult. Right now the approach that appears to be having the most success is encasing the mRNA in a lipid nanoparticle for delivery to cells.

Bringing mRNA drugs to market involves designing chemically modified mRNA that is more stable (resistant to nucleases) and less visible to immune cells than unmodified mRNA. These modified mRNA molecules are called “nucleotide analogs” because they are similar but different from naturally occurring nucleotides.

**IN THE PIPELINE**

**Moderna Therapeutics** (Cambridge, MA) has received nearly $2 billion to fund ongoing mRNA drug development. The company now has five different products in Phase I clinical studies. Four of these are vaccine candidates: two against different strains of influenza virus, one against Zika virus, and one against an undisclosed target in partnership with **Merck** (Kenilworth, New Jersey). A mRNA-based vaccine uses lipid nanoparticles to deliver the instructions for making a particular viral protein to a cell. The cell then makes the viral protein and displays segments of it on its surface, activating an immune response to fight infection.

The fifth drug for which Moderna has initiated clinical trials — in partnership with **AstraZeneca** (Cambridge, UK) — is a mRNA that codes for the protein known as vascular endothelial growth factor, or VEGF. This protein promotes the growth of blood vessels, and may help to improve blood supply in cardiac tissue after a heart attack, or in diabetic wound healing.

**CureVac** (Tubingen, Germany) is focused on mRNA vaccines as well, with a prostate cancer therapeutic vaccine in Phase II clinical studies. Therapeutic vaccines train the patient’s immune system to recognize a specific cancer associated protein, priming immune cells to attack the tumor that bears those proteins. CureVac also has a mRNA-based rabies vaccine in Phase I clinical studies, with several more infectious disease and therapeutic cancer vaccines in preclinical development.

Other companies to watch in this space include:

- **BioNTech** (Mainz, Germany): Phase I studies completed on a mRNA-based therapeutic vaccine for melanoma; preparing to enter clinical studies on therapeutic cancer vaccines for head and neck cancer and personalized vaccines.

- **Arcturus** (San Diego, CA): Preclinical development of mRNA drugs to treat protein deficiency disorders.

- **RaNA** (Cambridge, MA): Preclinical development of mRNA drugs to treat protein deficiency disorders.

mRNA drugs show much promise and we will continue to closely follow this area for new developments. Next week, we’ll continue our discussion of nucleic acid-based therapeutics as we look at additional types of RNA and DNA based drugs.
RNA Therapeutics March Onward

TAking STEPS WITH ANTISENSE
With their high specificity and relative low manufacturing cost, RNA therapeutics may be tomorrow’s biotech sweetheart. In fact, chances are good that previously “undruggable” targets that cannot be accessed by small or large molecule drugs, are now within reach. However, the main roadblock continues to be delivery—getting the RNA drug where it needs to be, in high enough concentrations, to be effective.

Last week we looked at the emerging class of nucleic acid-based drugs known as messenger RNA therapeutics. This week, we’ll continue our discussion by examining the RNA therapeutic known as antisense. Let’s march!

RNA RELAY
Messenger RNA (mRNA) is a molecule that carries a copy of the protein-making instructions from DNA in the nucleus of a cell to the ribosomes located just outside of the nucleus. The information contained in mRNA is used by the ribosomes to assemble a protein. Without mRNA relaying the instructions, there would be no protein produced.

ANTISENSE EXPLAINED
Antisense drugs are short (between 10 and 30 nucleotides), synthetic pieces of nucleic acid whose sequence is complementary to the mRNA that codes for a disease-associated protein. When the antisense drug enters a patient’s cells, it binds to the disease-causing mRNA. This binding triggers an enzyme called RNAse H to destroy the double-stranded antisense-mRNA hybrid. Our bodies recognize that double-stranded RNA as a mistake and destroys it. Without the mRNA, the disease-associated protein simply is not made — stopping disease in its tracks.

The history of antisense in drug development is quite fickle. Over the years the inability to get antisense drugs to accumulate at therapeutically effective concentrations in the target tissue was the undoing of many clinical trials. But the love affair with antisense renewed itself in 2013 thanks to the FDA approval of Ionis Pharmaceuticals’ (Carlsbad, CA) Kynamro for the treatment of familial hypercholesterolemia.

Kynamro targets apolipoprotein B, a key component of LDL cholesterol, to lower cholesterol levels:

MEET INTRONS & EXONS
Exondys 51 (Sarepta Therapeutics; Cambridge, MA) and Spinraza (Ionis and Biogen; Cambridge, MA) are recently-approved antisense drugs that work by altering how the cell processes pre-mRNA — an immature template of mRNA. Pre-mRNA is a long strand of RNA from which specific pieces are cut out or added in before becoming the mature mRNA that is able to relay the protein-making instructions from DNA to the ribosomes.

Think of pre-RNA as a master grocery list that includes all of the most common foods you buy over the year. Envision mRNA as your adjusted grocery list that includes only what you need to buy for this week.

When pre-mRNA is first produced, it contains two types of sections that are either cut out or left in depending on the instructions:

- **Introns**: regions cut out of the final mRNA; grocery items you delete from your master list because you don’t need them this week.
- **Exons**: regions included in the final mRNA; grocery items you keep from your master list because you need them this week.
Duchenne's muscular dystrophy (DMD) and spinal muscular atrophy (SMA) are diseases in which errors occur in the processing of pre-mRNA, resulting in proteins that are not fully functional. Harking back to our grocery list analogy, this means your weekly grocery list is incorrect. Some wrong items were left on and some right were left off, resulting in a failed shopping trip.

Antisense drugs alter the way pre-RNA is processed by either including or excluding exons to make a more functional protein. Antisense technology fixes the weekly grocery list.

In DMD, the wrong items were left on during the list-making process. Exondys 51 is an antisense drug that binds to the “wrong item” to ensure it stays off. In reality, it cuts a mutated exon out of the dystrophin gene. By cutting out this exon, DMD patients produce a more functional dystrophin protein than the disease-associated version. The dystrophin protein maintains muscle integrity.

SMA patients don’t produce enough of a protein called spinal motor neuron (SMN) because it is missing an exon; the right item was left off during the list-making process. Spinraza fixes this error, in other words, the antisense drug glues itself to the exon which needs to stay on so it can remain on the list. This results in the production of a full-length mRNA and a more functional SMN protein.

THE FUTURE OF ANTISENSE

The future of antisense looks bright. By targeting the right tissues (the most promising targets are in the liver) and developing more stable formulations, we can expect to see more success stories. Check out some of the antisense drugs currently in clinical development:

The complex yet fascinating world of antisense marches forward. They promise several key advantages, including high specificity, low manufacturing costs, and the potential to target previously “undruggable” targets. We here at the WEEKLY look forward to reporting on new approvals in this arena.
THE SKINNY ON DNA TESTING

23andMe (Mountain View, CA) recently found itself back in the limelight after the disease risk section of its mail-in DNA kit received an OK from the FDA. The Silicon Valley biotech had to halt sales of its direct-to-consumer genetics testing back in 2013 after regulatory officials grew concerned over marketing claims and the possibility of consumers misinterpreting the test results. 23andMe rebooted a limited part of their genetics section in 2015 after a preliminary go ahead, and last month marked a full return with the approval of their Genetic Health Risk (GHR) reports.

In this issue, we’ll get the skinny on the science driving 23andMe.

TERM OF THE WEEK: SNP GENOTYPING

23andMe’s moniker is a nod to the 23 pairs of chromosomes that make up the human genome. Recall the human genome is all the DNA found in a human cell. The tests, which are available to anyone with a mailbox and a credit card, rely on a technique called SNP genotyping.

“SNP” (pronounced snip) stands for “single nucleotide polymorphism” — a change in a single DNA nucleotide (A, C, G, or T) that occurs at a specific position in the genome. An example of a SNP is demonstrated in the image below: within a specific gene sequence the most common variant for individuals is a “T” at a particular location, but in some individuals the “T” has been replaced by a “C” SNP.

Many SNPs have no significant impact on an individual’s health, but other SNPs are associated with disease susceptibility; having one variant instead of another makes someone more likely to get a particular disease.

SNP genotyping is characterizing the SNP profile of an individual — finding out which A, C, G, or Ts are in positions differing from the norm. In the case of 23andMe, that information is used to assess disease risk, carrier status for certain genetic diseases, wellness information, and ancestry.

A CHIP OFF THE OLD GENE

23andMe’s core technology involves the use of DNA microarrays, also known as DNA “gene chips.” The technology relies on the very specific base pairing rules followed by double-stranded DNA: A’s always pair with T’s, and C’s always pair with G’s.

A DNA microarray is simply a tiny piece of glass or silica that has had a microscopic checkerboard etched into it — each square is about 11 micrometers (10-6 meters) by 11 micrometers big, just big enough to hold one single-stranded piece of DNA.

Each square represents one gene, or gene variant, within the human genome. The entire gene sequence need not be present — typically, between 25 and 60 bases are used (though we are only showing 6 in our image above). Computer software keeps track of the location of gene sequences within the array. In this manner, the specific variants within each DNA sample can be identified.
EASILY CONFUSED: GENOME SEQUENCING VS. SNP GENOTYPING

The term *DNA sequencing* is often mistakenly used when the correct term is *SNP genotyping*.

- **DNA sequencing** means to determine the order of every single base pair in a given gene (gene sequencing) or in an entire genome (whole genome sequencing).
- **SNP genotyping** means to identify single base changes between a given gene sequence and a reference sequence.

MUTATIONS & SUSCEPTIBILITY GENES

To qualify as a diagnostic test in the eyes of the FDA, genetic testing companies must show that a specific DNA sequence can confirm whether one has (or doesn’t have) a particular disease.

For example, Huntington’s disease is caused by a known mutation in the huntingtin gene. If a specific mutation is detected, a doctor can say with certainty whether the patient has or will develop Huntington’s disease. It is a dominant genetic disorder, meaning that one copy of the disease-associated gene variant is enough to cause the disease (recall that we have two copies of each gene, one from each parent).

Most genetic disorders are recessive — meaning two copies (one from each parent) of the disease-associated gene must be present to actually develop the disease. If someone has only one copy of the gene variant that causes cystic fibrosis, they are deemed a “carrier” — they themselves do not have cystic fibrosis, but they have a 25% chance of passing the disease on to their child if the other parent is a cystic fibrosis carrier.

Mutations in susceptibility genes, in contrast, do not necessarily mean the patient has or will develop the disease, it only means the patient is *at risk* for developing the disease. One well-characterized susceptibility gene is apolipoprotein E (ApoE). The ApoE4 variant of the ApoE gene is associated with a *higher risk* of Alzheimer’s disease (AD). Those with two copies of ApoE4 may have as much as 20 times the risk of developing AD; however, some individuals with two copies *never develop the disease* according to statistical studies of different ApoE variants. On the other hand, another variant — ApoE2 — may *reduce the risk* of developing AD.

By identifying which ApoE gene variant an individual has, researchers can say whether or not that person has an average risk, a higher than average risk, or a lower than average risk of developing AD — but they cannot definitively diagnose or predict AD onset.

23andMe now offers 10 Genetic Health Risk tests that look at susceptibility genes, including ApoE4. Part of the FDA’s earlier concerns about consumer misinterpretation involved the distinction between susceptibility and diagnosis — making sure consumers understood the test results did not, for example, diagnose them with AD, but merely reported their susceptibility in the form of a statistical risk factor.

23andMe also offers carrier status tests for more than 40 different conditions. They also provide information on gene variants associated with “wellness” — such things as sensitivity to caffeine and lactose intolerance, as well as non-health-related traits such as dry or wet earwax and skin pigmentation.
MORE THAN A SNP

Direct-to-consumer genetic testing is only a part of 23andMe’s business. Since its founding in 2006, the company has been collecting genetic data on patients along with their self-reports of symptoms and health status — about 85% of users from the 23andMe database have opted in.

The company has also initiated research efforts around specific diseases such as inflammatory bowel disease (in partnership with Pfizer; New York, NY) and Parkinson’s (in partnership with Genentech; South San Francisco, CA), wherein they recruited patients with those diseases to participate in identify SNPs more common in those populations. In 2015, the company announced it would begin to do its own drug discovery research and launched 23andMe Therapeutics.

From new options for consumer genomics to breakthroughs in drug discovery, we expect 23andMe to stay in the news for some time to come.
OLD WAY OF FIGHTING BACTERIA RENEWED

One of the greatest public health challenges of the 21st century is antibiotic resistance, which occurs when a few bacteria in a given population develop a genetic mutation that enables them to survive — even in the presence of antibiotics.

How do bacteria become drug resistant? Suppose a particular antibiotic inhibits an enzyme required for bacterial replication. If one bacterium mutates so the enzyme has a slightly different shape, the antibiotic is no longer effective. The mutated bacterium lives on and continues to replicate, even as all the others die off. Over time, this resistant strain becomes dominant, spreading from person to person, remaining unchecked and thriving. It is not uncommon for a strain of bacteria to become resistant to several different antibiotics, giving rise to the term multi-drug resistant bacteria.

In this issue of the WEEKLY, we’ll take a look at an entirely novel approach to fighting bacterial infections — bacteriophage.

TERM OF THE WEEK: BACTERIOPHAGE

A bacteriophage — also referred to as a phage — is a virus that infects bacteria. By attaching to a bacterium’s surface, a phage punches holes in the membrane and injects its own genetic material inside. The phage then replicates inside of the bacterium, creating so many new viruses that the bacterium breaks open, releasing newly produced viruses, which can then go on to infect other bacteria, continuing the cycle.

The word “bacteriophage” is derived from the Greek word phagein — “to devour.” So we can think of bacteriophage as, literally, devouring bacteria — a potentially very useful trait! Typically each phage is specific for a type of bacteria, meaning that if adapted for therapeutic use, researchers can select viruses that will only attack harmful bacteria, and leave the many strains of “friendly” bacteria that make up our gut microbiome alone. And since each type of phage has coevolved for millennia with its chosen strain of bacteria, each adapting and changing in response to the other, resistance is much less likely to evolve as has been the case for antibiotics. Likewise, humans have safely coexisted with bacteriophage for a long time, suggesting that there should be few safety issues with their use as therapeutics.

Scientists have known about the bacteria-devouring ability of phage for about 100 years, but with the advent of antibiotics in the late 1920s, medicine’s focus shifted to these new wonder drugs because they were easier to manufacture and test in controlled settings. Now that antibiotic resistance is emerging, so too is a renewed interest in bacteriophage, which are now starting to be manufactured and tested in a standardized way for the first time. Let’s take a look at some of the biotech companies delving into the world of bacteriophage-based therapeutics.

THE COCKTAIL APPROACH

The first multicenter clinical trial examining the use of bacteriophage as antibacterial treatments was initiated in 2015 by French biotech Pherecydes (Paris, France). Preparative work for the trial began in 2013 as researchers established protocols for producing phage that met good manufacturing practice guidelines. The researchers are studying two different “cocktails” of bacteriophage — mixtures of different bacteriophage that have shown activity against different substrains of a particular bacteria in the lab. The first contains 13 different phages targeting P. aeruginosa; the second, 12 phages that target E. coli. Both are being evaluated for the treatment of burn wound-associated infections.

Other companies testing phage cocktails in human patients include:

- **AmpliPhi Biosciences** (Richmond, VA): Phage cocktail AB-SA01 is in Phase I clinical testing for antibiotic resistant S. aureus in two different clinical settings: chronic rhinosinusitis as well as acute and chronic wound and skin infections.

- **TechnoPhage** (Lisbon, Portugal): Phage cocktail TP-102 targets bacteria associated with chronic ulcers, respiratory and skin infections.

- **Intralytix** (Baltimore, MD): Completed Phase I studies using a bacteriophage for the treatment of infected wounds.
**THE ENGINEERED APPROACH**

Creating a phage cocktail can be a challenging biomanufacturing problem. An alternative approach to combining beneficial characteristics of different viruses is to genetically engineer a synthetic virus that combines the properties of multiple phages into a single genome.

For example, scientists could insert genes into a phage genome to increase the range of bacteria subtypes an individual phage can attack, yet still maintain the specificity that prevents the phage from raiding friendly bacteria. Researchers could also add in genes to make the bacteriophage’s antibacterial response even stronger. Companies with engineered bacteriophage in preclinical development include **Synthetic Genomics** (San Diego, CA) and **EnBiotix** (Cambridge, MA).

**THE COMPONENT APPROACH**

A third approach to tapping into the therapeutic power of bacteriophage lies in isolating the component or components that make them toxic to bacteria. For example, in order to inject their genome into bacteria, phage must essentially punch a hole in the membrane, which is itself very damaging to the bacteria. The viral protein that creates these tears in the membrane are called “lysins” — enzymes that essentially chew holes in the bacterial cell wall. **ContraFect** (Yonkers, NY) has completed Phase I clinical studies of its drug CF-301 — a lysin — for the treatment of *S. aureus* bloodstream infections.

Although still in the early days of clinical testing, bacteriophage offer the possibility of being a safe and effective solution to the current antibiotic resistance public health crisis.
Viruses Blasting Cancer

ENGINEERING VIRUSES TO ATTACK
Getting bacteria-eating viruses to combat antibiotic resistance isn’t the only way viruses are being hacked to defend team homo sapien. This week, we’ll turn our attention to another benevolent use of viruses: cancer-fighters known as oncolytic viruses.

ONCOLYTIC VIRUS PRIMER
Oncolytic viruses are an immunotherapy — a type of therapy that harnesses the power of a patient’s immune system to combat a disease. Getting a virus to trigger the immune response to fight cancer is no easy task, the process involves engineering the virus to selectively infect and kill cancer cells. Oncolytic viruses are created in the lab by genetically modifying existing viruses in at least two ways:

• Making the virus safe by removing genes that cause the virus to make people sick
• Engineering viral surface proteins so the virus recognizes and binds to the cell receptors of cancerous cells, disregarding the healthy, non-cancerous cells

The oncolytic virus follows the same life cycle as any virus—once inside the human body it hunts down, attaches to, and enters its host cell. In this case, the host happens to be cancer cells! The virally infected cancer cells are destroyed via the process of cell lysis—as the oncolytic virus multiplies inside of the cells, it causes the cancer cells to burst open which kills them. Spewing from the burst cells are new infectious viruses that further target remaining tumor cells. The presence of a replicating virus also activates the immune response, so the cancerous area is further attacked.

Additional modifications may also be made to the virus, depending on the characteristics of the targeted cancer. For example, an oncolytic virus might be modified to produce proteins that stimulate the immune system or directly attack the tumor.

Most oncolytic viruses are tested both as “stand-alone treatments” and “in-combination with other immunotherapies” — such as checkpoint inhibitor therapies — to help fully activate the immune response against cancer. Let’s take a look at the unique features of a selection of oncolytic viruses on the market and in development.

INSIDE OF IMLYGIC
Viruses can be thought of as very simple packages of genetic material — DNA or RNA — encapsulated in a protein package. Like the human genome, viral genomes code for proteins required by the virus. Some of these proteins enable the virus to make copies of itself (replicate), or to evade the human immune response. It is often necessary to modify the viral genome in order to safely use a virus as a therapeutic, but how?

Amgen’s (Thousand Oaks, CA) Imlycic is the only FDA approved oncolytic virus, aiming to attack melanoma. The virus used in Imlycic is a modified herpes simplex 1 virus. The modifications made to Imlycic to ensure safety and efficacy include:

• Deletion of viral gene ICP34.5. This gene codes for a protein that enables the virus to replicate in human cells by blocking a human protein known as PKR. PKR prevents viral replication. It is less active in most tumor cells, so this makes the virus able to selectively replicate in tumor cells.
• Deletion of viral gene ICP47. This gene codes for a viral protein that thwarts the immune response by turning off a process called antigen presentation. Normally, one of the key ways the immune system “knows” to attack a virally-infected cell is by recognizing antigens (or fragments of viral proteins) displayed on the infected cell's surface. Turning this process off helps the virus evade the immune system. Turning it back on prompts the immune system to attack virus-infected tumor cells.
• Activating the earlier expression of the viral gene US11, resulting in increased viral replication in tumor cells.
• Insertion of a gene for the human protein GM-CSF, which activates the immune system, aiding in the overall immune response toward the tumor triggered by viral infection.

Taken together, these modifications create a virus that selectively replicates in tumor cells, resulting in their...
direct destruction as well as activation of a host immune response targeting the virus-infected tumor cells.

**ONCORUS ON THE OFFENSE**

Oncorus (Cambridge, MA) is also developing a modified herpes virus, ONCR-001, for the treatment of cancers, including the notoriously difficult-to-treat brain cancer, glioblastoma. Like Imlygic, ONCR-001 has been modified to selectively target tumor cells. Unlike Imlygic, ONCR-001 retains all viral genes needed for viral replication.

So, how is safety maintained in the presence of an oncolytic virus that is actively replicating? Oncorus scientists have figured out a clever way to take advantage of a key difference: the types of microRNAs produced by healthy cells vs. cancer cells.

MicroRNAs are a type of “regulatory RNA” that promotes the degradation of a target messenger RNA (mRNA — the RNA that gets turned into proteins). This means that different target sequences will be recognized in healthy cells vs. cancer cells. By engineering sequences that microRNAs from healthy cells will recognize, Oncorus scientists can ensure the viral mRNA will be destroyed in any healthy cells it infects. No viral mRNA, no viral proteins, no virus. Because these microRNAs are not present in tumor cells, ONCR-001 is able to produce viral proteins and new viral particles freely when it infects those target cells. ONCR-001 has shown strong ability in fighting glioblastoma in preclinical models.

**THE GENESIS OF GENELUX**

San Diego-based Genelux is adapting the vaccinia virus as an oncolytic virus for the treatment of a variety of solid tumors. Vaccinia is the scientific name for the cowpox virus — the virus that is used as a vaccine for smallpox. Because of its decades-long use as a vaccine, researchers have confidence the virus is safe to use in humans, although the modified version must still undergo safety testing.

Their lead product, GL-ONC1, selectively replicates in tumor cells and tumor-associated blood vessels, directly killing tumors while cutting off their blood supply. The company is also developing oncolytic viruses with genes for “therapeutic payloads” — proteins that will boost the patient’s immune response to the cancer, or even therapeutic antibodies that will then be produced inside of the cancer cell. This approach is a clever response to the fact that due to their relatively large size, most therapeutic antibodies are not able to completely penetrate solid tumors. Using an oncolytic virus to penetrate the tumor and deliver genes instructing the tumor itself to make the antibody could be a game-changing work around.

Finally, Genelux is also creating engineered virions that incorporate “imaging” proteins. For example, GL-ONC1 delivers a gene for a fluorescent protein directly to tumor cells. As the virus replicates inside of the tumor, the fluorescent signal increases. In preclinical animal testing, this has allowed non-invasive detection and imaging of tumor progression and regression in real time, and may one day be a powerful guide to physicians monitoring cancer patients. GL-ONC1 has successfully completed Phase I and is preparing to enter Phase II for a variety of solid tumors including peritoneal carcinomatosis.

**ONCOLYTIC PIPELINE**

With the approval of Imlygic in 2015, biotech companies, investors, and regulatory officials have recognized the strong potential of oncolytic viruses to treat cancer. The race is on to get the next oncolytic virus through the clinic:

<table>
<thead>
<tr>
<th>Company</th>
<th>Indication (Clinical Studies Phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncolytics Biotech (Calgary)</td>
<td>Head &amp; neck (P2 completed); Brain, lung, colorectal, prostate, pancreatic, ovarian, melanoma (P1)</td>
</tr>
<tr>
<td>SillaGen (Seoul)</td>
<td>Liver (P1)</td>
</tr>
<tr>
<td>PoCThera (Oxford)</td>
<td>Oesophageal cancer. other carcinomas (P1)</td>
</tr>
<tr>
<td>Virtu Biologics (Glasgow)</td>
<td>Mesothelioma, melanoma (P1)</td>
</tr>
<tr>
<td>Oncow Therapeutics (Helsinki)</td>
<td>mesothelioma, ovarian (P1)</td>
</tr>
<tr>
<td>Viralytics (Sydney)</td>
<td>Melanoma, prostate, bladder, and lung cancers (P1/2)</td>
</tr>
</tbody>
</table>

We expect to see this new therapeutic class become increasingly common, opening up novel approaches for a whole range of cancers.
Nanobodies: These Are Not Your Mother’s mAbs

THE DRUG KINGPINS

Monoclonal antibodies (mAbs) are the undisputed drug kingpins. In 2013, the mAb market raked in $75 billion in combined sales, covering a whole range of indications from cancer and infectious disease, to autoimmune disorders, and even high cholesterol.

Despite the success, mAbs have one chink in their armor: they cannot enter cells due to their large size, hampering their range as therapeutics. To date, mAbs can only target proteins on the surface of cells, such as receptor proteins, or proteins circulating in the bloodstream, such as inflammatory cytokines. The development of cell-penetrating mAbs would open up a world of therapeutic targets and patient benefits.

Let’s review the fundamentals of therapeutic antibodies and explore a new type of therapeutic antibody that may be able to go where no antibody has gone before.

MAB RECAP

Antibodies are proteins naturally produced by our immune system to help defend against foreign invaders such as viruses and bacteria. Each antibody produced has a unique shape that enables it to recognize unique targets (antigens) which are typically proteins on the surface of pathogens. By binding to these pathogens, antibodies act as a flag to alert the rest of the immune system to attack.

Antibody therapeutics also rely on the ability of antibodies to interact with a specific target. Scientists have developed antibodies that recognize and bind proteins on the surface of tumor cells, thereby alerting the immune system to attack the tumor. Antibodies are also selected for their ability to inhibit (stop the activity of) a particular protein. For example, the breast cancer drug Herceptin (Genentech, South San Francisco) inhibits the activity of the growth factor receptor HER2 by preventing it from interacting with the growth factor HER2:

By developing antibodies that can enter cells, this inhibitory power can be used against targets inside of the cell. Let’s take a look at the newest contender.

NANOBODIES

Scientists at Ablynx (Ghent, Belgium) are developing a new type of therapeutic antibody from an unlikely source — camels and llamas, members of the biological family Camelidae. These antibodies are structurally and functionally very similar to human antibodies, with a few important differences that could add up to something big!

Like all antibodies, Camelidae antibodies work because they have a specific shape that enables them to recognize and bind to a specific target. However, they are a tenth the size of other mammalian antibodies — giving rise to the moniker “Nanobodies.” Nanobodies have the ability to recognize targets hidden inside of cells. Their small size may potentially also enable them to cross the challenging blood brain barrier, or penetrate the interior cells of tumors – two activities that conventional antibody therapies lack.

In addition to their small size, Nanobodies also exhibit a less complex structure overall. Because of this, they have been successfully produced in bacterial cells. If Nanobodies can be scaled-up, it would significantly
reduce production costs as compared to standard antibody production in mammalian cells. Preliminary studies in mice also suggest that Nanobodies can be maintained in the stomach and intestine — opening up the possibility of oral delivery for some indications such as Crohn’s disease.

**IN THE CLINIC**

We can expect to see the first Nanobodies on the market this year. In February of 2017, Ablynx submitted an application to the European Medicines Agency for the first of its Nanobody therapeutics, caplacizumab. Caplacizumab is being tested for the treatment for a rare disease known as acquired thrombotic thrombocytopenic purpura (aTTP), a blood-coagulation disorder that results in extensive microscopic clots forming in small blood vessels throughout the body. The disease is triggered by excess von Willebrand factor (vWF), a protein that initiates blood clotting. Caplacizumab inhibits vWF, thereby preventing clot formation.

The company has two more Nanobody products in Phase II clinical testing: Vobarilizumab, which reduces the activity of interleukin-6 (IL-6). IL-6 is a protein that stimulates the immune response; inhibiting the immune system may prove a useful treatment for autoimmune disorders. Vobarilizumab is being tested for the treatment of rheumatoid arthritis and lupus, in partnership with AbbVie (North Chicago IL). Next up is ALX-0171, which binds the fusion (F) protein on the surface of the respiratory syncytial virus (RSV). The F protein enables RSV to lock onto lung cells. ALX-0171 is expected to interfere with the interaction of F protein and lung cells, thereby preventing RSV infection.

In partnership with Boehringer Ingelheim (Ingelheim, Germany), Ablynx is also entering the oncology space with Phase I testing of a Nanobody that inhibits the vascular endothelial growth factor (VEGF) protein. VEGF is a growth factor secreted by tumor cells to encourage the growth of blood vessels into the tumor, a process called angiogenesis. By inhibiting VEGF and angiogenesis, the flow of blood and nutrients into the tumor is stopped, essentially starving the tumor. It is hoped that Nanobodies may be even better angiogenesis inhibitors than monoclonal antibodies have proven to be, due to their enhanced tumor-penetrating abilities.

As Nanobodies continue to be tested for safety and efficacy, a whole new kingdom of potential antibody targets may begin to emerge.
FOUNDATIONS OF EPIGENETICS

Genetic mutations — changes in the order of the A, C, G, and T nucleotide bases that make up a gene — have been the primary focus of cancer researchers over the last several decades. By sussing out mutations involved in regulating cell growth and division, scientists better understand the molecular range of different cancers and consequently develop more targeted and effective therapeutics.

In recent years, another type of genetic variation has captured the attention of researchers: epigenetic modifications. Best characterized in cancer, epigenetic changes are also thought to play a role in a range of other diseases, including autoimmune disease, cardiovascular disorders, diabetes, neurodegenerative disorders such as Alzheimer’s disease, and potentially even male infertility.

In this WEEKLY, we’ll tell the epigenetics story and discuss how it’s being used to develop new treatments.

TERM OF THE WEEK: EPIGENETICS

Epigenetic modifications are changes to DNA that do not alter the actual gene sequence; they are chemical modifications to the DNA itself. These changes typically affect gene expression, or how often the gene is read by the cell. Epigenetic modification can occur either directly to the nucleotide bases themselves (A, C, G, or T) or to the histones, which are small proteins that package and order DNA.

One of the most common types of epigenetic modification is methylation — the addition of a methyl (CH3) group to cytosine (C) nucleotides. The end result: methylation reduces or even blocks gene expression.

A second type of modification is called acetylation — the addition of an acetyl group (CH3 CO) to the histones. Acetylation loosens the association of the DNA with the histones, making the DNA more accessible to the enzymes used in gene expression, ultimately increasing protein production.

Deacetylation — the removal of an acetyl group — increases the association or “grip” of the DNA around the histones. Deacetylation makes the DNA less accessible to enzymes used in gene expression, thereby decreasing the production of proteins.

ADDING IT ALL UP

Epigenetic modification is a normal part of development. This is in part why different genes are expressed in the heart than, say, the liver — the two different tissue types contain the same genome, but tissue-specific differences in epigenetic modification lead to differences in gene expression in the two tissues.

Problems may arise, however, if variations in epigenetic modifications result in changes to gene expression. If a cell or tissue type begins to make too much of a protein that activates cell growth, for example, the cell could begin to divide too often — potentially leading to cancer. Alternatively, a cell could begin to make less of a protective protein, for example, a “tumor suppressor” protein (a protein that deactivates cell division), and again cancer could ensue.

Epigenetic medicine seeks to identify disease-associated differences in epigenetic modifications, and to develop drugs to restore the epigenome to that of healthy cells.
BREAKING IT DOWN

Epigenetic drugs are small molecule drugs that target epigenetic regulators, or proteins that write, read, or erase epigenetic modifications.

- **Writers** are the enzymes that make the chemical modifications — methylation or acetylation as described above — to DNA molecules or histone proteins.
- **Erasers** are enzymes that remove these chemical groups.
- **Readers** are the proteins that detect and respond to these modifications, causing the DNA to be more or less tightly wrapped around the histone protein. Any one of these proteins could be inhibited or activated to affect changes in epigenetic modifications.

OLD SCHOOL: WRITING & ERASING

The disease that has been best classified in terms of epigenetic variations is cancer. Currently, there are five epigenetic drugs on the market to treat cancer, and more in development. Those on the market fall into two categories:

- **Histone deacetylase (HDAC) inhibitors:** HDACs are erasers — they remove acetyl groups from histone proteins, resulting in increased expression of associated genes. Three HDAC inhibitors have been approved by the FDA: Zolinza (Merck; Kenilworth, NJ) and IstoDox (Celgene; Summit, NJ) both treat cutaneous T-cell lymphoma, and Farydak (Novartis; Basel, Switzerland) for the treatment of multiple myeloma. HDAC inhibitors in development include:
  - **DNA-methyltransferase (DNMT) inhibitors:** DNMT’s are writers — they add methyl groups to DNA, resulting in decreased expression of associated genes. Two DNMT inhibitors have been approved by the FDA: Vidaza (Celgene) and Dacogen (Otsuka; Tokyo, Japan). Both drugs are used to treat myelodysplastic syndrome and acute myeloid leukemia.
- **Histone-methyltransferase (EZH2) inhibitors:** EZH2’s are also writers — these enzymes transfer methyl groups to histone proteins. One EZH2 is associated with over activity in a number of different cancers. There are no EZH2 inhibitors currently approved, but several are in development, including Constellation Pharmaceuticals* (Cambridge, MA) CPI-1205 in Phase I for advanced B-cell lymphomas, and Epizyme’s (Cambridge, MA) tazemat, currently in Phase II for non-Hodgkin lymphoma, certain genetically-defined solid tumors, and mesothelioma.

THE NEW CLASS: READERS

A class of proteins called “Bromodomain and Extra Terminal motif” (BET) proteins are reader proteins. They recognize and bind to specific patterns of acetylation on histone proteins. Upon binding, they recruit additional proteins that regulate gene activity. Irregularities in histone acetylation, then, may send the wrong message to a BET protein. By inhibiting the interaction between BET protein and histone proteins, researchers have found that they can prevent incorrect messages from being received by the BET proteins. Currently, there are no BET inhibitors (BBI) approved, but several are in clinical development. The farthest along is Resverlogix’s (Calgary, Canada) apabetalone, which is in Phase III testing for atherosclerosis and associated cardiovascular disease. Additional BBIs in clinical development are shown in the table below:

<table>
<thead>
<tr>
<th>Company</th>
<th>Indication (Clinical Studies Phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merck (Kenilworth, NJ)</td>
<td>Hematological malignancies (PI)</td>
</tr>
<tr>
<td>Bristol-Myers Squibb (New York, NY)</td>
<td>Solid tumors (PI/PI)</td>
</tr>
<tr>
<td>Innovent Pharmaceuticals (Wilmington, DE)</td>
<td>Hematologic malignancies (PI/PI)</td>
</tr>
<tr>
<td>AbbVie (North Chicago, IL)</td>
<td>Various cancers (PI)</td>
</tr>
<tr>
<td>Constellation (Cambridge, CA)</td>
<td>Hematologic malignancies (PI)</td>
</tr>
<tr>
<td>FCRIRA Therapeutics (Watertown, MA)</td>
<td>Hematologic malignancies (PI)</td>
</tr>
<tr>
<td>Gilead Sciences (Foster City, CA)</td>
<td>Lymphomas, breast and prostate cancer (PI/PI)</td>
</tr>
<tr>
<td>Daiichi Sankyo (Tokyo, Japan)</td>
<td>Solid tumors, hematological malignancies (PI)</td>
</tr>
<tr>
<td>Roche</td>
<td>Solid tumors (PI)</td>
</tr>
</tbody>
</table>

Epigenetics promises to change the way we look at the human genome. Scientists have made great strides in understanding how epigenetic modifications contribute to both health and disease; however, a complete understanding of these modifications is still very much a work in progress. As that work develops, researchers will undoubtedly uncover new drug targets and approaches to disease management. Stay tuned!
A Skin Cell With Stem Cell Diversity?

INDUCED PLURIPOTENT STEM CELLS SHOW PROMISE

Imagine being able to reprogram one of your own skin cells to produce a functioning nerve cell or section of cardiac tissue. This may sound like science fiction — but the groundwork for this to become a reality is already in the works as researchers expand their ability to create and manipulate induced pluripotent stem cells.

In this WEEKLY, we’ll get the details on what these multifaceted cells are all about and discover their therapeutic potential.

STEM CELL PRIMER

Stem cells are unspecialized cells that have the ability to develop (differentiate) into 1 of 200 cell types in the body. There are two general classifications:

• Embryonic stem cells (found only in developing embryos) can become any cell type within the adult body. These are pluripotent stem cells.

• Adult stem cells (found in the organs of an adult) can only become certain cell types. Typically, these cell types come from the organ in which they are derived from.

Due to their ability to differentiate (change into) into any cell type in the adult body, pluripotent stem cells show the most promise as a therapeutic. The idea is they can be induced in the lab to form a specific type of adult tissue and then transplanted into a patient who needs that tissue.

For example, someone who damages their spinal cord may potentially benefit from a transplant of replacement spinal cord tissue. In fact, these types of clinical trials are currently ongoing and show promise. However, transplanting tissue derived from an embryo carries the same risks as an organ transplant — rejection by the immune system. Thus, patients undergoing this type of therapy must receive immunosuppressive drugs, which carries its own set of risks.

What could be a possible alternative that solves the rejection issue?

TERM OF THE WEEK: INDUCED PLURIPOTENT STEM CELL

An induced pluripotent stem cell (IPSC) is a type of pluripotent stem cell that can be generated directly from adult cells. In theory, this means that a scientist could create a stem cell treatment using cells from a patient’s own body by following these steps:

• Remove a skin or other easily accessible cell type from the patient.

• Manipulate the cell in the lab to produce an induced pluripotent stem cell.

• Add the required growth and differentiation factors to get the induced pluripotent stem cell to differentiate into the desired tissue type.

This newly differentiated tissue could then be transplanted back into the patient’s body without fear of immune rejection since it is derived from their own cells!

AN EASIER RECIPE

The development of IPSCs was so significant that the scientists at Cambridge University (Cambridge, UK) and Kyoto University (Kyoto, Japan) who figured out how to create them received the 2012 Nobel Prize in Medicine. Their discovery was based the observation that when pluripotent stem cells differentiate into specialized cell types, certain genes are deactivated or switched off. They wondered if the reverse might also be true — if by reactivating or turning on those same genes, they could arrive at pluripotency. Through a series of experiments, they hit upon the correct combination of genes to reactivate, and succeeded in inducing pluripotency.

RESEARCH APPLICATIONS

The best way to decipher a disease is to examine the affected cells from a patient — for example, a blood sample from a leukemia patient or a tumor biopsy from a breast cancer patient. What if the affected tissue is impossible or dangerous to access — such as brain or cardiac tissue? IPSCs to the rescue! To better understand Alzheimer’s, researchers are creating IPSCs from Alzheimer’s patients’ skin cells, which are then induced to become brain cells. In this manner, the disease models reflect the genetics of an Alzheimer’s patient without the
need to directly access their brain cells. These patient-based disease models are being used as both a way to better understand the disease process as well as for drug discovery research.

Companies developing IPSC-based drug discovery platforms include:

- **Evotek’s** (Hamburg, Germany) collaboration with **Celgene** (Summit, NJ) to use their IPSC platform to discover and develop new drugs for a range of neurodegenerative disorders, including Alzheimer’s, Parkinson’s, and ALS.
- **Axiogenesis’** (Cologne, Germany) collaborations with **Metrion Biosciences** (Cambridge, UK) to develop IPSC-derived heart muscle cells and neurons for drug discovery applications.

**CLINICAL APPLICATIONS**

Ultimately, scientists and physicians want to use patient-derived IPSCs for individualized treatments as described above. Currently, there is only one ongoing clinical trial using IPSCs, taking place at the **RIKEN Institute** (Wako, Japan) for the treatment of wet age-related macular degeneration. The scientific community is closely watching this trial — if IPSC treatments prove to be safe and effective, they will revolutionize an already revolutionary field.
BIOTECH PRIMER AT BIO 2017

Biotech Primer will be headlining a few events at the BIO 2017 Annual International Convention in San Diego, CA next week. Will you be there? If so, please join us!

• Learn the fundamentals of biotechnology in our all-day class BioBriefing: Biotech for the Non-Scientist on Monday, June 19th. SOLD OUT
• What are the most popular topics in the WEEKLY? Attend Readers’ Choice: Biotech Primer WEEKLY Top Topics on Tuesday, June 20th at 10:45am to learn about genome editing, immunotherapy and much more. Event open to BIO 2017 registrants.
• Be sure to visit us at booth #5225 to pick up your free copy of our book, The Biotech Primer: An insider’s guide to the biotech and pharma industry!
• Come by our booth for a pair of tickets to the PABNAB party on Wednesday night. One pair per person, until all tickets are gone.

BIOTECH PRIMER’S BIOTECHNOLOGY FOR NON-SCIENTISTS COURSES

What can you expect to learn when you take a class with us? Starting with the basics, we build upon this base to deliver more complex, industry-relevant knowledge about disease, diagnostics and drugs.

Some of my favorite topics to teach include the genetic variation, genomics, and immunology sections of the course. Starting with the concept of the gene as the basic unit of human inheritance, I love to surprise my students with information on just how highly similar we all are at the level of DNA—but how in some cases, very small differences can lead to a fatal disease state. We then discuss how advances in next generation whole genome sequencing technologies are enabling us to better identify disease specific mutations, and how this information is already being used to develop better diagnostics and drugs. In the immunology section, I give an overview of how our immune system works and explain how biotech companies have leveraged this knowledge to develop cutting-edge therapies.

This is a BIO2017 affiliate event and registration is now closed because we have sold out. However, we deliver this type of course in various locations and can customize it and bring it in-house to your team. For a list of upcoming classes click here. To learn more about our in-house classes, contact Stacey at Franklin@BiotechPrimer.com.

READER’S CHOICE: TOP TOPICS FROM BIOTECH PRIMER WEEKLY

On Tuesday, June 20th, I will be giving a talk, Readers’ Choice: Biotech Primer WEEKLY Top Topics, that highlights the most popular Biotech Primer WEEKLY topics over the past year. Two of the topics chosen by our readers are summarized below:

• **Genome editing**: we often refer to a person’s genome—the complete set of genes—as a blueprint for life. Sometimes, there are errors in this blueprint. Wouldn’t it be life changing if we could edit and correct these errors? We might soon be able to! Researchers have developed tools that make it possible to cut an individual’s DNA at a specific site, and either disrupt or correct the gene sequence at that location. The first of these therapies are now entering clinical trials. Come learn more about how this technology works!

• **Immunotherapy**: the term “immunotherapy” covers a wide range of topics, including vaccinations, monoclonal antibody therapeutics, checkpoint inhibitor therapies, CAR-T, and antibodies that deliver toxic compounds directly to cancer cells. During Tuesday’s talk, I’ll give a detailed overview of how each of these different types of immunotherapies work.

Please join me on June 20th, from 10:45am-12:15pm at the San Diego Convention Center in room 10. This talk is...
only open to BIO 2017 full registrants. FYI: Last year the room was filled to capacity and many were turned away at the door. Get there early and get your seat!

NO SCIENCE SINCE HIGH SCHOOL? WE CAN HELP!

Biotech Primer has published a book titled *The Biotech Primer: An insider’s guide to the biotech and pharma industry*—though some refer to it as “Biotech for Dummies.” The 200-page book explains the science behind the biotech industry, and includes a glossary of commonly used terms. Similar to our classes, *The Biotech Primer* starts out with the fundamentals of biology used by researchers and progresses to how those basics are employed to create therapeutics. The text is written to be understood by all — even those who have not taken a science class since high school. The illustrations and cocktail fodder (so you can impress your friends at your next party) keep things interesting. Stop by booth #5225 to pick up your free copy!

Excerpted below are a few paragraphs from Chapter 8: The Science of Discovery:

VALIDATING THE TARGET

Once a potential drug target has been identified, researchers will try to validate the target by determining whether the target plays a key role in the disease process and whether targeting it is likely to be both safe and effective. Target validation is a very important step in the drug discovery process, since research and development gets progressively more expensive—if a drug is unlikely to be successful, millions of dollars can be saved if this is realized early on.

Target validation will most often include cell-based assays (in vitro testing) and animal models (in vivo testing). Since the goal of many therapeutic interventions is to inhibit the activity of the selected target, many validation assays attempt to measure the effects of inhibition. In some cases, a selected target may play a role in disease progression – but if it is inhibited, another cellular protein will simply take its place, nullifying the potential therapeutic effect of an inhibitor. In other cases, inhibiting a selected target may have the desired therapeutic effect—halting cancer cell growth, for example—but may also result in unexpected side effects, such as the death of healthy cells.

One of the most popular ways of testing the effects of inhibition in cell-based assays is through the use of RNAi, described in detail in the chapter “From Gene to Protein.” RNAi is an effective way to quickly determine the results of blocking the production of a particular protein, thus mimicking the effects of a strong inhibitor.

If the cell models show promise, the researchers will move on to animal models, most likely designing experiments using so-called “knockout” mice—mice in which a particular gene has been disrupted. Researchers can ask similar questions to those asked in the cell model, but on the scale of the whole animal: do the experimental mice still get cancer, Parkinson’s disease, diabetes, or heart disease when the target gene is silenced or absent? The animal model also provides valuable information about targeting safety that might not be addressed in cell models because it is possible to examine the effects of gene targeting on the whole organism.

COCKTAIL FODDER

Proteins are easily broken down by the body’s digestive system, therefore patients receiving biologics (large molecule drugs) do not take them orally, but rather as injections.
UNDERSTANDING THE DISEASE PROCESS

Greetings from BIO 2017! It’s been a busy week here at the Bio International Convention here in San Diego, CA. At our convention booth, this year’s giveaway was our book written especially for non-scientists: The Biotech Primer: An insider’s guide to the biotech and pharma industry. If you weren’t at the convention to stop by and get a copy, please enjoy this excerpt below on cellular communication – a topic fundamental to understanding both normal and diseased cellular processes, and a process that is modulated by many drugs on the market today.

CELLULAR COMMUNICATION

In multicellular organisms, cells must communicate with each other. Since cells don’t have mouths, ears, or access to email, they must rely on chemical messengers. A chemical message – for example, a hormone – is released by one cell, and received by a second cell – the target cell. The target cell receives the message through proteins inserted into its membrane known as receptors – proteins that control the passage of molecules and the flow of information across the membrane. When the signaling protein binds its receptor, the receptor changes shape and transduces (converts from one form to another) the chemical message across the membrane to the cell interior. This process of cellular communication is known as signal transduction. The most common end result of signal transduction, and a key step in cell decision making, is the switching on, or off, of protein production – more commonly called gene expression.

Another class of membrane proteins that aid in cellular communication is channel proteins. These proteins act as molecular gates that allow the passage of small molecules and ions, for example, glucose and sodium, across the membrane in response to a stimulus, such as an electrical current in the case of ions or insulin signaling in the case of glucose. In neurons, ion transport between cells serves as a principle means of signal transduction. The influx of calcium ions (Ca++) into a neuron results in the release of neurotransmitters – chemical messengers specific to the nervous system. Different types of neurotransmitters regulate a variety of brain functions, including muscular activity, memory and learning, and mood regulation.

The regulation of blood sugar levels by the protein hormone insulin is an example of cellular
communication. After you eat, beta cells in your pancreas sense increased blood glucose and respond by releasing insulin into the bloodstream. Insulin molecules attach to specific insulin receptors on muscle cells and, in doing so, deliver a signal to the inside of the muscle cell to send glucose channels to the membrane, resulting in glucose uptake. In this way blood glucose levels are kept constant.

**CELL SIGNALING: A CLOSER LOOK**

Some cells send signals while others receive signals, but most cells do both. The signals are chemical hormones, such as adrenaline, or proteins, such as insulin. They are produced within specialized cells (the signaling cell) and released to find their target cells. The signal is often called a ligand. In some cases, the signaling cell and target cell may be the same cell. The target cell may be in direct contact with the signaling cell, or it may be in a different part of the body and receive a signal that has been transported through the bloodstream.

Alternatively, the signal and target may be in close proximity and the signal can be transported by diffusion through the intracellular space. After receiving a signal, the target cell responds in a manner that is determined by the nature of the signal received.

**GROWTH FACTOR SIGNALING**

Growth factors are proteins that signal a cell to multiply. For instance, epidermal growth factor (EGF) stimulates the proliferation of skin cells during wound repair. Cells are constantly exposed to many different growth factors, and the particular ones they respond to depends on their cell surface receptors. Skin cells, as well cells covering the gut, lung and breast, have or express receptors for epidermal growth factor (EGF), while nerve cells express receptors for nerve growth factor (NGF).

After receiving the initial growth factor signal, the enzymatic activity of the internal portion of the growth factor receptor is activated. The particular type of activity switched on is protein kinase activity – or the ability to transfer a phosphate group from one molecule to another. These types of receptors are sometimes referred to as receptor tyrosine kinases (RTKs), because they selectively transfer phosphate groups to the amino acid tyrosine on the recipient protein. This transfer, in turn, causes a slight shape change in the protein which received the phosphate group, typically leading to the activation of that protein’s own kinase activity. This newly activated protein kinase then goes on to activate yet another kinase protein, and so on, in what is referred to as a signal transduction cascade. The last element in this cascade to be phosphorylated is typically a protein called a transcription factor. Once phosphorylated, the transcription factor enters the nucleus, where it binds to the DNA at a particular location, activating expression of a specific gene.
Defects in the growth factor signaling process are associated with different types of cancer. A major challenge in oncology lies in understanding the complex signaling pathways that trigger cell division and determining what has gone wrong in each type of cancer. Once these signaling pathways are understood, it is possible to develop targeted therapies for the particular cancer.
FOUR MOLECULAR VARIANTS EXPLAINED

Hearing your doctor utter the words HER2-positive, HR-positive, triple-negative, or BRCA mutation can be devastating — even for the most resilient person. Simply put, breast cancer is a complex disease. A diagnosis can be derived from any combination of the factors listed above — or, none at all.

The National Cancer Institute (Bethesda, MD) has outlined four molecular subtypes of the disease. Each subtype is categorized by the cancer’s hormone receptor (HR) status and the level of expression from the HER2 gene. These cellular distinctions lead patients on different treatment journeys because the cancer subtype determines the drugs used in a treatment plan.

In this WEEKLY, we present a quick primer on the science behind HER2-positive, HR-positive, triple-negative, and the BRCA gene.

HER2-POSITIVE

HER2-positive (HER2+) breast cancer patients — about 20% of all breast cancer cases — have the most highly effective therapies available on the market. HER2+ cancer cells produce, and therefore present, larger than normal numbers of the HER2 receptors on their cell surface. These HER2 receptors capture growth factors, which trigger the cell to grow and reproduce more rapidly than normal. Mutations are more likely with rapid reproduction and thus, a tumor is born.

The overexpression of the HER2 receptor is a result of having extra copies of the HER2 gene — known in the world of genomics as gene amplification. Gene amplification events are thought to be caused by mutations that occur after a person is born — it is not an inherited form of cancer.

Genentech’s (South San Francisco, CA) Herceptin is a monoclonal antibody that binds to and blocks the activity of the HER2 receptor on cancer cells. When the HER2 receptor is blocked, the HER2 growth factor can no longer bind and send a growth signal to the cell, so the cancer cells stop dividing. The presence of an antibody on the surface of HER2+ breast cancer cells also signals the patient’s immune system to attack that cell.

Kadcyla, also made by Genentech, is an antibody-drug conjugate — a monoclonal antibody that delivers a highly toxic drug directly to HER2+ breast cancer cells. Kadcyla binds the HER2 receptor like Herceptin, but also delivers a toxic payload (which is actually attached to the monoclonal antibody). As a normal part of the cell’s lifecycle, cell-surface receptors get internalized or “taken up” by the cell on a regular basis. When Kadcyla is attached to a receptor that gets internalized, the toxic payload is released from the antibody and kills the cancer cell internally.

HR-POSITIVE

About 70% of breast cancer diagnoses involve a significant number of receptors for either estrogen or progesterone, making them hormone receptor positive (HR+). HR+ cancers may respond positively to treatments that block either the action or the production of estrogen. In some cases, these treatments may continue to be used for up to five years after initial treatment in order to prevent recurrence.

Two common types of medication for HR-positive breast cancers are Tamoxifen and aromatase inhibitors. Both types of drugs may also be prescribed as a preventive treatment in women who are at high risk for breast cancer. In fact, Tamoxifen is named on the World Health Organization’s List of Essential Medicines, a list of the most important medications needed in a basic healthcare system.

Tamoxifen works by inhibiting the estrogen receptor and was originally discovered by AstraZeneca (London, U.K.). On the other hand, aromatase inhibitors block the production of estrogen by inhibiting an enzyme whose activity is required for estrogen production. The different aromatase inhibitors on the market include Arimidex (AstraZeneca), Femara (Novartis; Basel, Switzerland, and Aromasin (Pfizer; New York, NY).

Selective estrogen receptor degraders (SERDs) are drugs that bind to estrogen receptors and cause them to be degraded. Fewer estrogen receptors mean that the cells receive growth signals from estrogen. Currently, there is only one selective estrogen receptor degrader approved — Faslodex, marketed by AstraZeneca. A second SERD,
Elacestrant, is in Phase I clinical testing by Radius Health (Waltham, MA).

Another new class of therapies for estrogen-receptor positive breast cancer are small molecule inhibitors of cellular enzymes known as cyclin-dependent kinases (CDKs). CDKs promote the development and division of cancer cells and inhibiting CDKs help to arrest cancer growth.

The first CDK inhibitor, Ibrance (Pfizer) was approved in 2015. Kisqali (Novartis) was approved in March of 2017, and Eli Lilly’s (Indianapolis, Indiana) Abemaciclib is in Phase III clinical development.

TRIPLE-NEGATIVE
Triple-negative breast cancers lack receptors — they are estrogen-receptor negative, progesterone-receptor negative, and HER2-negative. Since there are no receptor drug targets, this subtype is challenging to treat and there are no targeted therapeutics to date. If detected early enough, triple-negative breast cancer may respond well to chemotherapy.

THE BRCA GENE
BRCA stands for “BReast CAncer susceptibility gene” and everyone has the BRCA 1 and BRCA 2 genes. The job of BRCA is to scan cellular DNA for damage and trigger DNA repair processes when mutations are found. BRCA genes are passed down from one generation to the next — a good thing, unless the version passed down is a mutated variation.

Mutated BRCA1/2 genes are non-functioning, so they cannot locate DNA damage, nor can they enlist DNA repair. Testing positive for BRCA1/2 mutations may indicate there is an accumulation of DNA damage, which may eventually lead to cancer. BRCA is normally active in breast and ovarian cells, which is why certain mutations in BRCA1/2 are associated with a significantly increased risk of developing breast or ovarian cancer. It must be stressed that BRCA1/2 mutations in and of themselves do not cause cancer; they simply make it more likely to occur.

A new class of drugs known as PARP1 inhibitors gives hope to women whose breast cancer is associated with non-functioning BRCA genes. PARP1 is a second type of DNA repair protein. By inhibiting this pathway, DNA damage becomes so extensive that the cancer cells commit “cell suicide” (or apoptosis.) When the cell in question is a cancerous cell, apoptosis is a very good outcome.

The first FDA approved PARP1 inhibitor drug, Lynparza (AstraZeneca) was approved for BRCA associated ovarian cancer in December 2014. Clovis Oncology (Boulder, CO) achieved the second FDA-approval of a PARP1 inhibitor, Rubraca, in December 2016, and Tesaro (Waltham, MA) garnered the most recent approval in March 2017.

Not all triple-negative breast cancers are BRCA associated, but many BRCA associated cancers are triple-negative. For this reason, triple-negative breast cancer patients may find hope in PARP1 inhibitor drugs.

Breast cancer is a complex disease, and a better understanding of its molecular causes has enabled researchers to develop more effective therapies. As our understanding of the disease continues, we can expect to see additional novel therapeutics.
MEDS FOR THINNER BLOOD CAN EQUAL FEWER CLOTS, BUT HOW?

The FDA's recent approval of Portola Pharmaceuticals' (South San Francisco, CA) new blood thinner drug Bevyxxa paved the way for the prevention of blood clots in patients hospitalized for conditions such as heart failure, stroke, and pulmonary disease. The medical term for blood clot is venous thromboembolism (VTE), but if we take it apart:

- “venous” means relating to a vein or the veins.
- “thrombo” is a blood clot.
- “embolism” involves the lodging of an embolus, a blockage-causing piece of material, inside of a blood vessel.

Hospitalized patients are at high risk for VTE because of their restricted mobility — not being able to move causes blood to pool and collect in the body. An especially dangerous type of VTE is deep vein thrombosis — blockage of a vein that is deep within the body, as opposed to near the surface of the body. If a portion of a deep vein thrombosis breaks off, it may travel to the lungs, causing a potentially fatal pulmonary embolism.

An estimated 24 million people are hospitalized annually due to VTE, so let's find out how blood clotting is activated and learn the science of Bevyxxa.

A CLOT IN THE DARK

In healthy people, blood clotting is activated when tissue or a blood vessel is damaged, and involves specialized blood cells known as platelets — also known as thrombocytes. Either type of aforementioned damage results in activating platelets, which then form an initial “plug” at the site of injury. At the same time, proteins known as clotting factors are also activated. Clotting factors work together to produce a protein called fibrin, which is a fiber-like protein that forms a network of strands that, together with the platelets, form a clot at the site of injury. Clot formation in response to injury prevents excessive bleeding and enables healing to begin.

VTE occurs when blood clots form in the absence of an injury. These clots may break free and migrate to another part of the body, where it may interfere with blood circulation and impair organ function. If this occurs in a major organ such as the lungs, brain, or heart, critical injury or death may result. The clots may also grow to a size large enough to block the flow of blood in the blood vessel in which it originally developed.

Risk factors for VTE may be acquired (including older age, major surgery, prolonged immobilization, certain type of cancers, pregnancy and hormonal contraceptives) or inherited.

MECHANISM OF ACTION: BEVYXXA

Bevyxxa and other drugs that prevent the formation of blood clots belong to a class of drugs called anticoagulants which thin the blood. Bevyxxa works by directly inhibiting one of the key clotting factors, Factor Xa. This differs from older anticoagulants such as warfarin that works by inhibiting Vitamin K, which is required for complete activation of clotting factors.

Some key benefits of direct Factor Xa inhibition include faster onset, less interaction with other medicines or certain foods, and fewer bleeding events observed during clinical trials, leading to a better safety profile. Bevyxxa is the first oral Factor Xa inhibitor to be approved, and has been approved for use for up to 42 days. These attributes mean Bevyxxa can be prescribed to a patient to continue taking the anticoagulant after release from the hospital.

Patients were selected for treatment with Bevyxxa based on increased levels of “D-dimers” in their blood. D-dimers are degradation products of fibrin, the key protein component of blood clots. When our body breaks down blood clots, D-dimers are produced. Thus, having higher than normal blood levels of D-dimers is a sign that higher levels of blood clots are present.

AN ANTICOAGULANT U-TURN

Anticoagulant drugs can be life-saving; however their inhibitory effects may need to be reversed due to major bleeding, or in the case of an emergency surgery. Portola’s andexanet alfa, currently in late stage development, reverses Factor Xa inhibition. Andexanet alfa works by irreversibly binding Bevyxxa, preventing it from binding clotting Factor Xa. If Bevyxxa can’t
interact with and inhibit Factor Xa, it no longer prevents blood clotting.

**COCKTAIL FODDER: BLOOD THINNERS IN THE WILD**

Ever wonder how mosquitos and ticks are able to keep the blood flowing from their point of attack until they’ve had their fill? It turns out that their saliva contains a natural anticoagulant which prevents platelets from being activated. Fortunately, the effect is only temporary and localized to the site of the insect bite.

Foods that we eat may also impact blood clotting ability. For example, foods high in vitamin E such as almonds and hazelnuts, as well as spices such as cayenne pepper, garlic, ginger, and onion have some natural anticoagulant effects, while foods high in vitamin K such as leafy green vegetables, egg yolk, and soybeans may promote coagulation. For most healthy people, the relatively small amounts of these foods consumed in a normal diet would not have a significant impact on blood clotting; however, those on anticoagulant medicines may want to consult their physician about any possible dietary impact on their medicine’s efficacy.
CAN APPS PROGRAM BETTER HEALTH?

Digital medicine is defined by the field’s pioneer Dr. Eric Tool of the Scripps Translational Science Institute (La Jolla, CA) as “the ability to digitize human beings, by a variety of means (sequencing, sensors, imaging, etc.), fully exploiting our digital infrastructure of ever-increasing bandwidth, connectivity, social networking, the Internet of all things, and health information systems.” This new field is changing the way diseases from diabetes to substance abuse are prevented and treated.

“Digitizing human beings” may sound impersonal — but in fact the opposite is true. By enabling better access to individual health data, patients and physicians can create truly personalized health management plans. In this WEEKLY, we’ll take a look at this emerging biotech sector and the companies leading the way into the land of digital medicine.

THERE’S AN APP FOR THAT

There is a wealth of potential to delay the onset of diseases confronting our population, and a number of companies are developing digital medicine apps — similar to the ones you have already downloaded onto your phone such as your favorite music streaming app. Most of today’s digital medicine falls under the heading of “medication augmentation” — interventions meant to supplement rather than replace medication.

Chronic diseases such as diabetes can be managed much more effectively with “continuous intervention” — a day-to-day monitoring of the patient’s lifestyle choices and medication compliance. In many cases, these apps are highly sophisticated, clinically validated, and FDA-approved. The disease areas most commonly being tackled include Type 2 diabetes, chronic respiratory disease, chronic cardiovascular conditions, and mental health conditions.

Let’s unpack some of the digital medicine coming to an app store near you.

DIABETES

Diabetes management is a prime target for digital medicine intervention, as the number of people diagnosed with the disease has quadrupled over the past 35 years, from 5 million to 20 million (Centers for Disease Control).

WellDoc’s (Columbia, MD) BlueStar smartphone-based app is the first FDA-cleared mobile prescription therapy. The app allows users to enter data including levels of blood glucose, carbohydrates consumed, medications taken, exercise amount, hours of sleep, and perceived stress levels. BlueStar then makes personalized recommendations regarding diet, exercise, and medication, and even pinpoints the best times of day for the patient to test blood glucose levels. This information is easily shared with a physician.

In clinical testing, patients assigned the BlueStar app showed an average 1.9% drop in glycosylated hemoglobin (HbA1C) levels when compared to patients treated according to the current standard of care and no continuous intervention app. HbA1C is a reflection of average blood glucose levels over the past three months. Patients must receive a doctor’s prescription for the BlueStar app.

Another leader in digital therapeutics diabetes management is Omada Health (San Francisco, CA), whose interactive behavioral intervention program reduces the development of Type 2 diabetes in prediabetics through personal coaching via the integration of web, mobile, and smart devices. The goal is to help patients lose weight and increase physical activity. Although not FDA-approved, the platform is recognized by the Centers for Disease Control as an effective diabetes prevention tool.

CHRONIC RESPIRATORY DISEASE

In some cases, digital health companies are partnering with pharma companies to ensure better use of a therapeutic drug. Propeller Health (Madison, WI) has joined forces with GlaxoSmithKline for a “digitally guided therapy” platform for use with inhalers to treat asthma and chronic obstructive pulmonary disease (COPD).

The platform consists of sensors, provided by Propeller, that are attached to patients’ inhalers; a smartphone app for patient use; a website for physician use and data from a network of air-quality sensors. Propeller
monitors inhaler use, tracking patients over time and providing data on disease management as it relates to environmental factors (air quality). The Propeller system has four FDA clearances which allow the company to claim the system can be used to increase medication adherence, predict exacerbations, and reduce the frequency of symptoms and exacerbations in asthma and COPD.

HEART DISEASE

No discussion of digital medicine would be complete without a nod to the legendary story of Dr. Eric Topol using AliveCor’s (Mountain View, CA) mobile electrocardiography (ECG) device and app to diagnose a heart attack mid-flight. Dr. Topol was actually using a prototype model during his in-flight diagnosis; today, the Kardia Mobile device — essentially two sensor pads — is FDA-cleared and available for $99. After downloading the accompanying smartphone app, users can get an ECG reading in 30 seconds by opening the app on a phone placed nearby and placing their fingertips on the sensor pads. An irregular reading indicates possible atrial fibrillation, potentially indicating a heart attack.

ADDICTION & SLEEP

Pear Therapeutics (Boston, MA) is tackling substance abuse disorders with its digital therapy. Their lead product, reSET, is a smartphone app for patients with a clinician-facing web interface. It is designed to deliver behavioral therapy through a series of learning modules. The goal is to keep patients interested and engaged in their treatment between therapist visits. The apps have been clinically tested in five different trials. Patients who received reSET treatment in addition to standard addiction therapy showed better rates of drug abstinence. The app has been submitted to the FDA for approval.

Big Health (San Francisco, CA) has developed a digital therapy, Sleepio, to help with insomnia. The app consists of cognitive behavior therapy (CBT)-based exercises delivered by an online, animated therapist dubbed The Prof. When tested against an online version that included interaction with the Prof but lacked CBT-based activities, Sleepio was more effective at helping 75% of the participants to fall asleep.

THE FUTURE

The companies and apps described here are really just the tip of the iceberg of this new therapeutic world. Additional disorders for which digital therapies are being developed include obesity, hypertension, hyperlipidemia, smoking cessation, chronic pain, coronary artery disease, and even serious mental illnesses such as schizophrenia. The field is still in its infancy, but the dramatic benefit already seen by many adopters suggests a bright future.
PROPERLY FOLDING MISFOLDED DISEASE PROTEINS

Amicus Therapeutics (Cranbury, NJ) found itself in the news earlier this month when the FDA agreed to review the company’s new drug application for their investigational therapy to treat Fabry’s disease. The drug under consideration, migalastat, has already been approved by the European Medicines Agency. It belongs to a small, but growing class of therapeutics known as pharmacological chaperones that properly fold improperly folded proteins that cause disease.

Let’s take a look at which chaperones are on the dance floor and find out the steps they are taking to treat disease caused by proteins.

TERM OF THE WEEK: CHAPERONE PROTEIN

Chaperone proteins are proteins that assist in the correct folding and assembly of other proteins. Many of the proteins produced by our cells require chaperone proteins to ensure their correct molecular structure.

A pharmacological chaperone is a small molecule drug that targets specific misfolded proteins and encourages them to fold correctly.

Protein misfolding plays a role in many different rare diseases, including enzyme deficiencies like Fabry’s and the related Niemann-Pick disease, as well as Huntington’s disease, and some cases of amyotrophic lateral sclerosis (ALS). Some of the mutations in the genetic disease cystic fibrosis (CF) involve misfolded proteins. Diseases caused by misfolded proteins that disrupt cellular function are sometimes called proteopathies, where proteo = protein, pathy = disease.

MECHANISM OF ACTION: FABRAZYME

A type of lysosomal storage disorder, Fabry’s disease involves the inability to process certain types of lipids (fats), because they lack functional versions of critical enzymes, resulting in a range of symptoms, including kidney, heart, and skin disorders. The enzyme in question here, galactosidase, helps to break down glycolipids — lipids with a carbohydrate attached. Production of functional galactosidase enzyme is limited because of mutations in the galactosidase gene that cause the enzyme to be misfolded and therefore non-functional.

The only Fabry’s disease treatment on the market in the U.S. is Fabrazyme, which is made by Genzyme (Cambridge, MA). Fabrazyme is an enzyme-replacement therapy: since the patients don’t make enough functional galactosidase enzyme, scientists produce it in the lab using cells that have been genetically engineered to produce the enzyme, which is then purified and injected into patients.

MECHANISM OF ACTION: MIGALASTAT

Amicus’ drug migalastat, if approved, would be the first small molecule treatment for Fabry’s. The potential availability of swallowing a drug (vs. injecting) would give those with Fabry’s another drug delivery option.

In the lab, migalastat binds to and inhibits galactosidase. In the body, this high affinity is taken advantage of by migalastat binding to mutated galactosidase during the process of folding, where it then shifts the folding towards the correct conformation. The now correctly folded protein makes its way to a cellular compartment known as the lysosome, where it carries out its job of digesting lipids. The inside of the lysosome has an acidic pH, which causes migalastat to disassociate, leaving behind a functional galactosidase enzyme for the body to pick up and use.

Fabry’s is caused by a variety of different mutations within the galactosidase gene; not all of them are amenable to treatment with migalastat. Amicus scientists estimate that between 35% to 50% of patients will be responsive to migalastat.

MECHANISM OF ACTION: LUMACAFTOR

Another disease that can be traced to protein misfolding is cystic fibrosis. CF is a genetic disease caused by one of several possible mutations in the gene encoding the “cystic fibrosis transmembrane conductance regulator” (CTFR) protein. The CTFR protein is critical for the production of sweat, digestive fluids, and mucus.

The most common mutation, responsible for about two-thirds of CF cases, results in a protein that is so misfolded, it never makes it to the cell surface where it is required to do its job. Vertex Pharmaceuticals’ (Boston,
MA) drug lumacaftor serves as a pharmacological chaperone for these proteins, assisting them with correct folding so that they can make it to the cell surface. Lumacaftor is one piece of the CF puzzle; it is often used in combination with other therapies to fight various aspects of the disease.

**MORE DANCE CHAPERONES**

Instead of creating pharmacological chaperones, another approach to getting mutated proteins to fold correctly is to stimulate diseased cells to produce greater amounts of natural chaperone proteins. This can be done by identifying small molecules that induce cells to express heat shock proteins, a common class of cellular chaperones (described below). Two companies following this approach are Orphazyme (Copenhagen, Denmark) and Chaperone Therapeutics (Research Triangle Park, NC).

Orphazyme’s lead product, arimoclomal, has completed Phase II clinical testing for amyotrophic lateral sclerosis (ALS) associated with mutations in the gene for superoxide dismutase 1 (SOD1) enzyme; sporadic inclusion body myositis (sIBM), a rare muscular atrophy disease; and Niemann-Pick disease, a lysosomal storage disorder similar to Fabry’s disease.

Chaperone Therapeutics has a drug in preclinical development for Huntington’s disease, which is associated with disordered folding of the huntingtin protein.

**COCKTAIL FODDER: SHOCKING THE CHAPERONE**

The largest family of naturally-occurring chaperone proteins are called “heat shock proteins” because they were first discovered as part of a cellular response to heat shock — exposure to a higher than normal temperature. These proteins were later discovered to be induced in response to other types of cellular stress such as ultraviolet light exposure or wound healing. It’s thought that these cellular stressors can disrupt protein folding, and the production of heat shock protein chaperones can help to counteract the disruption.

Pharmacological chaperones that activate or mimic these protective proteins may prove to be the fresh new approach that can make a difference in a whole range of different diseases.
Putting The CAR-T Before The Horse

THE STORY BEHIND CAR-T

The hottest cancer therapy in the pipeline — chimeric antigen receptor therapy (CAR-T) — got a big boost last month when an FDA advisory panel unanimously recommended approval of the treatment for children and young adults with a severe form of leukemia who have run out of other options. Developed by Novartis (Basel, Switzerland), this elegant hack of the immune system is one of many horses in the race for a FDA approval, with Kite Pharmaceuticals (Los Angeles, CA) and Juno Therapeutics (Seattle, WA) rounding out the pack. Let’s take a moment to review these revolutionary therapeutics and understand how they attack cancer.

TERM OF THE WEEK: KILLER T-CELLS

CAR-T therapy is modeled after a cell in the immune system known as the killer T-cell. The job of a killer T-cell is exactly what the name implies — to kill dangerous cells. They target diseased cells in the body via their receptors: each one has a uniquely shaped receptor, and will recognize its intended target because the shape of its receptor “matches” or fits into a uniquely shaped surface protein found only on diseased cells. Once the Killer T-cell “docks” onto its target, it injects an enzyme which triggers death. The result: no more bad cells.

WHY CAR-T?

In theory our immune system should recognize the unique proteins presented on all diseased/cancerous cells; however there are two main reasons this doesn’t always happen:

• Early on in the tumor development, the cell composition is similar enough to healthy tissue that it can be overlooked by the immune system.

• Later as a tumor progresses, it releases chemical signals that suppress the immune response, helping it to evade detection. This trickery is known as the tumor microenvironment and once again the dangerous cancer cells can pass by undetected.

So what’s a scientist to do?! Figure out a way to train killer T-cells to ALWAYS recognize and destroy cancer cells... enter CAR-T.

HOW TO TRAIN AN IMMUNE SYSTEM

Killer T-cells are “trained” to go after early and late stage cancer by having their physical structure altered. This alteration is accomplished by fusing an antibody with the receptor of a killer T-cell to create a chimeric molecule — or the “C” in CAR-T.

Training day begins by having killer T-cells drawn out of a patient’s body and isolated in the lab. Next, scientists deliver a gene to the T-cells that encodes the chimeric receptor. This receptor consists of two parts:

• A targeting domain: This is the part of the chimeric receptor that will be outside of the T-cell. It is composed of an antibody that will recognize and dock onto a unique surface protein of the patient’s cancer.

• An activation domain: This part of the receptor will be triggered once the targeting domain is engaged. It will signal to the killer T-cell to:
  1. Stay alive.
  2. Make copies of itself.
  3. Release signaling molecules called cytokines. Cytokines are chemical signals that activate other white blood cells to join the fight against the tumor.
  4. Kill the target cell.
The T-cell/antibody hybrid is now a CAR-T therapeutic. It is then multiplied in the lab and infused back into the patient’s body. Once inside, the CAR-T locks onto its cancer target, replicates, sends out cytokines, and kills the designated cancer cells. The CAR-T will continue to replicate and kill any and all cancer cells recognized by the initial antibody component, with the goal of eliminating the disease.

**WHAT’S IN A NAME?**

Chimeric antigen receptor therapy broken down:

- **Chimeric:** Composed of components from two distinct parts, such as an antibody and a killer T-cell.
- **Antigen:** A protein that is recognized by an antibody, such as a protein on the surface of a tumor cell.
- **Receptor:** A protein that is embedded in a cell membrane and transmits signals to itself in response to being activated, for example a T-cell receptor transmits signals to the T-cell when it docks onto its target.
- **Therapy:** A treatment meant to manage or cure a disease.

As these therapies begin to move from clinical trials into clinical practice, the treatment of cancer will truly be revolutionized, offering new hope to patients and their families.
CRISPR/CAS9 UPDATE

As CRISPR/Cas9 adds new indications to its resume, legal battles over its IP continue to be waged in the US and Europe.

On the clinical front, CRISPR/Cas9 entered its first human trial at Sichuan University (Chengdu, China) last fall for metastatic lung cancer, and is widely expected to do so in the U.S. by the end of the year. This past March, a team of scientists at Oregon Health and Science University (Portland, OR) announced that they had successfully edited a gene linked to severe heart defects in human embryos.

On the patent front, CRISPR technology and its applications were discovered by two different research teams, one at University of California, Berkeley, and another at the Broad Institute (Cambridge, MA). Both have filed patents on various aspects of the CRISPR/Cas9 system. The Broad Institute had granted an exclusive license to Editas Medicine (Cambridge, MA), while Berkeley had granted licenses to Caribou Biosciences (Berkeley, CA), CRISPR Therapeutics (Basel, Switzerland and Cambridge, MA), Intellia Therapeutics (Cambridge, MA), and ERS Genomics (Dublin, Ireland). In February, the U.S. Patent Office ruled in favor of the Broad Institute and its licensee, while in March the European Patent Office ruled in favor of U.C. Berkeley patents. The legal battle is certainly far from over.

With all of these new developments making waves in the industry, let’s review the basics.

CAS TO THE RESCUE

CRISPR was originally discovered as a key component of the bacterial immune response. Bacteria, like people, are plagued by viral infections, and bacteria have evolved clever ways to attack invading viruses. In the 1980’s, scientists observed an interesting pattern in bacterial genomes: repeating, palindromic sequences, with unique sequences referred to as “spacers” between the repeats. They dubbed these regions a tongue twister of a name, “clustered regularly interspaced short palindromic repeats,” or CRISPR. Scientists also noticed CRISPR sequences were always located near a gene that coded for an enzyme that cut DNA. This enzyme became known as Cas, short for “CRISPR-associated”.

In the mid-2000’s, scientists realized the “spacers” matched DNA sequences of invading viruses — the bacteria were storing away bits of invading viral DNA between its own bacterial CRISPR sequences! These bits of viral DNA create a “genetic memory” of the virus, enabling the bacteria to fight back if reinfected.

Reinfection triggers the following steps:

- Viral DNA present in the spacer sequences is copied into viral RNA.
- The DNA-cutting enzyme Cas is made, and attaches itself to the viral RNA produced from the spacer sequence.
- This newly minted viral RNA/Cas complex finds its “match” on the invading viral DNA.
- The Cas enzyme is now positioned to cut up viral DNA, destroying the invading virus.

USE IN HUMANS

In 2013, researchers adapted this bacterial defense for use in human cells. Human cells were engineered to contain both specially-designed RNA and Cas genes. When these human cells produce the RNA/Cas complex, the dynamic duo is ferried to its complementary DNA target. Once in position, Cas goes to work cutting the DNA. The particular Cas protein chosen for this work was one discovered in Streptococcus bacteria, Cas9 — hence the moniker CRISPR/Cas9.

The ability to cut human DNA in precise locations is an exciting innovation because of what the cell does next.

BREAKING & FIXING

Cas9 creates double-stranded breaks (DSB) in the specified DNA sequence. DSBs cut both strands of the double-stranded DNA helix. Think of DNA as a two-lane bridge that, after experiencing an earthquake, has a section break off and fall into the water below.

DSBs activate two repair pathways to fix the break in the DNA:

- Non-Homologous End-Joining (NHEJ) closes the gap between the break by joining the two sections back together—visualize pushing the two sides of the bridge together, leaving the fallen section in
the water. An unintended byproduct of NHEJ is the possibility of sequence error, much like the sections of the bridge not lining up properly. If the repair occurs in the middle of a gene, the minor error can be enough to disrupt gene function and halt the production of the corresponding protein.

- **Homology Directed Repair** (HDR) relies on a highly similar (homologous) DNA segment to repair the break—visualize the missing bridge section built elsewhere and helicoptered in to fill the break.

By engineering double-stranded breaks to occur at specific locations, scientists activate the NHEJ or HDR cell repair pathways. By activating the NHEJ pathway, scientists can disrupt a disease-associated gene, preventing the production of a protein that causes the disease. By activating the HDR pathway, a short sequence of DNA is delivered with CRISPR/Cas9 to correct the mutated sequence, perhaps allowing a missing protein that causes disease to be made. In both scenarios cures for many different types of diseases may be realized.

**CRISPR IN THE CLINIC**

A clinical trial for metastatic lung cancer, initiated last fall by Chinese researchers at Sichuan University use CRISPR/Cas9 to disable the PD-1 gene in T-cells. The PD-1 gene produces the PD-1 protein, which is located on the T-cell’s surface. When the PD-1 protein is activated, the T-cell doesn't function. When the PD-1 protein is deactivated, the T-cell functions. Aggressive cancers take advantage of this on/off switch turning PD-1 on, effectively shutting down the T-cell. By turning PD-1 off, the T-cells can't be suppressed—freeing them up to attack cancer cells.

**COMING SOON**

A U.S. clinical trial of CRISPR to disrupt PD-1 in T-cells is expected to begin before the end of 2017. This two-year study is funded by the Parker Institute (San Francisco, CA).

A number of private companies also have plans for CRISPR/Cas9 clinical trials that include both gene disruption and gene correction. The table below summarizes some key players in the genome-editing arena and their approaches to applying CRISPR. *In vivo* means the therapy will take place inside the human and *Ex vivo* means the treatment will be performed in cells taken from the body and then injected back into the patient.

As these and other potential treatments move through clinical trials, the world will be watching to see if this revolutionary technology will live up to the hype and change the way we prevent and treat disease.
YOUR INNER IMMUNE WORKINGS
What do monoclonal antibodies, CAR-T therapy, and checkpoint inhibitor treatments all have in common? They are immunotherapies, or therapies that activate the immune system to fight or prevent a disease. While an activated immune system can help save a life, an overactive immune system can attack the body it is charged with protecting. This over-activity is the basis for autoimmune disorders.

The biotech industry has elegantly hacked the immune system — a highly complex network of signaling molecules, cells, and tissues — to make some of the leading immunotherapies such as Abbvie’s (North Chicago, IL) Humira that battles psoriasis or Merck’s (Kenilworth, NJ) Keytruda that fights different cancers. Let’s discover how the immune system operates and find out how our best and brightest are applying the immune approach to disease treatment.

IMMUNE SYSTEM PRIMER
The immune system is devoted to protecting us from foreign invaders. These include viruses, bacteria, parasites, fungi, and even cancer cells. The immune system consists of many different players, all working together as a team. The first defense consists of physical barriers, such as the skin and mucus membranes, which attempt to thwart these pathogens from entering our bodies in the first place. If these barriers are breached, then our cellular defense mechanisms kick in – first in the form of non-specific immunity, and then in the form of specific immunity.

NON-SPECIFIC IMMUNITY: MACROPHAGES AND NEUTROPHILS
Non-specific (or innate) immunity fends off pathogens at the cellular level. The troops are specialized white blood cells (WBC). Most WBC in the body are non-specific, meaning these foot soldiers attack in the same fashion without stopping to consider the specific characteristics of the enemy.

Macrophages are one type of non-specific defender that freely circulate in the bloodstream. When they encounter a bug, they engulf it – essentially eating it. Macrophages have the ability to differentiate between good and bad bacteria and virus based on a special receptor called a PAMP (Pathogen-Associated Molecular Pattern) found only on the bad ones. Other non-specific defenders include neutrophils (which also recognize the PAMP and engulf invaders) and natural killer cells, which inject the protein granzyme B into invaders, triggering cell death.

Once activated, these non-specific defenders release “inflammatory cytokines,” or signaling molecules that switch on other immune cells. The inflammatory response is kicked into gear, ensuring a rapid and comprehensive retaliation.

SPECIFIC IMMUNITY: T-CELLS AND B-CELLS
When non-specific defenses are unable to rid the body of pathogens, it’s time for back up. Waiting for the call are T-cells and B-cells, which make up your specific (or adaptive) immunity. These cells are highly specialized to recognize unique targets, called epitopes, thanks to their distinctly shaped receptors. Once the B-cell or T-cell receptor binds to the pathogen’s epitope, they are activated. Each T- or B-cell recognizes only one unique epitope.

Activated T-cells divide rapidly and produce three types of descendants: killer T-cells, helper T-cells, and memory T-cells. All recognize the same target as the originally activated T-cell.

- Killer T-cells roam the body in search of their pre-programmed epitope, and seal the deal by injecting granzyme B, triggering cell death.
• Don’t let the label “helper” fool you – helper T-cells are critical, they release inflammatory cytokines that activate antibody-producing B-cells, killer T-cells, and macrophages to respond en masse. The human immunodeficiency virus (HIV) only infects helper T-cells, and in so doing completely cripples the immune response.

• Memory T-cells don’t defend against an initial infection, but if these cells encounter the same epitope a second time, they are very quickly converted into killer T-cells and helper T-cells, ensuring a rapid response.

Activated B-cells also reproduce rapidly and produce two types of descendants: plasma cells and memory B-cells.

  • Plasma cells secrete antibodies – proteins that recognize and bind to any bacterium or virally-infected cell that bears a matching epitope. The binding action triggers other immune cells, such as killer T-cells or macrophages, to sweep in and destroy the invader attached to the antibody.

  • Like memory T-cells, memory B-cells lie in wait, preparing for future attacks instigated by the same foreign invader.

APPLIED IMMUNOLOGY

Immunotherapies use strategies from specific immunity to their advantage. Monoclonal antibody therapies are developed by selecting antibodies that recognize and bind to a disease-specific epitope. The classic example, Genentech’s (South San Francisco, CA) therapeutic antibody Herceptin, binds to the HER2 epitope which is present at high levels on the surface of 25% of breast cancer patient’s tumors. This compels white blood cells, such as killer T-cells and macrophages, to attack the tumor. Herceptin acts just like a naturally-occurring antibody, only it is produced in the lab. Chimeric antigen receptor T-cell (CAR-T) therapies, which to date are still in clinical testing, are T-cells whose receptors have been engineered to recognize and destroy cancer.

CHECKPOINTS ON THE CASE

The body has natural checkpoints to prevent inflammatory disorders; this screening process stops T-cells and B-cells from mistakenly killing its own tissues. These checkpoint proteins send a “stop attacking” signal to the T- and B-cell when they encounter their body’s healthy cells. Many types of cancer cells have evolved to express these checkpoint proteins, tricking the T and B-cell into thinking the cancer is a healthy cell. Checkpoint inhibitor therapies prevent the cancer from activating their checkpoint proteins, enabling the immune system to more fully go after the tumor.

THE OTHER SIDE OF THE COIN: AUTOIMMUNE DISEASE

The immune system prevents us from falling deathly ill as it responds to constant microbe exposure. However, an overactive immune system can cause serious problems, potentially leading to autoimmune disease. Chronic inflammatory disorders such as Crohn’s disease, rheumatoid arthritis, and psoriasis wreak havoc by activating white blood cells to target innocent cells in the body and release inflammatory cytokines to sustain the barrage.

Biologic drugs that treat these disorders—like Humira (AbbVie, North Chicago, IL), Enbrel (Amgen, Thousand Oaks, CA), and Rituxan (Genentech, South San Francisco, CA)—work by shutting down key parts of the response. Humira and Enbrel inhibit a specific inflammatory cytokine known as TNF-alpha. Both of these drugs are approved for a range of inflammatory diseases. Rituxan, approved for rheumatoid arthritis, works by reducing the number of B-cells that target the synovial tissue of joints.

From one side of the coin to the other, the immune system continues to both challenge and reward the industry as new pathways and targets are discovered. A delicate balance of the body’s toughest fighters, understanding and optimizing the immune system is central to the immunotherapy paradigm.
DECODING THE GUT-BRAIN AXIS

There is no shortage of microbiome-focused startups in biotech right now. The link between the gut microbiome — the entire collection of microbes living in the gut — and diseases such as inflammatory bowel disease are well-established. New research has made it clear, however, that the gut microbiome also impacts neurological health, leading to the phrase “the gut-brain axis.” Let’s explore this connection and examine the early efforts by a few innovative biotechs to translate these new discoveries into the clinic.

GUT MICROBIOME PRIMER

The human microbiome is the complex collection of microbes (mostly bacteria, but also includes small numbers of fungi and viruses) that reside on and inside the human body, including our skin, mouth, nose, respiratory tract, and digestive tract (gut). The microbiome is huge — microbial cells outnumber human cells by a ten to one ratio! Human cells are much larger than bacteria cells, however, so don’t worry — you’re still mostly human. For every 100 pounds that you weigh, it is estimated that about two pounds of that weight come from bacteria.

Most of us think of bacteria as harmful and certainly some types are; however, those that have evolved with humans to become part of the human microbiome are either neutral — causing no harm — or beneficial. Scientists are busy trying to better understand and characterize these beneficial bacteria and the role that they play in human health.

The gut contains the largest number of bacteria, as well as the greatest diversity of bacteria, when compared to other parts of the body. Thus much of the attention directed towards the human microbiome has been focused on the gut microbiome in particular, which continues to surprise us with its influence on diseases such as obesity, diabetes, and, increasingly, brain disorders.

TRAIL BLAZERS: AXIAL & THE MAZMANIAN LAB

Axial Biotherapeutics (Cambridge, MA) is focusing much of its initial attention on work originally done at microbiologist Sarkis Mazmanian’s lab in the California Institute of Technology (Pasadena, CA) — mostly relating to the gut-brain axis’ role in Parkinson’s disease (PD) and autism.

Parkinson’s Disease

Parkinson’s disease (PD) is a chronic and progressive movement disorder, according to the Parkinson’s Disease Foundation. Symptoms include tremor of the hands, arms, legs, jaw, and face; slowness of movement; rigidity of the limbs and trunk; and impaired balance and coordination. These symptoms are caused by the malfunction and death of neurons that produce the neurotransmitter dopamine. PD affects nearly one million people in the U.S., and the cause is unknown. About 75% of PD cases are accompanied by gastrointestinal disorders such as constipation, which provided an initial impetus to examine a possible connection between gut health and the disease.

A key molecular characteristic of PD is the aggregation of a protein called alpha-synuclein (αSyn) within cells of the brain and gut. Researchers in the Mazmanian lab used a strain of mice that overproduce αSyn to study the disease. One group of the αSyn mice were bred in a completely sterile environment to create “germ-free” (GF) αSyn mice. The other αSyn mice had the normal collection of gut bacteria. On a series of tests designed to assess motor skills, the GF αSyn mice performed significantly better — suggesting that even in mice that overproduced the αSyn protein, the presence of certain microbes are required for the disease to progress.

Further work suggested that a molecule called butyrate, produced by certain gut bacteria, can enter the brain and activate an immune response, leading neurons to malfunction or die.

There is reason to believe that this connection is also at work in humans with PD. In collaboration with Rush University (Chicago, IL) gastroenterologists, Mazmanian lab researchers transferred fecal samples from PD patients into the GF αSyn mice. Fecal transplants are an established way to “reset” the gut microbiome of...
the recipient to make it match that of the donor. After transplantation, the mice began exhibiting symptoms of PD. Transfer of fecal matter from healthy people did not trigger these symptoms. These experiments suggest that the gut microbiome is a major contributor to the disease process in PD patients.

Of course, these promising early stage findings still need to be translated into human therapeutics. This may be easier than traditional neurological approaches because it is much easier to deliver drugs to the gut than to get them to cross the blood-brain barrier. Following up with a targeted approach to modulate the production of butyrate and other inflammatory compounds produced in the gut may bring the first truly effective PD therapy into the clinics.

**Autism Spectrum Disorder**

Autism spectrum disorder (ASD) is a developmental brain disorder characterized by impaired social interaction, communication, and restrictive and repetitive behavior. These symptoms impact a child’s educational, social, emotional, and physical development. More than 3.5 million Americans live with an autism spectrum disorder according to the [Autism Society](#). The cause is unknown, although genetics is thought to play a role.

Similar to PD, a significant portion of ASD patients exhibit gastrointestinal problems. The Mazmanian lab demonstrated that feeding ASD mice a specific strain of bacteria called *B. fragilis* — a part of the human microbiome — altered the mouse microbiome and reduced some of the ASD-like behaviors such as anxiety and repetitive behavior, and increased levels of communication with other mice. These experiments suggest a possible probiotic therapy for autism.

**CARB LOADING WITH SYMBIOTIX**

Another early-stage company making headlines in the gut-brain axis space is Symbiotix (Boston, MA). Focusing on multiple sclerosis (MS), their lead candidate is a carbohydrate molecule produced by *B. fragilis*. This therapy increases the production of regulatory T-cells, which are a class of T-cells that “turn down” an overactive immune response by releasing anti-inflammatory signaling molecules. Symbiotix is preparing to enter clinical trials with an orally-administered product for the treatment of MS and inflammatory bowel disease.

The emerging work described here gives credence to the old expression “think with your gut.” In addition to the diseases discussed above, scientists are discovering links between the gut microbiome and other brain disorders such as anxiety and depression. As the story continues to unfold, we are likely to see new therapeutics based on restoring the balance that millions of years of human-microbe co-evolution has fine-tuned.
Back to school means shopping for new school supplies, adjusting to a new schedule, and making sure all required vaccinations are up to date. Every state requires school-age children to be vaccinated against certain infectious diseases including tetanus, hepatitis B, measles, mumps, rubella, polio, pertussis (whooping cough), and chicken pox. Vaccination policies are highly effective at eliminating many types of sickness from the most perfect incubator — the classroom.

In this WEEKLY, we’ll go to the chalkboard to learn the basic science of vaccines.

**BASIC SCIENCE**

The idea behind vaccination is simple: by exposing someone’s immune system to a harmless version of a pathogen, we can train it to recognize and respond to the bug in the wild. After an initial exposure to a virus, our immune system creates memory cells which are then ever-ready to spring into action and attack the same disease later on down the line. Vaccination is required because creating these pathogen-specific memory cells takes a few weeks — a length of time that is long enough for a virus to do serious damage to the body.

**ABCS OF VACCINES**

There are a few ways to create a vaccine and below we list the most common methodologies.

- **Inactivated vaccines:** The most obvious type is the inactivated vaccines — the use of heat or chemicals to kill the pathogen. Inactivated vaccines produce a dampened immune response in comparison to other vaccine methods and often require “booster” shots. Inactivated vaccines include polio, influenza, and pertussis vaccines.

- **Live, attenuated vaccines:** A live, attenuated vaccine simply means that the weakened (not killed) pathogens are unable to cause disease. Attenuation occurs by a process called “passage,” or growing viruses at temperatures slightly lower than the human body or in cells different from the human host cells. Under these conditions, the virus accumulates mutations that make it better able to survive in a new environment, but when injected into a human, it is no longer virulent. Attenuation may also be more direct and occurs when genes associated with pathogenesis or replication are removed in the lab. In general, attenuated vaccines induce a strong and long-lasting immune response. Examples of live, attenuated vaccines include measles, mumps, rubella, chicken pox, and polio.

- **Subunit vaccines:** In some cases, just one protein from the virus can be enough to induce an immune response. These are called subunit vaccines and are typically made using recombinant DNA techniques to produce the desired protein. Advantages of subunit vaccines include easier production and a better safety profile for patients. Examples of subunit vaccines include pertussis and hepatitis B.

- **DNA vaccines:** DNA vaccines are the next frontier in vaccine development. Rather than delivering a whole pathogen or pathogen subunit, DNA vaccines deliver just a gene. Once inside the body, the patient’s own cells reproduce the pathogenic protein. If successful, this technology would mimic a natural infection and elicit a strong immune response. The technical challenge that remains to be solved is the delivery of the pathogenic gene. Ichor Medical Systems (San Diego, CA) and Inovio Pharmaceuticals (San Diego, CA) are both developing electroporation-mediated DNA delivery systems to solve this problem.

**TERM OF THE WEEK: HERD IMMUNITY**

Herd immunity means a significant portion of a population has immunity to a particular pathogen. There is little opportunity for an outbreak, so even those who cannot be vaccinated such as immunocompromised individuals, pregnant women, or newborn babies are unlikely to become infected despite their unprotected state.

**A UNIVERSAL FLU VACCINE?**

Unlike most other vaccines, you must get the flu shot every year in order to be afforded protection. Current flu vaccines work by mounting an antibody response against two large proteins on the surface of the virus —
hemagglutinin (H) and neuraminidase (N). The catch? The structure of those two proteins changes every season due to a high mutation rate. Once the structure changes, the immune system no longer recognizes it, and the body must be retrained. This is also why the vaccine is only 60-70% effective — when formulating each year’s vaccine, scientists attempt to predict the influenza strains that will be circulating in winter, and they are seldom 100% correct.

For many years, scientists have talked of producing a universal flu vaccine. Recent advances in vaccine technology have led to some promising developments. **BiondVax** (Ness Ziona, Israel) has identified nine epitopes — short sequences of proteins that elicit an immune response — that do not vary much between different strains of the virus. These sequences were combined to make one recombinant protein referred to as Multimeric-001 (M-001). The hope is this combination of epitopes will invoke a strong immune response which will be protective over several flu seasons. M-001 is in Phase II clinical testing.

Researchers at **Crucell Vaccine Institute of Janssen Pharmaceuticals** (Leiden, Netherlands) have discovered an antibody that recognizes and binds a portion of the HA protein that doesn’t mutate very rapidly. Studies in animals suggest treatment with this antibody significantly reduces the amount of active virus present. Human clinical trials are in the works.

A clinically tested and FDA-approved universal flu vaccine is still years away, but these early results are promising. The new vaccine would most likely need to be administered every five or 10 years, rather than annually—but the real advantage will be its ability to protect against a range of different influenza strains, inching closer to 100% efficacy. And since most of us have suffered through a bad case of the flu, we can all agree that a universal flu vaccine cannot come soon enough!
THE FLASH OF THE FIRST CAR-T

Last week’s much anticipated FDA approval of the first chimeric antigen receptor T-cell (CAR-T) therapy for acute lymphocytic leukemia hails as the first gene therapy on the US market.

Classified as a “cell-based gene therapy,” Novartis’ (Basel, Switzerland) Kymriah works by removing patients’ T-cells, using a viral vector to introduce a gene that will allow the T-cells to recognize and kill cancer, and then infusing these modified T-cells back into the patient. Recall T-cells are found in the blood and fight disease.

Along with its significant potential, Kymriah also carries serious risks. Its approval came with a boxed warning because of the potential for “cytokine release syndrome (CRS),” also referred to as a cytokine storm, which has caused fatalities in clinical trials of other CAR-T products.

Making CAR-T safer while maintaining efficacy are goals of next generation CAR-T. Let’s explore cytokine storms and find out how scientists aim to circumvent this roadblock to fighting cancer.

TERMS OF WEEK: CYTOKINE & CYTOKINE STORM

Cytokines are small proteins which play an important role in relaying messages from one cell to surrounding cells and tissue. Cytokines serve two main functions involving white blood cells:

• Activate additional white blood cells to fight off pathogens
• Stimulate white blood cells to move towards sites of inflammation

Cytokine signaling makes for a very quick and strong immune response. Usually, the response is kept in check, and dissipates when the bad cells have been eliminated.

However, in some cases, this positive feedback loop — activated cells releasing still more activating cytokines — spins out of control, resulting in a cytokine storm. Acute inflammation with accompanying symptoms such as high fever, swelling, and nausea can occur. In severe cases, serious tissue damage and death can result — for example, lung failure induced by excessive amounts of fluids and cells moving into the lungs.

A cytokine storm is the adverse event most associated with CAR-T treatments and next generation CAR-T treatments are being developed to have built-in controls to regulate cytokines so the storms can be stopped.

AN ON/OFF SWITCH

In first generation CAR-T, maximum activation occurs. With a full cytokine barrage, there is no way to tamp down the cytokine response.

In next generation CAR-T, a small molecule drug may be co-administrated with the therapy. The drug’s function is to activate CAR-T to fight cancer, or turn it “on.” If a cytokine storm ensues, the small molecule drug can be immediately withdrawn — the “off” switch — essentially deactivating CAR-T and stopping cytokine release.

This second iteration of CAR-T is made possible by a handful of companies who are designing drugs that will act as an “on/off switch” to control CAR-T. Bellicum Pharmaceuticals (Houston, TX) is developing a CAR-T product, BPX-601, that uses small molecule-activation. BPX-601 entered Phase I clinical trials in February 2017. Intrexon (Germantown, MD) has a similar product in Phase I clinical development.

GETTING BISPECIFIC

Juno Therapeutics (Seattle, WA) is developing a CAR-T product that uses a sort of two-step verification process. It turns out that tumor cells have many proteins on their surface — so it is a challenge to find a distinct protein that is also unique to any given cancer cell. Instead of relying upon finding that one special protein, why not target a more common one and use another protein to double check the work?

Juno’s approach: bispecific chimeric antigen receptors. This means each engineered killer T-cell has not one, but two chimeric antigen receptors (CARs).

• One CAR is activated in the presence of a protein found on the surface of cancer cells. Once activated this CAR-T cell would produce more copies of itself, release cytokines, and attack the tumor.

• A second CAR called an inhibitory CAR (iCAR), is activated in the presence of a different protein found only on healthy cells — NOT on cancer cells. If an
iCAR is activated, an inhibitory signal is sent to the first CAR, preventing the CAR-T from working. Simply put, one CAR finds the target protein while the other iCAR verifies the cell is “unhealthy” via another protein. This bispecific CAR-T, now in preclinical development, aims to eliminate the off-target effects and decrease the amount of cytokines released.

OXYGEN DEPRIVED

Earlier this year, Cellectis (Paris, France) published a paper describing work they’ve done to engineer CAR-Ts with an oxygen-sensitive domain.

Under normal cellular conditions, this domain signals the CAR-T to remain inactive. Under low oxygen, or hypoxic conditions, this domain sends an activation signal to the CAR-T. Since most solid tumors have a hypoxic environment, an oxygen-sensitive CAR-T should be activated within the tumor but not outside of it.

INTELLIGENT DESIGN THROUGH DECOYS

It turns out that CAR-T activation results in cytokine production because part of the CAR-T is actually inside the cancer cell, where it interacts with other proteins in a cytokine signaling pathway.

Scientists at the Blood Research Institute of Blood Center of Wisconsin (Milwaukee, WI) have developed decoy molecules that interfere with the protein-protein interactions in the pathway. These decoy molecules are short protein fragments called peptides. By binding to proteins in the cytokine pathway, the signal to produce more cytokines is blocked.

The decoy peptides reduce cytokine production by ~70%, which is likely enough to prevent the immune response from spinning out of control. In the words of Laura Savatski of the Blood Research Institute, “By reducing cytokine production by CAR-T cells, you prevent a cytokine storm from happening. So instead of dealing with a problem at the back end of the therapy, you solve it at the front end through intelligent design.” Decoy molecules are currently in preclinical development.

With some of the best minds in the biopharma industry working on CAR-T design, this landmark FDA approval is likely to be just the first shot in a treatment revolution.
BLOOD CANCER: MULTIPLE MYELOMA
Plasma cells are the antibody-producing cells of our immune system which happen to play a critical role in our defense against infections. In multiple myeloma, plasma cells begin to grow and divide in an uncontrolled manner, forming a cancerous mass known as a plasmacytoma. Marrow — which produces plasma — no longer functions in our defense, it simply takes up space inside the bone.

What does biotech have in store to fight multiple myeloma? Let’s find out the treatments on the market and the up-and-comers in development.

EASILY CONFUSED: PLASMA CELLS VS BLOOD PLASMA
Plasma cells are specialized white blood cells that produce infection-fighting antibody proteins. Most plasma cells are found in the bone marrow.

Blood plasma is the straw-colored liquid component of blood that holds blood cells in suspension, made up of water (95%), proteins, glucose, clotting factors, electrolytes, hormones, carbon dioxide, and oxygen.

PICKING APART PLASMACYTOMA
Plasmacytoma formation can lead to a host of problems with recognizable clinical symptoms. Instead of producing normal disease-fighting antibodies, plasmacytoma cells produce abnormal antibodies called M proteins, which don’t provide any benefit to the body and crowd out normally functioning antibodies. And because all blood cells are formed in the bone marrow, overproduction of plasma cells can also crowd out normal blood-forming cells. This can lead to anemia, caused by a shortage of oxygen-carrying red blood cells; increased bruising and bleeding due to a reduction in clot-promoting platelets; and an increased risk of infection due to lower levels of healthy infection-fighting white blood cells.

Although multiple myeloma is classified as a blood cancer, it has a significant impact on bone health. As the plasmacytoma grows, bone-forming cells called osteoblasts are suppressed. At the same time, production of a substance that activates bone-reabsorbing cells, osteoclasts, is increased. The resultant damage to the bone structure creates soft spots or lesions which may extend from the inner bone marrow to the outside surface of the bone. Bone lesions result in significant pain and increase the risk of fracture. Bone destruction also releases excessive calcium into the bloodstream, leading to a range of symptoms including confusion, nausea, and loss of appetite. Excess blood calcium, combined with high levels of M protein, also contributes to impaired kidney function seen in multiple myeloma patients.

UNMASKING MULTIPLE MYELOMA
There is no one diagnostic test for multiple myeloma. Blood and urine tests to detect some of the symptoms listed above such as low blood cell counts, elevated blood calcium levels, and impaired kidney function may suggest multiple myeloma. These tests are followed by a bone marrow biopsy for confirmation.

Most cases of multiple myeloma have no known cause, although some research suggests that regular exposure to herbicides, insecticides, petroleum products, heavy metals, and asbestos increases the risk of developing the disease. And although there is not a specific gene yet associated with multiple myeloma, abnormalities in chromosome structure or number are associated with the disease.

ON THE MARKET
Once considered incurable, there are now a number of effective treatments for multiple myeloma, and several more are in the pipeline.

Darzalex (Johnson & Johnson; New Brunswick, NJ) and Empliciti (Bristol Myers Squibb; Princeton, NJ) are both monoclonal antibody therapeutics approved to treat multiple myeloma. They work by recognizing and binding to proteins on the surface of multiple myeloma cells, activating the patient’s immune system to destroy those cells.

Ninlaro (Takeda; Osaka, Japan) is a small molecule proteasome inhibitor therapy. A proteasome is a specialized compartment within the cell that gets rid of damaged proteins by digesting them. If the proteasome is inhibited, damaged proteins build up within the cell.
This triggers a process called apoptosis — essentially, cell suicide. In other words, the cancer cell kills itself. Farydak (Novartis; Basel, Switzerland) is a small molecule “histone deacetylase (HDAC) inhibitor.” HDACs are enzymes that modify chromosomes (strands of DNA that contain our genes) and influence how often specific genes are activated. Some cases of multiple myeloma are associated with changes in gene activation. By inhibiting HDACs, Farydak can correct this changed gene expression.

**IN THE PIPELINE**

Two novel drugs in the multiple myeloma pipeline are Mivebresib (AbbVie; North Chicago, IL) and Selinexor (Karyopharm Therapeutics; Newton, MA).

Similar to Farydak, Mivebresib influences the activation of specific genes by inhibiting a group of proteins called Bromodomain and Extra Terminal motif (BET) proteins. In some types of cancer, genes are activated or deactivated inappropriately due to BET activity. By inhibiting BET, Mivebresib may restore normal gene activity to these cells. Mivebresib is currently in Phase I clinical testing for multiple myeloma.

Selinexor helps to increase the number of tumor suppressor proteins present in the nucleus of cancer cells. These proteins help to protect against cancer by detecting DNA damage and promoting apoptosis in those cells that have high levels of DNA damage. In many types of cancer cells, tumor suppressor proteins are transported out of the nucleus, where they can no longer do their job of detecting DNA damage. By blocking this transport, Selinexor enables tumor suppressor proteins to do their job of triggering apoptosis in cancer cells. Selinexor began Phase III clinical testing for myeloma in June 2017.

CAR-T therapies are also in development for multiple myeloma. Bluebird Bio (Cambridge, MA), in partnership with Celgene (Summit, NJ), and Nanjing Legend Biotech (Nanjing, China) have announced promising results in early phase CAR-T trials for multiple myeloma.

Multiple myeloma is a complex cancer. In recent years, a better understanding of the disease has led to the approval of several new therapeutics. In the coming years, we can look forward to additional approvals as novel therapeutics move through the pipeline.
From Fantasy To Reality: Xenotransplantation

TRANSPLANTING ORGANS FROM ANIMALS INTO HUMANS

Every ten minutes, a new person is added to the national transplant waiting list. A little more than 75,000 people are active waiting list candidates — meaning they are medically eligible for transplantation according to the Organ Procurement and Transplantation Network. Over the past decade, the gap between organ supply and demand has continued to grow; an estimated 20 people a day die as they wait for an organ transplant.

The idea of xenotransplantation — transplanting organs from animal donors to humans — has been the subject of discussion for decades in science circles. Now, with the tools provided by modern biotech, we may soon see this seemingly science-fiction idea become a life-saving reality. Let’s explore the past, present, and future of xenotransplantation.

THE PAST

The dream of xenotransplantation has been around for over a century. In 1905, a French scientist inserted portions of a rabbit’s kidney into a child suffering from kidney failure. The patient’s kidney function improved; however long-term follow-up was not possible as the child died of pneumonia within a few weeks. In the early 1960s, a small group of critically ill patients at Tulane University received kidney transplants from chimpanzees. None of these patients survived long-term, and the establishment of working cadaver organ procurement programs put xenotransplantation on the back burner until 1984 when “Baby Fae” captured the public’s imagination as the first infant to receive not only a heart transplant, but a xenotransplant, when she received a baboon heart. The transplant operation itself was successful; however, the baboon heart was rejected by Baby Fae’s immune system 21 days after surgery.

THE PRESENT

A few key challenges have prevented xenografts from solving the problem of organ shortages for patients in need. They include:

• Determining the correct animal donor, taking into account both anatomical and ethical considerations.

• Preventing immune rejection: Immunosuppressive drugs need to be given at a higher dose for xenotransplant recipients than for those receiving organs from human donors. High doses of immunosuppressive drugs can lead to serious infections.

• Lingering concerns that viral genes present within animal genomes, which do not harm the animal, could be harmful to humans.

Today, pigs are considered to be the best potential animal organ donor for humans, due to their availability and the size of their organs closely matches human organ size. Let’s see what biotech is doing to address the two remaining challenges.

THE FUTURE

Cambridge, MA-based eGenesis is using CRISPR/Cas9 genome editing to tackle the problems of immune rejection and potential interspecies virus transfer — referred to as “porcine endogenous retroviruses (PERVs)” in pigs. CRISPR can be used to “knockout” (remove) undesirable genes, or to “knock-in” (add) desirable gene sequences.

Last month, eGenesis scientists published a paper in which they described their successful use of CRISPR genome editing combined with a cloning technique called “somatic cell nuclear transfer” to produce dozens of healthy pigs whose genomes no longer contain PERVs. The pigs were produced using the following steps:

• Use CRISPR to inactivate PERV sequences present in an adult pig’s DNA.

• Transfer the modified DNA into a pig egg whose own nucleus has been removed. This technique is known as somatic cell nuclear transfer.

• The egg, now containing a PERV-free pig genome, is stimulated with an electric shock to trigger cell division.

• After several rounds of cell division, an early-stage embryo is formed, which can then be transferred into a surrogate mother to complete development.

This was the first time that as many as 25 genes had been simultaneously and precisely deactivated with
CRISPR — a significant accomplishment and a major step towards making xenotransplants safe.

More work still needs to be done, however, before pig-to-human heart transplants are routine. In addition to deleting PERV genes, scientists will need to deactivate pig genes that help to trigger immune rejection and add in gene sequences to help the human immune system recognize the transplant as safe.

As for the cloned pigs, the next step will be to see if the changes made to their genome will be transmitted to subsequent generations because breeding pigs are much easier than cloning them. And speaking of pig breeding, Smithfield Foods (Smithfield, VA) — better known for producing pork products for consumption — has thrown its hat in the ring by establishing a bioscience unit to be used for supplying pig parts for medical use, ultimately including organs for xenotransplantation.

eGenesis is not the only company using genome editing in the pursuit of xenotransplants. Synthetic Genomics (La Jolla, CA), in collaboration with United Therapeutics (Silver Spring, MD), is also working on modifying pig genomes to make their organs safe for human transplant.

We are still several years away from making xenotransplants a routine clinical reality. The next step will be testing genetically modified pig hearts in baboons. But with two of the leading genomics pioneers behind eGenesis and Synthetic Genomic — George Church and Craig Ventor, respectively, this is certainly an area to keep close tabs on.

COCKTAIL FODDER: ORGANS FOR SALE?

The only place one may buy an organ legally is in the country of Iran; however, citizenship is required in order to lessen transplant tourism. Australia and Singapore have legalized monetary compensation for living organ donors, in order to cover associated medical expenses and compensate for time of work.
USING MEDICAL IMAGING TO INVESTIGATE DISEASE

Medical imaging — using various modalities to take a snapshot of the body’s interior structure — has been around since 1895, with the discovery of X-rays by Wilhelm Roentgen.

X-rays are a type of electromagnetic radiation (more on that later!) that are able to pass through soft tissues such as skin, fat, and muscle — but not bone. When an X-ray beam is aimed at the body, it passes through the soft tissue but is blocked by the bone, casting a shadow to create an X-ray image on a piece of film. The introduction of X-rays revolutionized medicine, making it possible to accurately diagnose broken bones and to identify the location of harmful objects such as bullets inside of patients’ bodies.

Newer technologies such as PET and CT scans supplement X-rays, aiding in the diagnosis and management of a disease. Let’s find out how these pictures are worth much, much more than a thousand words.

TERM OF THE WEEK: ELECTROMAGNETIC SPECTRUM

Electromagnetic radiation is a combination of electric and magnetic fields from various wavelengths that move through space and carry energy. The electromagnetic spectrum encompasses the following:

- Radio: Low frequency, long wavelength
- Microwaves: Medium frequency, medium wavelength
- Infrared: High frequency, short wavelength
- Visible: Moderate frequency, moderate wavelength
- Ultraviolet: Very high frequency, very short wavelength
- X-ray: Extreme high frequency, extremely short wavelength
- Gamma: Ultrahigh frequency, ultrashort wavelength

We can only see a small portion of the electromagnetic spectrum — visible light. The remaining types of energy, from low frequency radio waves to high frequency gamma rays, can be created, manipulated, and detected for various applications, including medical imaging technology.

CT VS. PET

Medical X-rays, as described above, work by beaming electromagnetic radiation at the body from an outside source (the X-ray machine). “Positron emission tomography” — a PET scan — in contrast, assesses bodily function from the inside out. A radioactive tracer is injected and seeps through the body and attaches itself to certain tissues, then the gamma rays emitted by the tracer are detected by the PET scan. The radioactive tracer (also called a radiopharmaceutical) consists of a “carrier molecule” that is bonded tightly to a radioactive atom. The carrier molecule used has an affinity for the part of the body to be imaged.

CT scans — “computerized tomography” — are essentially 3D X-rays. X-rays are taken from many different angles, creating cross-sectional images or “slices.” These cross-sections are then processed by a computer to produce a 3D anatomical image. A contrast agent — which is used to enhance the body’s structure or fluids — is typically given to patients prior to a CT scan, making the soft tissues denser, therefore enabling the agent to block X-rays and thus become visible in the CT.

This combination of both types of tomography — the use of waves to penetrate the body to create images — is very useful when information about both metabolism (PET) and anatomy (CT) are required to study disease.

PARKINSON’S & ALZHEIMER’S

Radioactively labeled glucose — fluorodeoxyglucose (FDG) — is widely used for metabolic imaging studies in PET scans because they detect deficits in brain activity that may show signs of neurodegenerative diseases such as Alzheimer’s or Parkinson’s.

Since all types of tissue use glucose for energy, increased uptake of glucose-loving FDG indicates more metabolic activity. In Alzheimer’s or Parkinson’s disease, a brain PET scan that uses FDG as the tracer may detect lower than normal FDG consumption in certain brain areas, meaning there is a reduction of metabolic
activity in those regions — a possible indication of the early neurodegeneration.

Although FDG-based PET scans have emerged as a useful tool in neurodegenerative disease, they are not capable of clearly differentiating one type of neurodegeneration from another. To do that, disease-specific radiopharmaceutical detection agents have been developed.

DaTSCAN (GE Healthcare; Chicago, IL) is a radiopharmaceutical that binds to cells in the brain that release dopamine, a chemical messenger. Loss of this type of brain cell is associated with Parkinson's disease (PD). PET imaging with DaTSCAN enables physicians to visualize the loss of dopamine-associated neurons, and to diagnose PD.

Preclinical efforts are now being directed at the development of radiopharmaceuticals for the detection of alpha-synuclein, a protein that is elevated in the brains of PD patients. Alpha-synuclein elevation is thought to occur earlier in PD progression than dopamine signaling irregularities; thus, the ability to detect it could enable earlier diagnosis and intervention. This is considered such an important goal in the field that in 2016, the Michael J. Fox Foundation announced a $2 million dollar award to the first team that successfully develops a PET tracer for the visualization of α-synuclein. Leaders in this race include AC Immune (Lausanne, Switzerland) and ICBI International (La Jolla, CA).

Three radiopharmaceuticals — Vizamyl (GE Healthcare), NeuraCeq (Piramal Imaging; Berlin, Germany) and Amyvid (Eli Lilly; Indianapolis, IN) — have been approved for the detection of beta-amyloid protein plaques in the brain, which could indicate the presence of the Alzheimer's disease (AD). They are approved for clinical use in PET scans, however they are not considered to be stand-alone diagnostics. In other words, a negative scan may be used to rule out AD, but a positive result is not enough on its own to diagnose.

The second type of abnormal protein cluster found in AD patients’ brains are tau proteins. PET tracers for the detection of tau are in development by several companies, including AC Immune (Phase I), Piramal Imaging (Phase I), Merck (Kenilworth, NJ; Phase I), Roche (Basel, Switzerland; Phase I), and Janssen (Raritan, NJ; preclinical). Challenges that must be overcome when developing new radiopharmaceuticals for targets inside of the brain include developing an agent that will cross the blood-brain barrier and be extremely target-specific.

**DETECTING CANCER**

Tumor cells consume glucose at a much higher rate (approximately 200 times higher) than healthy cells. This difference makes FDG-based PET scans a reliable method for detecting and monitoring cancer as follows:

- After the body is injected, tumor cells consume the radiolabeled glucose (FDG) much more rapidly than non-tumor cells.
- The PET machine detects the gamma rays emitted by FDG.
- Areas of high activity are referred to as “hotspots” and may indicate the presence of a tumor.

PET scans may be used as part of an initial diagnosis, and as a way to monitor treatment. Tumors shrink in response to therapy while the spreading of cancer to other parts of the body can point to metastases.

**NOT SO RADIOACTIVE**

The prospect of being injected with a radioactively-labeled imaging agent may give some patients pause. However, the total radiation dose received by that patient is equivalent to the exposure caused by two routine chest X-rays according to the National Institute of Biomedical Imaging and Bioengineering. This is due largely to the rapid decay of the radioactive label used for PET tracers. Within a few hours of administration, there is no detectable radioactivity in the patient’s body. Patients are encouraged to drink plenty of fluids after a PET scan to aid in flushing any remaining radiopharmaceutical out of the body.

The ability to see inside of a patient’s body, directly measuring metabolic activity or anatomical structure, is critical to detecting, monitoring, and treating diseases. With new discoveries in the field of imaging we can hope to catch signs of disease sooner rather than later.
Circadian Rhythm & Disease

AND THE BEAT GOES ON

Earlier this week, the 2017 Nobel Prize in Physiology or Medicine was awarded to three American scientists (Jeffrey Hall and Michael Rosbash, of Brandeis University, and Michael Young, of Rockefeller University) for their work in deciphering the molecular basis of circadian rhythm – the 24-hour cycle that governs the inner workings of all life on Earth. Although the work that garnered the Prize was done over twenty years ago, its implications to human disease and new therapies are still being worked out today. In this issue of the Weekly, we’ll take a look at this Nobel science.

CIRCADIAN RHYTHM GOVERS MORE THAN 24 HOURS

Overnight shift workers, students pulling an all-nighter to cram for a final exam, and business people rushing between time zones all share one thing in common: significant disruption to their circadian rhythm. This roughly 24-hour activity cycle responds primarily to light and darkness and is found in most living organisms—people, plants, animals, and even some microbes. Disruption in the cycle can cause more serious consequences on top of a few days of disorientation. Abnormal circadian rhythms are correlated with insomnia, diabetes, increased cardiovascular events, some types of cancer, Parkinson's disease, and more.

In this issue, we’ll take a closer look at how circadian rhythms are regulated, examine some links with various disease states, and find out how drug discovery efforts are taking this important phenomenon into account.

PROTEINS ON THE CLOCK

The circadian rhythm is regulated by a combination of environmental and internal cues. Environmental cues are based on light and dark, while internal cues are simply “biological clocks.” These biological clocks are groups of interacting proteins in cells throughout the body. The most extensively characterized biological clocks are appropriately dubbed Clock proteins. The Clock proteins interact with each other and regulate levels of expression of other proteins throughout an approximately 24-hour period. This is why most people feel disoriented after traveling through several time zones, even if they get an adequate amount of sleep. These tiny molecular clocks are signaling that it is later (or earlier) than the local time—and a whole range of physiological functions from body temperature to heart rate, blood pressure, and alertness levels respond accordingly.

Clock proteins are regulated by a “master clock” in the brain. Technically referred to as the suprachiasmatic nucleus, this master clock consists of about 20,000 nerve cells just above the region where the optic nerve crosses into the brain. This location, called the optic chiasm, explains why many of these neurons are sensitive to light.

ON THE MARKET: HETLIOZ

People who are totally blind cannot receive the proper light input to control their master clock. As a result, many suffer from non-24-hour sleep-wake disorder. It becomes virtually impossible to fall asleep at standard times, which severely impacts their ability to function professionally and socially.

In July 2014, the FDA approved Vanda Pharmaceuticals' Hetlioz for patients with non-24-hour sleep-wake disorder. Hetlioz is an agonist; it binds directly to and activates the melatonin receptor. Melatonin is a sleep-inducing hormone with levels increasing at the onset of darkness, linking it to the sleep-wake cycle. Since melatonin production is dysregulated in totally blind people, Hetlioz aims to normalize sleep patterns. Although melatonin itself is available in pill or liquid form over the counter, synthetic melatonin receptor activators such as Hetlioz are designed to be more stable.

IN DEVELOPMENT: RESET YOUR PRESET

Aptly-named Reset Therapeutics (South San Francisco, CA) is focusing on circadian-rhythm disruptions as they relate to a whole range of diseases including type 2 diabetes, Cushing's syndrome (elevated cortisol levels), high blood pressure, obesity, sleep apnea, cancer, and inflammation. Scientists at Reset have identified potential circadian-modulating compounds and are testing them in animal models of Cushing's syndrome and type 2 diabetes.
PRECLINICAL: SHUTTING DOWN THE CANCER CLOCK

A possible link between circadian rhythm dysfunction and cancer has caught the eye of drug development. One of the functions of the Clock proteins is to set restrictions on when cells can divide, so circadian disruptions may affect cancer-related cell division. Researchers at the University of California, Santa Cruz are zeroing in on a protein known as PASD1, which is expressed in many different cancer cell lines. PASD1 interacts with the Clock proteins, essentially interfering with their function and shutting them down. Early preclinical work suggests that the inhibition of PASD1 causes the Clock proteins to reactivate—making PASD1 a possible drug target.

TIMING IS EVERYTHING

Taking medicine at the same time every day is a good way to make sure a dose isn’t forgotten. For certain medications, however, the time of day the pill is swallowed may influence drug efficacy.

In most healthy people, blood pressure is tied to circadian rhythm, naturally dropping between 12:00 AM and 3:00 AM. In people with high blood pressure, however, this drop often doesn’t occur naturally. By taking blood pressure medication at night, patients can restore this normal decrease in pressure.

Dosage timing may also be important for certain types of cancer drugs. For example, PARP1 inhibitors work by shutting down a DNA repair enzyme in cancer cells. The shutdown results in so much DNA damage to the cells that they initiate a cell-suicide program known as apoptosis—the cancer cells pretty much end up killing themselves! Researchers at University of North Carolina (Chapel Hill, NC) have discovered that DNA repair enzymes are more active later in the day, translating into PARP1 inhibitors possibly being more effective if given in the morning; inhibiting the enzymes should be easier when they are not at their peak activity level.

Circadian rhythms govern more than the 24 hours of your day, and uncovering various mechanisms at the cellular level of the sleep-wake cycle might just open up new avenues to treat a whole array of diseases.
Vaccines: Powerful Simplicity

VACCINES: ELEGANT, POWERFUL SIMPLICITY

Anyone who’s suffered the aches and fever of influenza has good reason to value the simple flu shot. In fact, millions roll up their sleeves and literally take their medicine. The US Centers for Disease Control (CDC) (Atlanta, GA) estimates that about 146 million doses of influenza vaccine went to doctors’ offices, health departments, and the corner drugstore among other places, to help keep us flu-free during 2016-17. Drug companies keep up with this high demand by manufacturing these vaccines well in advance—six to nine months before the start of flu season in October.

The influenza vaccine is one of many life-saving vaccines that keep people healthy. This week, we look at different types of vaccines and how drug companies manufacture them.

JENNER’S NEEDLE IN THE HAYSTACK

Vaccines have been around a long time—dating from the late 19th century during the smallpox pandemic. This deadly disease killed or disabled hundreds of thousands of people just in England! In 1796, an English doctor, Edward Jenner, noticed that local milkmaids were immune to smallpox. Jenner observed that these women had all suffered from cowpox, a related but harmless virus. Jenner hypothesized that the cowpox imparted some type of immunity, so to test his theory Jenner took pus from a cowpox blister and inoculated his gardener’s small son, James, with the virus through shallow scratches. James developed a slight fever afterward, but when intentionally exposed to smallpox later, the little boy never became ill.

Jenner’s methods were a little rough (not to mention unethical by today’s standards), but his thinking was spot on. The idea behind vaccines is simple. First expose someone to a harmless version of a disease-causing microorganism, or pathogen. Amazingly, this “trains” their immune system to recognize and fight the germ. Exposure to the disease forces the body to create special white blood cells, known as memory cells, which combat any further exposure to the disease.

COCKTAIL FODDER: 40,000 SAVED!

According to the CDC, flu season runs from October through May in the US. The organization’s latest study estimates that the flu vaccine saved 40,000 lives in the US during a nine-year period.

TYPES OF VACCINES

Vaccines come in different varieties including: inactivated whole, live attenuated, and subunit vaccines. Each necessitates different manufacturing requirements.

Inactivated whole vaccine. Made with dead microorganisms (viruses or bacteria), these stimulate an immune response. Among the most famous is Dr. Jonas Salk’s polio vaccine, developed in 1955.

Live, attenuated (weakened) vaccine. These are created by reducing a pathogen’s strength so that they become harmless. Live vaccines tend to produce the strongest immune reaction.

Subunit vaccine. These vaccines use only one part of a pathogen, an antigen. The antigen provokes an immune response. One method of making subunit vaccines involves isolating a specific protein from a virus and administering only this protein.

Scientists are also currently developing DNA-based vaccines. These consist of a gene encoding a pathogenic protein as opposed to the protein itself.

The Whole Story

Most whole pathogen vaccines protect against viruses, such as rabies, not bacteria. But making any vaccine means first growing lots of virus. This “virus-farming” involves selecting and obtaining a strain of a particular virus, the seed strain, and choosing what to grow it in (the medium).

Where do manufacturers buy a pathogen in the first place? They come from one of two sources. Viruses (and other microorganisms and biological materials) are produced and housed in well established “culture collections,” such as the American Type Culture Collection (Manassas, VA.) Some companies or academic institutions also develop strains of particular viruses “in-house.” For the flu vaccine, new strains are selected annually based on the World Health...
Organization’s assessment of the virus’s evolution over the previous season.

Choosing the seed strain is only the first step of vaccine manufacture. Although dangerous and often deadly, viruses are powerless without a host—someone or something to grow on. Two of the most common “host-cell platforms” are chicken eggs and animal cell culture.

Chicken eggs? Yes, the incredible edible egg provides a fantastic growth medium for influenza and other viruses. (Side note: The CDC recommends that most people with egg allergies be vaccinated. However, it also suggests that those with severe, life-threatening allergies receive only certain vaccines.)

Some viruses thrive in certain types of animal cells. Two of the most commonly used in vaccine production include one from the kidney of the African green monkey—known as Vero cells, and one derived from the kidney cells of a cocker spaniel (MDCK cells). Though it is easier and quicker to scale up animal cell culture vaccine production, it is much more expensive than egg-based vaccine growth.

Regardless of medium, once there’s enough virus, the manufacturer needs to separate or isolate it from the host material. Isolation involves centrifuging and filtering to divide virus particles from the host cells.

Production of whole pathogen or inactivated vaccines involves the critical step of inactivation. This means disabling a virus’s ability to infect without eliminating the parts of the virus that trigger an immune response. Inactivation involves a variety of strategies, including detergent treatment, heat treatment, or exposure to UV light.

- **Detergent-treated**: Specific for envelope viruses, detergents break the chemical bonds that hold the virus’s envelop (outside surface) together disabling its ability to invade a host.

- **Heat, chemical, and pH-treated**: Viruses use proteins on their surface to infect host cells. Altering their shape destroys their ability to recognize and infect cells.

- **Ultraviolet light-treated**: A virus’s basic building blocks—their DNA or RNA—are destroyed by UV light. With no genetic code, viruses cannot make more of themselves. The last step in making vaccines is formulation. The inactivated virus gets mixed into a sterile water or salt solution along with stabilizers and preservatives. Some vaccines also contain adjuvants at this point. An adjuvant is a substance that boosts the immune response to a vaccine. Vials are then filled, inspected, labeled, and shipped. Most vaccines require refrigerated storage and shipping.

**ALIVE, NOT KICKING**

Producing a live, attenuated vaccine follows similar steps, without inactivation. In addition, it starts with a seed strain that has been rendered harmless. The new organism continues to grow but produces immunity without causing illness. Attenuated vaccines produce stronger, usually longer-lasting, immune responses than inactivated vaccines because they more closely mimic actual infection. Attenuated vaccines should not be given to people with weakened immune systems, such as cancer patients or the elderly.

Safe, attenuated vaccine strains are produced in a few different ways. Sometimes, it may simply be a related, but harmless virus that kicks in the immune response. Jenner’s vaccine, which was essentially the cowpox virus, is a classic example. Today’s smallpox vaccine uses a related virus, vaccinia. Another common method to produce vaccines is raising several generations of a clinical isolate, or a laboratory-pure version of a pathogen. Growing in non-human cells, it adapts to its new host, becoming less infectious over time. Examples include the measles, yellow fever, and poliovirus vaccine strains. Scientists can also use recombinant DNA technology to delete the portions of a virus’s genome that cause infection.

Vaccines have a simple premise, but the science and manufacturing that make them possible is complex. Different microorganisms require individual approaches. Through trial and error, microbiologists, virologists, and other scientists determine the best formulation.

In part two of this series, we’ll discuss other kinds of vaccines, including virus-like particle (VLP), polysaccharide, and further discuss subunit vaccines. We’ll also give an overview of how vaccines are tested and approved.
MORE ON THE POWERFUL, ELEGANT SIMPLICITY OF VACCINES

Last week, we overviewed vaccine development and manufacture, focusing on those that use whole pathogens to protect us from a disease. This week, we examine subunit and polysaccharide vaccines, which use different strategies to fight infection. We also take a brief look at the US Food and Drug Administration (FDA)’s vaccine approval process.

A PART IS SOMETIMES GREATER THAN THE WHOLE

The structure of viral cells is much simpler than that of our own cells. Despite the damage they can do, viruses consist only of one or more strands of DNA or RNA, encased in a protein shell such as in the viruses you see below.

This simple structure means that sometimes only part, or subunit, of a virus is enough to stave off infection. Different subunit vaccines use different bits of a pathogen. Often, they consist of nothing more than one of a virus’s surface protein. Subunit vaccines work because our immune systems recognize and respond readily to these surface proteins.

For subunit vaccines, drug manufacturers alter yeast, bacteria, or Chinese hamster ovary (CHO) cells to produce a protein by transferring the gene encoding the virus into them. These host cells make the viral protein, which the manufacturer then isolates and formulates into the vaccine. Subunit vaccines include those for hepatitis B and human papillomavirus (HPV).

Sometimes a viral subunit or subunits form what’s called a virus-like particle (VLP)—a protein structure whose shape closely mimics a virus with none of its genetic material. In these cases, the body’s immune system responds very robustly.

A subunit vaccine can also derive from toxins produced by dangerous bacteria. For example, Clostridium tetani (tetanus) secretes tetanospsmin, a neurotoxin that causes severe muscle spasms, potentially leading to death. The vaccine contains inactivated toxin, which helps develop antibodies that prevent future illness.

Because subunit vaccines contain none of a pathogen’s genetic code, they are generally very safe.

CARBS+PROTEIN=IMMUNITY (SOMETIMES)

In general, bacterial infections tend to be more difficult to protect against by vaccine than viral infections. That’s because the surface of some bacteria is covered in long chains of carbohydrate molecules. Called polysaccharides, they mask the bacteria’s proteins. This “cloaking device” means the body doesn’t recognize the threat and mount an immune response. But molecular biologists and other scientists have discovered that it’s possible to link polysaccharides to a harmless protein, thereby coaxing an immune response. These vaccines are known as conjugated polysaccharide vaccines because the carbohydrate is conjugated (connected) to a protein. The Haemophilus influenzae type B (or Hib) and some types of pneumococcal and meningococcal vaccines are made this way.

GETTING DOWN TO THE ESSENTIALS

The basics of immunization have been around over a century—use a disease-causing microbe, or just a part, against itself. However, the latest step in the evolution of vaccines takes a different tack, delving more deeply into the building blocks of life—DNA.

Instead of immunizing someone with a whole pathogen or fragment, a DNA vaccine injects only a small bit of a virus’s genetic code. Drug companies nestle the code in plasmids—small, circular DNA molecules within a pathogen. As you can see below, the “visiting” DNA prompts the host to produce the target viral protein and

DNA Vaccines Explained
consequent immune response within their own cells, but without an infection.

The key challenge for DNA vaccines is getting patients’ cells to accept the introduced DNA. So far, the most effective technique seems to be electroporation—delivering short pulses of electrical current to the patient with the vaccine. The electricity creates temporary pores in a patient’s cell membranes, enabling the DNA to enter.

The FDA has yet to approve any DNA vaccines for human use. The prospect of DNA vaccines, however, presents some important advantages, which includes producing a strong immune response and somewhat easier manufacturing. Producing large volumes of viral gene-containing plasmids still means growing lots of bacteria in which to reproduce the plasmids, but purifying and formulating these vaccines is more straightforward due to the relative simplicity of DNA’s structure. In addition, DNA vaccines don’t require refrigeration, extending their shelf life and transportation time.

Inovio Pharmaceuticals (San Diego, CA) currently has DNA vaccines for hepatitis B and C, and the Ebola, HIV, and Zika viruses in the early stages of clinical testing.

**TESTING, TESTING**

How does a new vaccine come to market? The FDA requires companies test vaccines for safety and effectiveness on human subjects. The process differs somewhat from clinical drug trials. Researchers test a new drug on sick volunteers to see if it makes them well; researchers administer an investigational vaccine to healthy volunteers to see if it prevents illness.

Clinical vaccine trials involve three distinct steps.

- **Phase 1** is typically a small study in which healthy volunteers get the vaccine. Doctors monitor them for side effects. If no unacceptable reactions occur, the vaccine advances to the next stage.
- **In Phase 2**, more subjects, typically hundreds, get the vaccine. The same number of subjects participate in the study without receiving the vaccine. Scientists refer to this second group as the control. Both groups are at the same level of risk for contracting the target disease. Researchers observe the “vaccinates” for two years or more to see if they contract the disease at lower rates than those in the control group. Researchers also monitor immune response by measuring levels of anti-pathogen antibodies in the participants’ blood.
- **For Phase 3**, even more subjects—often thousands—at high risk for the disease receive the vaccine and are monitored as described in Phase 2, from three to five years. This trial also includes a control group.

**COCKTAIL FODDER: VACCINATION ACTIVATION**

Getting a shot won’t make you sick. Sometimes, people feel mild symptoms such as fatigue, headache, and low-grade fever after receiving a shot. These are signs that an immune response is being activated.
Natural Born Cancer Killers

FURTHER DOWN THE CANCER TREATMENT ROAD WITH CARS

This past August, to much fanfare, the FDA approved the first chimeric antigen receptor (CAR) T-cell therapy for blood cancer. Called Kymriah (Novartis), it promises to revolutionize treatment by genetically altering a patient’s own cells to fight cancer. Less than eight weeks later, Kite Pharma, now a part of Gilead Sciences (Foster City, CA), had its new CAR T-cell therapy, Yescarta approved as well.

Meanwhile, biotech companies continue to push the boundaries of immunotherapy by creating engineered immune cells.

A CAR THAT DOES WHAT?

But first, what exactly is a chimeric antigen receptor? CARs are manufactured proteins that molecular biologists and others engineer to appear on the surface of a white blood cell such as a killer T-cell. This new, revved-up receptor then targets the white blood cell to attack cancer cells.

A CAR consists of:

• Targeting domain: This part of the CAR exists outside the white blood cell. It is composed of an antibody that recognizes and docks onto a specific cancer surface protein.

• Activation domain: This component kicks into gear once the targeting domain locks onto the intended cancer surface protein. In CAR-T cells, the activation domain signals T-cells to do three things: 1) make copies of themselves; 2) release signaling molecules called cytokines (proteins that prompt other white blood cells to attack the tumor); and 3) finally—the really good bit-- kill cancer cells.

Need reminding how CAR-T cells work? Check out our WEEKLY on the topic.

NATURAL BORN KILLERS

Our immune system inherently includes NK (natural killer) cells. They are the body’s first responders. At the first sign of illness, NK cells attack the infection for two reasons. First, pathogens lack surface proteins called MHC1 that the body identifies as normal. Second, the presence of abnormal proteins tells the body that the invader poses a threat.

Many immunologists believe that prompt action by NK cells helps eliminate cancer cells early on– before they grow into a serious problem. However, in the early stages of tumor development, there are often not enough red flags – abnormal proteins on the cancer cell surface – to tag them as dangerous. Engineering NK cells to display a CAR “trains” them to recognize and respond to tumor cells. Once activated, the CAR-NKs behave much like killer T-cells, releasing cytokines that bolster the immune response to the cancer cells--killing the nasty cells by injecting even nastier toxins.

HOME GROWN ISN’T ALWAYS BEST

CAR-NK cells have two important advantages over CAR-T cells: safety and accessibility. CAR-T cells must come from the patient’s own T-cells to avoid triggering graft-versus-host-disease (GVHD). This potentially deadly illness occurs when the patient’s immune system responds badly to foreign tissue. Donor NK cells, in contrast, don’t appear to cause GVHD.
Besides avoiding potentially life-threatening reactions in already very ill patients, medical professionals can obtain donor NK cells relatively easily—for example, from umbilical cord blood. Labs modify these donor cells to express a CAR, which can then be given to the patient. Removing and engineering a patient’s own T-cells, then transfusing them back into the patient is much more time-consuming. The ability to more easily use donor NK cells means that biotech companies can create “off-the-shelf” products for this type of CAR therapy more readily. In addition, the resulting lower production costs mean more available treatments.

**TEST-DRIVING CAR-NKS**

This past June, scientists at the *MD Anderson Cancer Center* (Houston, TX) started a Phase I/II trial of CAR-NK cells. The research focuses on patients with chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or non-Hodgkin lymphoma. The trial cells contain a “suicide” gene that is triggered by excessive inflammation. Researchers hope this built-in safety feature will reduce problems caused by overactive immune responses in patients from earlier trials of CAR-T cells.

Researchers elsewhere are also looking into the development of CAR-NK cells that treat other cancers—specifically targeting the HER2 protein in breast and ovarian cancers.

**CHOWING DOWN ON DISEASE**

Of course, NKs, modified or otherwise, aren’t the only white blood cells going toe-to-toe with cancer. Another approach involves the immune system’s scavengers or macrophages. “Macrophage” comes from Greek, meaning “big eater.” These cells kill invading or diseased cells by surrounding and digesting them. Leftover fragments of the alien cell’s proteins or antigens are displayed on the macrophage’s surface. These leftovers help activate some of the immune system’s other defenses, such as killer T-cells.

**MMM…CANCER?**

Researchers are now exploring the potential power of CAR-macrophages to destroy specific cancer cells. The enhanced macrophages will simultaneously activate other immune cells to also recognize and attack those same antigen-bearing cells. Like other macrophages, CAR-macrophages can penetrate solid tumors much more effectively than “plain old” T-cells.

If a typical T-cell does make it into a solid tumor, the cancer’s own defenses makes short work of it. In contrast, by modifying macrophages to treat solid tumors, doctors may be able to effectively get at cells inside the tumor. At the same time, the super-powered macrophages will “wake up” the patient’s suppressed T-cells to fight the cancer as well.

Preclinical data from *CARMA Therapeutics* (Philadelphia, PA) shows that its scientists can modify CAR-macrophages to recognize and engulf different types of solid tumor cells. They’ve also demonstrated that infusing cancerous mice with tumor-specific CAR-macrophages leads to long-term tumor control and longer survival. CARMA plans to begin clinical testing the effect of CAR-macrophages on specific ovarian cancers in 2019.

As biotech companies continue to translate these new applications of CAR into treatments, both patients and doctors can look forward to seeing an increase in the number of different types of cancer that respond to these cutting-edge immunotherapies.
You’re at the supermarket, puzzling over whether those peaches for the pie are ripe. Maybe you’re watching your child’s soccer team, and struggling to separate the Green Hornets from the Scarlet Knights. As if determining offsides isn’t hard enough! Or more seriously, you’re approaching a stoplight on a busy street and can’t tell if the signal is red or green. People with color vision deficiency (CVD), more commonly known as color blindness, face challenges like these all the time. That’s not to mention the inability to fully experience some of the world’s wonders—the blazing of sugar maples in the fall or the first flush of April’s flowers.

TERM OF THE WEEK: PHOTORECEPTOR CELL

Photoreceptors are cells in the eye’s retina that respond to light and convert its signals into information the brain uses to create visual representations of images. We have two kinds of photoreceptors: rods and cones. Rods work in low levels of light and detect only black and white. Cones enable us to see colors. Photoreceptors contain light-sensitive proteins called opsins. Opsins change chemically in response to light. Photoreceptors detect those changes, enabling them to convey visual information. Rods contain only one of these proteins, called rhodopsin. Cones, in contrast, contain three different opsins, one of which responds to red, green, or blue wavelengths of light. For most people, our opsins bring us the world in technicolor.

SEEING RED—OR NOT

The most common types of color blindness are inherited. In these cases, the genes for one or more cone opsin are defective and make abnormal proteins. Physical or chemical damage to the eye, optic nerve, or brain can also cause color blindness.

YES HONEY, THE DRESS IS PURPLE

Color blindness affects far more men than women. The National Eye Institute (Bethesda, MD) estimates that nearly one in 12 men are color blind, versus one in 200 women. This difference arises because genes for the different opsin proteins are on the X chromosome. Women have two X chromosomes; men only one. With two copies of each opsin gene, it’s unlikely both would contain mutations interfering with color perception.

By far, most cases of genetic color blindness come from genetic mutations that code for red or green-detecting opsins. Consequently, those affected have trouble distinguishing shades of red or green. Rather than responding to only clearly distinct wavelengths of light, the mutated opsin detects a broader range, essentially bleeding over into the neighboring detection wavelength. The brain detects color by comparing signals from two different types of cone cells. Too much overlap between signals interferes with a person’s perception of color.

While most people with color vision deficiency have trouble distinguishing colors in the red and green parts of the spectrum, other conditions exist, such as tritanomaly—reduced sensitivity to blue light. Much more rarely, two different color-detecting opsins are affected, which results in significantly reduced color vision. Even though people with this condition retain one-color-detecting opsin, the brain needs to compare signals from different types of cones to see color. The most severe and rare form of CVD is called achromatopsia—true color blindness. People with this condition perceive no color at all, and suffer from other vision problems as well.

IMPROVING THE OPTICS

There is no cure for color blindness at present. However, two remarkable adaptive technologies exist. A company called Enchroma (Berkeley, CA) produces glasses that help people with red-green color blindness perceive colors normally. They combine the latest in lens development with color perception neuroscience. Enchroma’s proprietary technology filters specific wavelengths to create sharp distinctions between red and green, significantly improving color vision. Available off-the-shelf online or at vision centers nationwide, the glasses can incorporate prescription lenses. Colormax (Timonium, MD) also markets color vision correction contact lenses and glasses that filter specific wavelengths of light. The lenses are custom-made and so require a thorough in-person exam. Colormax guarantees that the lenses will enable wearers to pass
the color vision tests required for certain occupations, such as firefighter, pilot, and railroad engineer.

**TINKERING WITH THE FAULTY GENES**

Scientists at **Adverum Biotechnologies** (Menlo Park, CA) are looking at biotech approaches to treating inherited forms of color blindness. Possible gene therapies could entail delivering a correct version of the mutated gene. Adverum’s work stems from gene therapy treatment of CVD squirrel monkeys at the University of Washington in Seattle, WA.

**PHILOSOPHICAL QUESTION: RED OR RED?**

How does a person know if the red they see is the same red someone else sees? Even among the majority of people, who see color “normally,” there are probably variations in color perception. That’s because the eye and brain work in concert to translate light into color. Add to that the physical differences in saturation, hue, and tone and the panoply of color names in the English language alone—crimson, blood, garnet, vermilion, maroon...and we’ll all be seeing red for a loooonnng time.
The Science Behind Opioid Addiction

THE SCIENCE BEHIND OPIOIDS
Concerns over the opioid epidemic continue to grow, with deaths from narcotic overdoses the leading cause of death in people under 50 last year. Nearly half of those deaths were attributable to prescription opioids. The directors of both the Center for Disease Control (Atlanta, GA) and the Food and Drug Administration (Silver Spring, MD) have called for urgent action on the crisis, and President Trump declared the opioid epidemic a public health emergency.

This stark reality highlights the dark side of a class of treatments serving a vital need. Opioid pain medications manage the severe short-term or chronic pain of millions of Americans. While these medications mitigate needless suffering, the US government, healthcare industry, and medical community are joining forces to battle against opioid abuse and addiction.

Here at the WEEKLY, we always wonder: What is the science behind the headlines? So, let’s talk about how pain medications work, the different types on the market, and the approaches to developing less addictive versions of opioid drugs.

OPIOIDS VS. NSAIDS
There are two main categories of pain medications, opioids and non-steroidal anti-inflammatory drugs (NSAIDs). Although these two categories of drugs work differently, they do share one thing in common: both are derivatives of natural products. NSAID commonly known as Aspirin was developed by Bayer (Leverkusen, Germany). It is a synthetic version of an extract from willow tree bark. Opioids are synthetic versions of opium and morphine, which come from poppy flowers.

Aspirin works by stopping an enzyme called cyclooxygenase 1 (COX-1). Once stopped, COX-1 can no longer produce prostaglandins and thromboxanes. Prostaglandins and thromboxanes are produced in response to injury or infection and cause inflammation, which is associated with symptoms of fever, swelling, and pain. Other NSAIDs, such as ibuprofen and naproxen, also work by inhibiting COX-1 or its sister enzyme COX-2.

Opioid pain medications, such as Purdue Pharma’s (Stamford, CT) Oxycontin and Endo Pharmaceuticals’ (Malvern, PA) Percocet, work by binding to mu receptor proteins on the surface of cells in the central nervous system (CNS) —think brain and spinal cord. While the CNS is tasked with relaying pain signals, opioids decrease the excitability of nerve cells delivering the message, resulting in pain relief—along with a feeling of euphoria in some users.

LESSENING THE PAIN
Short term medical used of opioid painkillers rarely leads to addiction when properly managed. Due to the euphoria-inducing effects of the drugs, long term regular use, or use in the absence of pain, may lead to physical dependence and addiction. And because regular use increases drug tolerance, higher doses are required to achieve the same effect, leading abusers to consume pain pills in unsafe ways such as crushing and snorting or injecting the pills. According to the Centers for Disease Control, 91 Americans die every day due to opioid overdose, which includes prescription opioids and heroin. At the same time, chronic pain is also a serious problem, affecting approximately 100 million U.S. adults, while millions of others suffer acute pain due to injury or surgery. The medical need for these drugs is very real despite the dark side.

The answer to developing less addictive drugs may be found in a drug that blocks pain without inducing euphoria. These new drugs will need a different mechanism of action than traditional opioid drugs, which bind to the mu receptors of cells inside the CNS. Cara Therapeutics (Shelton, CT) is developing drugs that bind to a different type of opioid receptor, the kappa opioid receptor. These receptors are present on sensory nerves outside of the CNS. Cara Therapeutics (Shelton, CT) is developing drugs that bind to a different type of opioid receptor, the kappa opioid receptor. These receptors are present on sensory nerves outside of the CNS. Preclinical studies suggest that targeting these receptors could be effective at reducing pain without driving addictive behaviors. Their lead candidate, CR845, is currently in Phase III clinical testing for post-operative pain and pruritus (severe itching), and in Phase II clinical testing for chronic pain. Cara Therapeutics is also developing compounds that selectively activate cannabinoid (CB) receptors outside of the CNS. It’s interesting to note that CB receptors inside the CNS are linked to marijuana’s psychoactive qualities. Cara’s lead CB receptor activator, CR701, is in preclinical development.
Hydra Biosciences (Cambridge, MA) is developing small molecule drugs that inhibit ion channels, proteins on the surface of nerve cells that help transmit pain signals. This plays a critical role in sending the pain signal to the brain, yet because it works on nerves outside of the brain, it has less of a potential for addiction. Hydra is currently in Phase II clinical studies of HX-100 for the treatment of painful diabetic neuropathy.

Centrexion Therapeutics (Baltimore, MD) lead candidate is a derivative of capsaicin, a naturally-occurring compound found in chili peppers. Capsaicin has pain relieving properties and is used as a natural remedy. Centrexion’s lead candidate, CNTX-4975, is a highly potent, synthetic form of capsaicin designed to be administered via injection into the site of pain. CNTX-4975 targets the capsaicin receptor, an ion channel protein on the surface of nerve cells. When CNTX-4975 binds the capsaicin receptor, the influx of calcium ions results in desensitization of the nerves, making them unresponsive to other pain signals. This effect can last for months, and only affects nerves near the site of injection. Centrexion recently announced positive Phase IIB results for CNTX-4975 in a study testing its effectiveness against pain in knee osteoarthritis. The drug is also in Phase II clinical studies for Morton’s neuroma, a sharp pain in the foot and toe caused by a thickening of the tissue around one of the nerves leading to the toes.

Earlier this year, Nektar Therapeutics (San Francisco, CA) announced positive Phase III results of their addiction-resistant opioid, NKTR-181. Like traditional opioids, NKTR-181 works by entering the brain and activating mu-opioid receptors. However, the molecule is designed to enter the brain much slower than other mu-receptor activators, reducing the feelings of euphoria while offering pain relief.

Researchers at Tulane University (New Orleans, LA) have developed a compound that is derived from the endogenous opioid, endomorphin. Endogenous opioids are chemicals produced naturally by the body that bind to and activate the mu-opioid receptors, resulting in pain relief and mild euphoria without the detrimental side effects associated with opioid drugs such as depressed respiration, motor impairment, and addiction. Scientists have tried before to develop safer pain medications based on endogenous opioids, but have not been successful due to the molecule’s instability. The Tulane team created a stable derivative of endomorphin that binds to the mu receptor in such a way that pain relief occurs, but not the negative side effects listed above. Clinical testing is expected to begin within a few years.

ANTIDOTE TO AN OVERDOSE

Overdosing can be fatal since respiratory failure occurs at high opioid concentrations. If an overdose is suspected, the individual should be treated as quickly as possible with naloxone—a “competitive antagonist” of the mu-opioid receptor. Simply put, a competitive antagonist binds the receptor without activating it. Since naloxone doesn’t activate the receptor, it doesn’t have any pain relieving or euphoria inducing qualities; rather, it prevents the opioid drugs from binding. It may also displace opioids that have already bound the mu receptor, aiding in the stoppage of an overdose.

COCKTAIL FODDER: RUNNER’S HIGH

Some folks love to run; others avoid it at all costs. This might be explained by inherent differences in sensitivity to the natural opioids called endorphins that are released during exercise. Not everyone experiences the “runner’s high”—feelings of calm and mild euphoria—in much the same way that not everyone experiences euphoric feelings from pain medications. These differences may help to explain why some people enjoy exercise and others don’t, and why some people get addicted to opioids—while others can take them or leave them.

With several of the opioid alternatives outlined here already showing success in clinical studies, there is hope on the horizon for patients who suffer from chronic pain but want to avoid the risk of addiction. Next week, we’ll explore the use of medical devices in pain management.
MEDICINE-FREE PAIN MANAGEMENT

Migraine relief without drugs? No “digestive issues” due to pain meds after surgery? Better still, no worry about addiction after that appendectomy or hip replacement? Sounds a bit science-fictiony, does it not?

The news reminds us nearly every day of the profound need for pain management without opioids. As you read last week, alternatives to analgesics such as morphine, codeine, or fentanyl exist. But science also takes us beyond medicine: researchers have developed devices that control pain and inflammation electronically. This drug-free approach is called electrostimulation—using low levels of electricity to zap pain.

THE BODY ELECTRIC

How does one feel pain in the first place? Pain, and more pleasant sensations (the taste of chocolate, for instance) come courtesy of the nervous system. This system serves as the wiring that enables the machines of our bodies to move, experience, and interpret the world.

Our nervous system has two main parts that work in tandem: the central and peripheral nervous systems. The central nervous system functions as the “switchboard” that sends and receives information from peripheral nervous system’s local lines. The peripheral nervous system is a vast network of nerves found everywhere in body.

The central nervous system is made up of neurons. These cells send and receive signals electrochemically, through signaling molecules called neurotransmitters. These chemicals are converted into electrical signals in the neuron. Here’s how: when a neurotransmitter reaches one edge of a neuron, the dendrite, it opens ion channels in the cell membrane. These tiny openings allow the positively charged sodium ions to pass through. These positive ions travel down the other side of the neuron through an extension called an axon. When the electrical charge reaches the end of the axon, it signals the release of other neurotransmitters, which then switch on other neurons and the chain of sensation unfurls.

SLAMMING THE GATE ON PAIN

As you no doubt noticed, pain perception is complex. Remember the last time you bashed your knee? Did you rub it? The Gate Theory of Pain says this rubbing action does temporarily reduce pain. In that moment, signals from your non-pain nerves block signals from your pain nerves. In other words, non-pain nerves prevent pain from reaching your brain.

Electrostimulation relies on the body’s ability to run interference on pain. It uses electricity to treat chronic pain by stimulating a patient’s peripheral nerves or spinal cord. In either case, a small pulse generator sends electrical pulses to the nerves or spinal cord, “shutting the gate” on pain.

Several companies already have electrostimulation-based devices on the market, including:

• Medtronic (Fridley, MN). Its Intellis spinal cord neurostimulation system gained FDA approval in September and European Union approval for managing chronic pain earlier this month. This small, implantable neurostimulator communicates...
wirelessly with a Samsung tablet that a doctor uses to adjust pain relief and monitor patients’ activity. More activity indicates that the patient’s level of pain is lessening.

• **Stimwave** (Pompano Beach, FL). This company’s latest device, the StimQ Peripheral Nerve Stimulator, was approved in August for chronic pain. Tiny enough to be inserted by needle, it communicates wirelessly with an external transmitter that delivers electrical pulses. The device is also safe to wear in MRI machines. This innovation makes neurostimulation available to people whose treatment requires ongoing imaging.

• **Abbott** (North Chicago, IL) also offers neurostimulation systems. Their most recent is the BurstDR system. Developed by St. Jude Medical in St. Paul, MN, this proprietary technology delivers bursts of electrical stimulation in a way that mimics the body’s natural response to pain.

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**THE LATEST BUZZ: NEW APPLICATIONS AND NEW TECH**

While neurostimulation can treat chronic pain, the technology may provide other therapies as well. Because nerve signals permeate the entire body, modulating them may work for other medical conditions. For example, neural signaling partially controls inflammation, the body’s response to injury. This suggests that neurostimulation may treat inflammatory disorders such as Crohn’s disease and rheumatoid arthritis.

Federal agencies, large pharmaceutical companies, and small start-ups are taking note. The **National Institutes of Health** (Bethesda MD) has established the Stimulating Peripheral Activity to Relieve Conditions (SPARC) program to fund the basic exploration of how peripheral nerves’ electrical signals control the function of internal organs. This effort may lay the groundwork for electroceuticals—the next generation of tiny neurostimulation devices.

In the private sphere, **GlaxoSmithKline** (GSK; Middlesex, U.K.) leads the charge. The corporation is heavily involved in creating a Nerve Atlas to map the nervous system’s role in disease. GSK is also investing in start-ups such as **SetPoint Medical** (Santa Clarita, CA), which has already produced clinical data supporting the use of electroceuticals on rheumatoid arthritis. Another of GSK’s notable investments in electroceuticals was the launch of **Galvani** (Stevenage, England) in 2016, in partnership with **Verily** (San Francisco, CA). Galvani is developing electroceuticals for chronic conditions including Type 2 diabetes, autoimmune, and endocrine disorders.

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**COCKTAIL FODDER: THE DOWNSIDE OF FEELING NO PAIN**

Feeling no pain sounds great, doesn’t it? Yet people born with congenital analgesia suffer greatly from injury and illness, because they never feel the pain associated with them. In a BBC interview, sufferer Steven Pete talked about breaking a limb roller skating as a boy, noticing it only when friends pointed out the bloody wound caused by the bone protruding from his leg! Fortunately, this inherited nervous system disorder is extremely rare, with only 20 or so cases known in the medical literature.
Zinc Finger Nucleases

CATCHING THE RIGHT BREAK

How are ZFNs made? To start, zinc finger proteins (ZFP) are sequence-specific, DNA-binding proteins that activate gene expression. They are engineered to recognize unique sites within the genome. While ZFPs do not have the ability to cut DNA on their own, scientists can fuse a ZFP together with a DNA-cutting enzyme called nuclease—the “N” in ZFN. The marriage of ZFP to nuclease creates ZFN.

How do ZFNs work? Zinc finger proteins bring the zinc finger nuclease to the engineered location of the genome and the nuclease cuts the specific location. The double-stranded break (DSB) or cut activates the non-homologous end joining (NHEJ) repair pathway, this most often results in a disruption of a gene, useful for gene “knockouts”. If a repair template is delivered at the same time as the break, the homologous directed repair (HDR) pathway kicks in. This method is useful to “knockin” a gene. This video explains a potential application for zinc finger nucleases in HIV.
Plants That Heal

NATURE’S MEDICINE CABINET
Where does medicine come from? Before it gets to your medicine chest? Before you purchase it from your neighborhood drugstore? Next time you’re hiking through a forest or gazing at your pretty screensaver of the Olympic Peninsula, think of this: the magic that relieves a throbbing headache or lowers your dad’s blood pressure may well have started with a plant.

Historically, humans have looked to the world around us for what we need. Plants in particular offer the power to feed, heal or potentially harm us. They produce an array of bioactive compounds to survive and thrive. Some of these substances are also biologically active in people too. Sometimes, years of hard work and testing turn one of these compounds into drug we can use.

DOCTOR MOM IN THE FOREST PRIMEVAL
Imagine early humans, scrabbling for survival out on the savanna somewhere. One of the toddlers develops a burning fever. Desperate, the mother remembers those berries along that new stretch of riverbank. She asks herself, “What if?” Let’s say the berries cure the baby’s malady. Voila—the first medicine, courtesy of Dr. Mom.

THE DIVINE FARMER
As long as humans have eaten plants, we’ve experimented with their health effects. The Chinese were likely the first to formalize their knowledge into what we call Traditional Chinese Medicine (TCM). According to Chinese lore, the mythical emperor Shennong, sometimes known as the Divine Farmer, commanded his herbalists to record the effects of medicinal plants approximately 5,000 years ago.

Ethnobotanists and others have examined many TCM plants for use in pharmaceuticals. Some have yielded ingredients regularly used in western medicine. They include:

Mahuang or Ephedra (Ephedra sinica). Mahuang was used to relieve asthma, or at least wheezing and congestion. Scientists now know that mahuang contains an alkaloid called ephedrine. Ephedrine has a long history in western medicine as well. It’s the active ingredient in many cold medicines, including Sudafed.

Doctors also prescribe ephedrine to prevent low blood pressure during spinal anesthesia.

Indian snakeroot (Rauwouofia serpentina) is also featured prominently in early Chinese and Indian medicine. It contains an indole alkaloid, reserpine. Studying this chemical helped neuroscientists learn how neurotransmitters such as serotonin or dopamine govern depression. Reserpine is prescribed for hypertension and psychosis. Mahatma Gandhi reportedly relied on reserpine to calm his nerves.

FROM FOREST TO PHARMACY
Plants beyond those used in traditional medicine continue to influence modern medicine. In fact, for the past 25 years, approximately one quarter of prescribed medicines in the US contain plant derivatives.

According to some historians, the modern pharmaceutical industry began as early as the mid-1800s. Others say the early 1900s. Regardless, the first commercial drugs derived largely from plant extracts. They include these old favorites:

- **Digitalis.** Cardiac glycosides from the foxglove plant stimulate cardiac muscles and are used to treat congestive heart failure. Careful! Every part of these funky, beautiful flowers is poisonous to dogs, cats and humans.

- **Aspirin.** Aka acetylsalicylic acid. Salicin, the bioactive ingredient behind this most basic medicine comes from willow tree bark. Many of us use it to manage pain, fevers and inflammation. My mom uses it to help prevent stroke.

- **Quinine.** This alkaloid is a product of bark from the native South American cinchona tree. In the past, quinine was famously used against malaria. It is currently used to treat patients with lupus or rheumatoid arthritis. For malaria, a derivative of another plant ousted the cinchona—artemisinin, which comes from sweet wormwood.

Through the 1930s, pharmacological research remained focused on screening natural products. Plants, bacteria and fungi were tested for their potential anti-cancer, anti-inflammatory or anti-bacterial properties.
In 1955, the National Cancer Institute began screening natural products for cancer treatment. This program yielded a potent anti-cancer compound, taxol. Taxol came from the bark of the Pacific yew, an evergreen native to the Pacific Northwest. In 1993, the FDA approved a modified version of taxol, Paclitaxel, for chemotherapy. Today, Paclitaxel is on the World Health Organization’s List of Essential Medicines.

Scientists these days often synthesize new drugs using organic chemistry or recombinant DNA techniques. These medicines specifically target areas in the human body where diseases originate. Many in the pharmaceutical and medical communities no longer consider natural products a primary source of new therapeutic compounds.

However, the plant kingdom remains a biological treasure trove. A 2016 study by England’s Royal Botanic Gardens Kew estimates our amazing planet supports about 390,900 species of plants. So far, researchers have screened only about six percent for biologic activity in humans. Who knows what pharmaceutical miracles may originate in this immense diversity?

A SUPPLEMENT IS NOT A DRUG

More and more, people use plant-based supplements like echinacea to combat their colds or valerian to help them sleep. Scientists are investigating some supplements for their medical potential. However, it’s vital to remember that the FDA does not consider supplements drugs. So, what are the differences between a supplement and a drug?

The FDA evaluates and monitors the development of prescription medicine in the US. The agency defines drugs as substances intended to diagnose, treat or prevent disease. They must pass clinical trials before being approved for consumer use. The process often takes twelve years or more and incredible resources—intellectual and monetary.

In contrast, the FDA has no power to regulate supplements. Under the Dietary Supplement Health and Education Act, the FDA must treat supplements like food. The law doesn’t require supplements be tested for safety, purity or quality. They include minerals, vitamins, amino acids and other biological substances. The general rule is that these products are considered safe until proven unsafe.

Some supplements are whole plant extracts, containing multiple bioactive ingredients. If a pharmaceutical company wants to develop a drug from a supplement, it generally needs to isolate and test a single compound from the whole plant extract. Companies such as Sanofi (Paris, France), Novartis (Basel, Switzerland) and Pfizer (New York, NY) are investigating plant-derived compounds in their drug discovery pipeline. One smaller company, Napo Pharmaceuticals (San Francisco, CA), isolated a compound from the tropical croton plant (Croton lechery). It won FDA approval as an anti-diarrheal for HIV patients in 2013.

Here are a couple plants and one fungus currently marketed as supplements. Remember, caveat emptor—guinea pigs are us.

White mulberry. Dried fruit from the white mulberry tree tastes a bit like raisins. The fruit contains fiber, protein and vitamins and deoxynojirimycin, or DNJ. DNJ is a glucosidase inhibitor that acts upon the sugar metabolism pathway to prevent a spike of blood-sugar levels after eating. DNJ may prove useful for diabetics.

Devil’s claw. This small desert plant is native to Southern Africa. In the early 1800s, Europeans introduced this plant at home, where its root became a popular remedy for pain and osteoarthritis in France and Germany. Harpagide, a glycoside from devil’s claw, appears to control inflammation. Devil’s claw has also gained some traction with dog and horse owners because those animals also experience relief after taking the herb.

Chaga. Fungal tea, anyone? Strange but true—look for a chaga concoction on the chalkboard of your local coffee shop soon. This fungus grows on hardwood trees in chilly, northern forests (Maine, Alaska, Canada, Siberia). Packed with antioxidant enzymes, phytosterols and other bioactive compounds, preliminary research suggests the ability of chaga extract to reduce inflammation, reduce fatigue and boost immunity. No verdict on taste though!

Next week, we’ll move from the forest and the garden to the lab—explaining key steps researchers take to identify and move potential medicinal compounds into clinical trials.
ON THE ROAD TO NEW MEDICINES

For most of the 20th century, we discovered new drugs by trial and error. Scientists investigated countless unrelated compounds in animals to see which improved disease symptoms. For instance, in the 1950s and 60s, British scientists at Boots Laboratories tested hundreds of unrelated chemicals on guinea pigs searching for an alternative to aspirin for treating pain and inflammation. This scattershot approach consumed vast amounts of time and resources, with limited success.

PICKING UP THE PACE—RATIONAL DRUG DISCOVERY

Beginning in the 1980s, scientists began to take a new tack in developing drugs. They adopted an approach known as rational drug discovery or mechanism-based drug design. In this method, researchers first seek to understand a disease at the cellular level, and ideally, identify its mechanism. Once the cellular mechanism becomes clear, scientists can identify a drug target: the molecule (usually a protein) involved in the illness that the 'as yet undeveloped drug' will hopefully act on. Although still labor and resource intensive, rational drug discovery tends to produce more highly-effective drug therapies than the old trial and error approach.

PROBLEMS WITH PROTEINS

Identifying a target molecule for a drug to act upon requires years of basic research on a disease’s biology. Most diseases stem from issues related to how our bodies produce proteins. Significant underproduction of a protein causes disease; for instance, little to no insulin production leads to Type 1 diabetes. In contrast, overproduction of a protein can also produce disease; too many of the growth factor receptor HER2 can result in certain breast cancers. Finally, errors, or mutations, sometimes affect protein function. This is the case when a mutated tumor suppressor protein no longer controls cell division and cancer develops. Understanding which proteins correspond to a particular disease means researchers can design drugs to go after that disease-causing protein specifically.

X MARKS THE MOLECULE

Researchers identify their molecular targets by answering one incredibly complex question: How does diseased tissue differ from healthy tissue? Scientists look at two biological clues: differences in gene sequences (mutations), and changes in the levels of specific proteins. For example, does a lung tumor biopsy show mutations absent in healthy lungs? Are levels of protein expression higher or lower in the diseased tissue? If the answer to either question is yes, scientists have a potential target for their ‘as yet to be developed’ drug.

TESTING, TESTING

In the quest for new medicines, as in the rest of life, few things are as simple as we hope. Just because a protein plays a fundamental role in an illness doesn’t always make it a good drug target.

Consider the following scenario: Researchers discover a specific protein target that affects the course of a disease—great! They then design a drug to inhibit said target—also great! Problem solved, right? No so fast. Sometimes a different protein can quickly take over the target’s function, nullifying the new drug’s hoped-for effect. It’s back to the drawing board for the disappointed scientists.

In other cases, inhibiting a specific target achieves a goal – say halting cancer cell growth – but results in unexpected side effects, such as irregular heartbeat. It’s crucial that scientists validate that when a target is altered, that change to the target is both safe and effective.
Researchers verify the suitability of target molecules by answering two important questions: does the target play a key role in the disease? and will targeting the molecule likely be safe and effective? Validation most often includes cell-based (in vitro) and animal (in vivo) testing. The goal of many drug therapies is to stop or slow a target's activity. Consequently, many tests, or validation assays, seek to find out what happens when a drug puts the brakes on a molecular bad actor.

One of the most popular testing methods is RNA interference (RNAi). This technique can rapidly block production of a particular protein. It does so by destroying the messenger RNA that codes for the protein. This destruction can quickly demonstrate what happens to a cell when protein production ceases, thus mimicking the effects of a strong inhibitor drug.

If a cell model shows promise, researchers move on to an animal model. Often, scientists use “knockout” mice – in which they have disrupted a target gene that codes for the target protein. Researchers pose similar questions to those asked in cell-based testing, but on the larger scale of the whole animal. Do the mice get cancer or Parkinson’s disease or heart disease when the target gene is tampered? Because in vivo testing allows researchers to examine whole-body effects, it provides valuable information about target safety that in vitro testing simply cannot.

**TERM OF THE WEEK: BIOMARKERS**

One important tool in drug discovery and development are biomarkers. Biomarkers are molecules that doctors and others use to measure normal or abnormal biological processes in the body. They can help show the effects of a disease or therapeutic intervention.

LDL cholesterol is a classic biomarker familiar to anyone over the age of 35. Numerous studies have linked it to heart disease. LDL cholesterol makes an ideal biomarker because of these three reasons: it circulates in our blood and can be obtained by a simple blood draw; it can indicate heart disease early on, since someone can have elevated cholesterol long before developing atherosclerosis; and it changes as the disease progresses. In developing drugs, researchers have used changes in LDL as a surrogate endpoint. Surrogate endpoints are outcomes that researchers can measure to determine relatively quickly whether the trial intervention benefits the participants.

In the case of heart disease, researchers establish a target cholesterol level in lieu of waiting years or even decades to demonstrate a change in disease progression. In many cases, measuring biomarkers has turned out to be key to running shorter, less expensive clinical trials. We know now that statins, which lower cholesterol, can increase longevity because patients have taken them for more than 30 years. Initial trials, however, simply showed a decrease in patients’ blood cholesterol.

Identifying and validating drug targets are only the first steps on the long road to safe, effective new medicines. Tune in next week to find out how researchers identify therapeutics that might make it into clinical trials.
WE WANNA NEW DRUG

“One that won’t make me sick/ One that won’t make me crash my car/ and make me feel three feet thick...” Huey Lewis is singing about love, but he voices very human concerns when it comes to the medicines that heal bodies and minds.

Last time, the Weekly explored how researchers identify drug targets—the molecules in our bodies that make good candidates for therapeutic action. It’s a painstaking but strategic process, and only the first step toward Huey’s mythical remedy.

THERAPEUTIC CHOICES

There are small molecule drugs and large molecule drugs. Small molecule drugs include medicines most of us know, such as aspirin and over-the-counter cold remedies. In contrast, large molecule drugs, also known as biologics, are often specialty drugs, delivered through injection by a healthcare professional.

Therapeutic choice hinges on a disease’s origins. If an illness originates outside the cell, a biologic is a logical choice since it is too big to enter the cell. Though more expensive to produce, biologics typically cause fewer adverse reactions than their smaller counterparts.

Small molecule drugs attack disease from within the cell. The downside? Their small size means they have the potential to also activate many off-target molecules. This collateral damage increases the chance of adverse reactions.

ASSAY DEVELOPMENT:
GLOW AND NO GLOW

To find the best drug candidate, scientists need to design easy-to-perform, large-scale, fast, and accurate assays. Think automation. Think high throughput screening. Scientists often work with tests that produce a fluorescent signal or color change because color is easy to measure, relatively inexpensive to design, and safe. Depending on the assay, a color change may or may not indicate a drug candidate works.

GLOW

Imagine “Enzyme Z” causes “Disease Z.” Its cure lies in shutting down, inhibiting “Enzyme Z.” Enzymes feature active sites, a kind of pocket that binds various small molecules. Researchers manipulate these small molecule inhibitors to light up—emit a fluorescent signal if they bind (shut down) the enzyme.

NO GLOW

Scientists use similar colorimetric tests for large molecule drugs such as antibodies. Let’s say that activated “Receptor X” turns on a particular gene that in turn causes cancer. Scientists can engineer cells so when Receptor X is activated, the cell produces a green fluorescence and when it is not activated no green appears. Researchers are hoping for “no glow” or in lab-speak, a “blank.” Blanks are the winners that go on to the next stage of drug development.
HIT TO LEAD

High throughput screening ideally results in several promising drug candidates or “hits.” To become “leads” that merit animal testing, hits must pass rigorous in vitro testing. On this leg of the drug discovery voyage, researchers ask three questions:

1. Is the hit safe?
2. Is the hit specific to the target?
3. Does the hit show promise to treat the disease?

If a potential lead is supposed to inhibit an enzyme—it should act on that particular enzyme. A lead with effects that are too general may result in a drug with dangerous adverse reactions. Therefore, researchers aim to develop a drug that inhibits the target at a concentration low enough to avoid interfering with related enzymes. This approach provides a therapeutic window within which the drug is effective and safe.

LEAD TO CANDIDATE

Biopharma companies typically put lead compounds through several rounds of optimization before they can become drug candidates for preclinical testing. Lead optimization is the process by which a drug candidate is designed via iterative rounds of synthesis. For small molecule drugs, this tweaking may include creating derivatives of the lead. Often made using computer-aided design (CAD), derivatives can help ensure the lead fits well with its target. Researchers then test each for improved potency, fewer side effects, solubility (making sure that the drug stays liquid and won’t form clumps) and shelf life, otherwise known as stability.

Of course, optimization for large molecule drugs differs because biologics are genetically encoded. Researchers have developed techniques to create mutations in proteins at specific locations. Again, the goal is to see if changes produce a better drug—maybe even one up to Huey Lewis’s rigorous standards. If so, researchers have at long last an official drug candidate, one ready for the rigors of preclinical testing in animals.
THE PROMISE OF GENE THERAPY UNFOLDS

In many ways, 2017 was the year of gene therapy in the United States. Patients and pharmaceutical companies celebrated the approval of not one, but three treatments for otherwise untreatable health conditions. Researchers have been working on developing safe, effective gene therapies for three decades. Early trials were plagued with safety issues. Consequently, the field remained on hold until researchers could address those problems with new and improved gene-delivery vehicles. This Weekly explores these innovative approaches to intractable illness and disease.

THE FIRST APPROVALS

The FDA approved the first gene therapies in the United States last year --Chymera and Yescarta--both chimeric antigen receptor T-cell (CAR-T) therapies. They deliver a gene to cancer patients' white blood cells in order to program them to attack specific cancer cells. CAR-T treatments involve removing a patient's white blood cells, programming them to contain a cancer-destroying gene, and then re-administering them to the patient.

TWO FIRSTS IN ONE

Just before year’s end, the FDA approved a therapy for a rare form of inherited blindness. Developed by Spark Therapeutics (Philadelphia, PA), Luxturna is the first gene therapy to target a genetic disorder. It's also first in another significant way. Unlike the new cancer treatments, patients receive Luxturna directly, via sub-retinal injection.

The blindness treated by Luxturna is known as “biallelic RPE65 mutation-associated retinal dystrophy.”

- Biallelic: Pertaining to both copies of a particular gene (allele)
- RPE65: A protein in the retina that helps convert light into the electric signals the brain interprets as sight
- Retina: Light-sensitive tissue in the eye
- Dystrophy: Wasting of tissue

The corrected version of the RPE65 gene helps repair patients' retinal health and vision.

ASTOUNDING IMPROVEMENT

Clinical trial participants’ vision loss ranged from mild to severe. The trials included patients from ages four to forty-four. For 93% of them (27 out of 29), Luxturna treatment improved patients' visual function, as shown by a “multi-luminance mobility testing (MLMT) score.” The MLMT measures the ability to navigate an obstacle course in low light.

COST OF A ONE-TIME CURE

Luxturna is priced at $425,000 per eye. Most patients need treatment in both eyes. Its expense comes in part from the nature of the treatment as a one-time, cure. Spark Therapeutics is discussing payment options with insurers to help allay the sticker shock. One plan in the works with Pilgrim Health, a large non-profit New England-based insurer, hinges on outcomes. Should the treatment fail to meet the intended outcome at certain intervals post-treatment, Luxturna will refund the treatment’s cost. Another proposal in the works would allow insurers to spread payments out over multiple years.

TERM OF THE WEEK: VECTOR

Scientists have adapted some viruses to transport therapies by tweaking them to target disease instead of causing illness. These souped-up microbes are known as viral vectors. They are the vehicles that make genetic therapy like Luxturna possible. The virus itself is simply a segment of genetic material— RNA or DNA—surrounded by a protein coat. Proteins on the surface of the vector (the modified virus) target proteins on a patient's specific diseased or malfunctioning cell surface. These viral vectors then incorporate DNA into the patient’s genome by “tricking” the patient’s cells into producing the DNA it delivered. Ultimately, this enables a patient to make the functional protein that he or she lacked.

Over a decade ago, gene therapy stalled because of safety concerns. Researchers could not initially control the insertion point of modified genes. In some cases, the introduced genes disrupted patients' other genes, causing serious illness. Researchers have now developed vectors that allow them to more precisely target where a therapeutic gene goes into a person's genome, making
the treatment much safer because it doesn’t interfere with the function of critical genes.

Types of Gene Therapy Vectors

The most commonly used type of viral vector for gene therapy applications are adeno-associated viruses (AAV). However, AAVs aren’t the only vector in town. Lentiviral vectors are also being tested in gene therapy clinical trials. The choice of vector depends in part on the target tissue and the size of the genetic payload. AAV vectors so far seem to be best at targeting eye and muscle tissues, while there is some evidence that lentiviral vectors are better at targeting blood and central nervous system tissues. Lentiviral vectors have a higher carrying capacity as well, so may be preferred for very large genes or in cases where the goal is to deliver more than one gene.

LOOKING AHEAD

Luxturna’s success raises hopes of cures for numerous other diseases caused by a defect in a single gene, including hemophilia, sickle cell anemia, Huntington’s disease, and other types of hereditary blindness. As the table below indicates, many companies have gene therapy treatments in clinical trials.

2018 may see more groundbreaking approvals as these treatments make their way through clinical testing.
Drug Discovery 301

**DRUG DISCOVERY 301**

Biotech Primer Weekly wrapped up last year by exploring the first two stages of drug discovery. We looked at how pharmaceutical companies identify drug targets, or the molecules (usually proteins) involved in an illness that an ‘as yet undeveloped drug’ will hopefully act on. Next, we examined how researchers develop those pharmaceutical candidates. Now we turn to what comes next for fledgling drugs on the arduous journey to FDA approval. First, though, a smidgeon of Latin.

**PRIMUM NON NOCERE**

This maxim from Hippocrates means “First, do no harm.” Before testing a drug on people, researchers must make sure it’s safe in two important ways. First, they assess a substance’s safety *in vitro*. This Latin phrase means “in glass”—that is, lab-grown cells. The cell type varies, but if possible, researchers use one that is relevant to the disease in question. For example, they may use lung cells to test a drug for respiratory syncytial virus (RSV).

*In vivo* testing (“in a living thing”) comes next. This area of preclinical testing assesses a drug candidate’s toxicity in at least two different species of animals, such as mice and guinea pigs. The animals receive more of the drug for longer than would human volunteers. Meanwhile, lab technicians watch the animals for adverse effects. Preclinical testing must follow the FDA’s Good Laboratory Practice (GLP) guidelines. These regulations help ensure scientific integrity and humane treatment of laboratory animals.

**HOMING IN ON THE SWEET SPOT**

Almost any substance—even water!—can be toxic in very high amounts. Consequently, drug developers aim for just the right dose—one that gives the desired effect with minimal unwanted consequences. Finding the sweet spot is the domain of pharmacokinetic (PK) and pharmacodynamic (PD) analyses. Think of pharmacokinetics as “what a body does to a drug” and pharmacodynamics as “what a drug does to a body.”

PK analyses typically measure:

- The time it takes to absorb a drug into the body;
- Maximum concentration (Cmax) of a drug in plasma and target tissues;
- Bioavailability (where in the body and at what concentration the drug ends up);
- Half-life, or how long a drug takes to reach half its maximum concentration in the body;
- Clearance, or the time for a drug to reach undetectable levels through excretion.

Pharmacodynamic analyses involve observing the biological repercussions of increasing amounts of a drug. Negative side effects include nausea, loss of appetite, fatigue, skin sensitivity, and changes in blood pressure or heart rate. Animal PK and PD studies give scientists an idea of what a safe and effective dose of a drug might be in people.

**HUNTING FOR MUTANTS**

Most drug candidates also undergo mutagenicity testing, which determines their likelihood of triggering mutations. Causing mutations indicates that a fledgling drug may be carcinogenic, hence often consigning it to the graveyard of pharmaceutical failure. One common screening for mutagenicity is the Ames test, which identifies chemicals that cause increased rates of mutations in bacteria.

Researchers can also assess a drug’s carcinogenic potential by examining test animals for tumors.
**Q/T TESTING**

Heart problems aren’t just bad for people, they’re bad for baby drugs too. So, companies try to rule out anything with potential cardiac side effects as early as possible. To find drugs that may be heart-unhealthy, researchers look at the “QT interval.” This is the time between the start of the Q wave and the end of the T wave in the heart’s electrical cycle. A lengthened QT interval suggests improper activity in a person’s ventricles and is a risk factor for sudden death. If an experimental drug binds to proteins on heart cells that enable the flow of calcium ions into the heart, they may extend a QT interval. Longer intervals generally seal a drug’s doom.

**ALTERNATIVES TO ANIMAL TESTING**

Currently, drug development necessitates in vivo testing to best understand how drugs act in a human body. *In vitro* testing simply can’t replicate Home sapiens’ complex physiology. However, two innovations may help reduce the animal testing involved in the search for new treatments:

- 3D tissue arrays: Companies such as Organovo (San Diego, CA) create tissue arrays that better mimic human physiology than flat layers of cells in tissue culture flasks.

- Computer modeling: Researchers at the University of California, San Francisco, and SeaChange Pharmaceuticals (San Francisco, CA) have developed software that reliably predicts small molecule drugs’ interaction with “off-target” molecules. These are cellular proteins other than the intended drug target. More off-target interactions mean a greater chance of undesirable side-effects.

**THE PAPER CHASE**

Once researchers amass enough safety data to ensure that a drug candidate will be safe for people, they submit an Investigational New Drug (IND) application to the FDA. The IND application contains short-term safety data, information on manufacturing the drug, and details about the methodology and design of clinical testing. If the FDA blesses the application, the drug candidate may enter Phase 1 clinical testing. Animal testing often continues in parallel with clinical (human) studies to collect long-term safety data, especially for medicines that treat chronic diseases.

**COCKTAIL FODDER**

For every 5,000 drugs tested preclinically, only about five show enough promise to justify submitting an IND application to the FDA. Once a drug clears the preclinical testing hurdle, it can move on to human testing. Tune in next week to learn more about this crucial process.
PHASE I AND II CLINICAL TRIALS

Every drug in clinical use today, from the latest CAR-T treatment to older cholesterol-lowering statins, share one thing in common: they have all successfully navigated the rigorous clinical trials process. This is no small feat, as only ~10% of the drugs that enter Phase I testing successfully emerge as marketed products. Those few drugs that show remarkable success in early clinical trials make headlines, and deservedly so. Recent drugs that have made a big splash include Amgen’s oncolytic virus drug Imlygic, which is restoring faith in therapeutic cancer vaccines, and AbbVie’s Venetoclax, which seems to melt away tumors in chronic lymphocytic leukemia patients.

This week, we’ll take a look at the first two phases of clinical trials.

TERM OF THE WEEK: ENDPOINT

Clinical trials measure endpoints, that is, major health outcomes. There are generally two types:

- **Clinical endpoints** refer to benefits such as survival, decreased pain, the absence of disease, or greater mobility.
- **Surrogate endpoints** substitute for clinical endpoints when they are impossible or impractical to measure. For instance, a clinical benefit, such as survival, hopefully, takes decades to observe. Researchers may instead look to shorter-term phenomena. For example, in studying a drug designed to prevent heart disease, they can monitor cholesterol levels instead of decreased fatality from heart attacks. Similarly, in some cancer treatments, reduced tumor size stands in for longer life.

The FDA requires that clinical protocols clearly define endpoints. They are front and center in the application companies submit to the FDA that request permission to study a new drug. This application is called an Investigational New Drug application or IND.

PHASE I

The FDA divides clinical studies into three main phases. Phase I usually tests drug safety in healthy volunteers, typically one hundred or fewer. In some cases, Phase I trials may use patients rather than healthy volunteers. For example, cancer drugs have a level of toxicity that we would not want to expose healthy volunteers to, but that toxicity is an acceptable risk for patients who may have no other options.

This first stage involves a number of different tests. First, volunteers take escalating doses of the drug under close observation. If and when they experience adverse effects, they stop taking the drug. This establishes the Maximum Tolerated Dose, or MTD, which becomes a benchmark for the remaining trials. The MTD helps assure investigators and subjects that the treatment is unlikely to be toxic.

Other studies look at pharmacodynamics (PD) and pharmacokinetics (PK). The first examines what the drug does to the body; the second, what the body does to the drug. These investigations help determine drug dosage. Variations in how people of different sizes, ages, and genetic backgrounds etc., will likely respond to a new drug make testing in different populations critical.

PHASE II

Phase II examines drug efficacy as well as continuing safety tests. Phase II trials involve larger groups of participants, all of whom are patients. Group size varies, depending on the target market. A drug being developed for Type 2 diabetes needs far more participants than one for a rare disease such as ALS.

Phase II studies are usually randomized, double-blind studies. This means that patients are randomly assigned the drug or the placebo and even the researchers involved don’t know who receives which. This helps guard against bias in determining who goes into which group. Otherwise, investigators may naturally want to ensure the sickest patients receive the promising experimental treatments.

A placebo is also known as the “standard of care group”. According to the National Institutes of Health (NIH), the standard of care is a treatment accepted by medical experts as proper for a certain disease and widely used by medical professionals. For example, in clinical trials for Amgen’s Repatha, researchers compared its safety and efficacy against statins, the current standard of care for cholesterol reduction. Repatha was demonstrated to
be as effective as statins, and had a better safety profile in some patients, which led the FDA to approve Repatha.

**THE POWER OF POSITIVE THINKING**

Investigators measure drug efficacy by monitoring predefined endpoints. They always look at effectiveness relative to a control group - people who receive either standard of care treatment or a placebo.

The “placebo effect” may stem from an expectation or belief that the “medicine” will work. The placebo control is very important since scientists have firmly established that placebos can significantly affect patient health. Recent evidence shows that people dealing with chronic pain can produce natural opioid painkillers in response to a placebo. More rarely, the reverse of the placebo effect – the “nocebo effect” – occurs. In other words, some patients expect a study drug will either not help or in fact harm them.

**COCKTAIL FODDER**

The first antibiotic, penicillin, was discovered in 1928 when microbiologist Alexander Fleming noticed the mold Penicillium secreted a substance that killed his bacterial cultures. Inspired by this story, biochemist Akira Endo, who was working at Sankyo Pharmaceuticals (Tokyo) in 1972, decided to screen Penicillium cultures in hopes of finding a chemical that would inhibit cholesterol. Endo’s quest paid off - he discovered a compound that eventually went on to be one for the first statin drugs approved in 1987.

Why would mold cells secrete an anti-cholesterol drug? For the same reason they secrete penicillin – to kill the bacteria that they are competing with for resources. Cholesterol is required for bacteria to build functional cell walls.

Next week, we’ll continue along the pathway to approval as we zoom in on drug development Phase III and IV.
**From Drug Development To Approval: Phase III**

**PHASE III IS NO GUARDIAN**

Our last Biotech Primer WEEKLY explored the riskiest part of the human clinical trials pathway: Phase II. About 70% of drugs that enter Phase II never make it out. Most often, it’s because they fail to demonstrate effectiveness. Even making it to Phase III is no guarantee of success – about 40% of drugs fizzle out during this period. In today’s WEEKLY, we look at the final stage of clinical testing and the innovative clinical designs that are pushing for faster drug approvals.

**CHOICES, CHOICES, CHOICES**

Phase III clinical trials continue to test a treatment’s efficacy and safety, but in still larger groups of patients. Bigger groups mean more statistically significant results. As in Phase II, the trials have traditionally been randomized, double-blind studies. If the investigational drug appears to work, patients are allowed to continue taking the medication after the trial ends, before regulatory approval. This is an example of what doctors and other medical professionals call compassionate use.

Choices, choices, choices... In addition to randomized, double-blind trials, researchers have other study designs at their disposal, which we will explore below.

**THE PATRIARCHS: PARALLEL VS Crossover Designs**

Parallel studies use the classic experimental design. Participants are divided into two groups. The treatment group receives the experimental drug. The control group receives either the current standard of care or a placebo.

Investigators sometimes decide to conduct a crossover study. Here, both groups receive the medicine and then the placebo or vice versa. The treatments are separated by a washout period in which patients are taken off the study medicine (or placebo) to eliminate any effects.

Both parallel and crossover studies include a baseline period before patients take the drug or placebo. It allows researchers to gather the initial health information against which they will compare changes observed during or after the study. For example, when assessing a new cholesterol medication, researchers might take baseline cholesterol levels.

**A CROSSOVER STUDY’S STRENGTH**

A crossover study’s strength lies in its ability to capture differences in response to a drug versus a placebo in the same patient. This eliminates the inevitable individual variations among subjects in the experimental and control groups in the more traditional parallel study.

**THE NEW KID IN TOWN: ADAPTIVE DESIGN**

Adaptive studies offer more flexibility than traditional designs, and are gaining in popularity as more efficient in bringing new drugs to the market. They allow investigators to modify the trial design as they go, rather than spending time and money pursuing drug formulations or dosages that ultimately prove ineffective. For example, researchers may separate participants into different dosage groups. At a pre-specified time, they may note how patients respond to different doses. If one dose seems more effective, the researchers will conduct the rest of the trials using only that dosage. Researchers set the trial protocol, which lays out the adaptation schedule and processes before the trial begins.

One prominent example of adaptive design is I-SPY 2 study (Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging and Molecular Analysis). This trial, being conducted by 20 different cancer research centers across the US, examines up to 12 different breast cancer therapies at once. Drugs that show promise proceed to Phase III, allowing space for other drugs to enter the trial. “Graduates” of the I-SPY 2 study include AbbVie’s (North Chicago, IL) Veliparib and Merck’s (Kenilworth, NJ) MK-2206.

The 21st Century Cures Act promotes the use of adaptive trial design. This benefits volunteers, as
Researchers assign more patients to study groups taking medicine that demonstrates promise. It's good news for non-study patients too, who stand to benefit from new treatments getting to market faster.

NEW APPROACH TO FIGHTING CANCER: BASKET DESIGN

The design of clinical trials for new oncology drugs is changing to reflect our increasing understanding of cancer as a disease driven by mutations. Traditionally, trials test drugs on a specific illness, say, lung or breast cancer. In contrast, basket trials classify cancers according to the mutation they exhibit, not the tissue they affect. For example, the FDA initially approved the drug Zelboraf for melanoma patients with a mutation in their “BRAF” gene. Researchers at Memorial Sloan Kettering Cancer Center (New York, NY) explored whether Zelboraf could work against other cancers with the same genetic signature – the BRAF mutation. The results indicated that Zelboraf also effectively treated BRAF-mutated non-small cell lung cancer, Erdheim-Chester disease, and Langerhans cell histiocytosis. These last two, rare blood cancers, illustrate a key benefit of basket trials: they may offer patients with rare cancers better access to clinical trials, depending on their tumor’s genetic signature.

NEW APPROACH TO FIGHTING CANCER: UMBRELLA DESIGN

These trials reflect the fact that scientists classify most cancers as specific subtypes based on the mutation involved. An umbrella study may look at patients with one type of cancer, for example, lung cancer. However, researchers divide participants into subgroups based on the particular mutation behind their lung cancer, and treat them with drugs designed to target the subtype specifically. This allows researchers to identify patients most likely to benefit from the new drug.

One ongoing umbrella study is the National Cancer Institute's Alchemist Lung Cancer Enrichment Marker Identification and Sequencing Trials (ALCHEMIST) study. In it, researchers sequence patients' tumors and assign them to different treatment groups according to the subtype mutation.

Phase III is the final hurdle for drug candidates. If a drug “survives,” things are looking good for developers and patients alike. It’s not a slam dunk yet though, so next week we look at the approval process and what comes next.
PHARMA FINISH LINE: FDA APPROVAL

Last week, we focused on the final stage of clinical testing, Phase III trials, where drug developers assess the safety and efficacy of their drug in large patient groups. At the end of Phase III, drug developers face the moment of truth: does the study data support claims that the new drug is both safe and effective? If the answer is yes, then it's time to submit either a New Drug Application (NDA) for small molecule drugs, or a Biologics Licensing Application (BLA) for large molecule drugs. This week, we'll examine the approval process, including various pathways for expedited approval, and touch on post-approval safety studies also known as Phase IV.

UNDER THE FDA MICROSCOPE

Once a company submits either an NDA or BLA, the FDA takes about a year for review—which seems governmentally slow unless you consider that most applications run 100,000 pages or longer. There are three possible responses:

- **Approval letter**: Ta dah!
- **Approvable letter**: Close, and this is how to get the cigar! FDA requests correction of minor deficiencies, labeling changes, or post-approval studies.
- **Not approvable letter**: Thanks for playing! FDA elaborates on deficiencies in the application and why the drug is not approved. For many drugs, the development, testing, and approval journey is straightforward. Difficult, but straightforward.

THAT SPECIAL SOMETHING

Certain drugs are given special consideration throughout the FDA approval process. These particular therapies are eligible for regulatory designations that speed up the review and get a product to market more quickly. They must first meet two criteria. A drug must target a serious condition that likely results in death or significantly impairs daily living. Examples include cancer and Alzheimer’s disease. The drug also needs to address a major, unmet medical need. That is, either no medicine exists, or current therapy has safety issues. The FDA’s special designations include:

- **Accelerated Approval** allows drugs to go forward using surrogate endpoints instead of clinical endpoints. Surrogate endpoints, such as lowered blood pressure or reduced tumor size predict rather than demonstrate clinical benefits. In these cases, pharmaceutical companies must run post-market studies to verify the anticipated effect.
- **Priority Review** means the FDA will aim for a decision within six months.
- **Fast Track** is based on preclinical or clinical data that suggests the product addresses a specified unmet medical need. The designation enables developers to communicate more often with the FDA. The agency provides guidance on clinical trial design and process, which helps resolve questions or issues quickly. These designees also qualify for Accelerated Approval, Priority Review, and Rolling Review—which allows developers to submit each section of an NDA or BLA as they finish, rather than all at once.
- **Breakthrough Therapy** designates drugs that may greatly improve patient health. The bar is set high to join this privileged group, though. It requires preliminary clinical evidence of effectiveness. Once granted, Breakthrough designees receive Fast Track advantages, as well as intensive guidance on their development program as early as Phase I. They also get an “organizational commitment involving senior FDA managers,” according to the FDA website.
- **Orphan Drugs** are often found in the above categories. Companies develop them for rare diseases, those which affect fewer than 200,000 Americans. These include hemophilia and Gaucher’s Disease, a genetic disorder that causes skeletal and neurological issues. Prior to the **Orphan Drug Act of 1983**, the industry had no financial incentives to work on therapies for such small populations. Orphans lacked commercial sponsors, “parents,” to shepherd them through lengthy and expensive trials. The orphan drug legislation provides the following incentives:
  - Federal tax credits of up to 50% off research costs
  - Increased protection from generic competition
  - Waivers of FDA application and product fees (amounting to hundreds of thousands of dollars)

From Drug Development To Approval: Phase IV
COCKTAIL FODDER: PDUFA

The Prescription Drug User Fee Act (PDUFA) requires drug developers to pay a fee to the FDA to help fund the work necessary for timely approvals. The “PDUFA date” of a drug is the date by which the FDA has committed to review its application. For 2018, the PDUFA fee required when submitting an NDA or BLA that requires clinical data is $2,421,495.

FINISHED, NOT DONE

Phase IV generally refers to post-market studies, which companies undertake after a drug is approved and at the pharmacy. Drugs that were approved using a surrogate endpoint are monitored to confirm clinical efficacy. For all drugs, safety is monitored and confirmed. Phase IV includes informal studies of doctor and patient reports, which sometimes reveal unanticipated side effects. Such “surprises” aren’t uncommon in brand new drugs, because so many more people take them once out on the market versus the small number of patients in clinical trials.

For instance, after the anti-clotting medicine Plavix (Bristol-Myers Squibb; New York, NY) was approved, doctors found it less effective in some people than expected. Further probing found that the drug wasn’t fully activated in about 14% of patients because their bodies produced a less active form of the liver enzyme that activates Plavix. Fortunately, a genetic test can quickly reveal whether a patient can benefit from Plavix. This unlooked-for result spurred the FDA to require that the Plavix label carry a black box warning – a warning outlined in a black box, which is the strictest warning put on the labeling of prescription drugs or drug products by the FDA.

The rigorous path from preclinical testing to approval usually takes a decade or longer, with no promise of success. According to the Biotechnology Innovation Organization, approximately one in ten drugs entering clinical testing ultimately make the grade. The highest rate of failure occurs in Phase II; only 31% of drugs proceed to Phase III. Of those that make it to the final Phase III, a little more than half - 58% - are considered safe and effective enough for an NDA or BLA submission. From start to finish, only 10% of the drugs that begin Phase I reach the market.

Despite the low odds, innovative companies continue to bring new drugs to market every year. Stay with us as we continue following their stories in 2018.
**Market Access**

**MARKET ACCESS PRIMER**
For the last few weeks we here at Biotech Primer have tracked the progression of a drug candidate from the lab to the marketplace, where only the fittest survive. Winning at clinical trials means earning an official regulatory approval. Congratulations! But as any seasoned drug developer will tell you, the game has only just begun. Ensuring newly-approved drugs are available to all patients is the next hurdle.

In this WEEKLY we'll explore market access for innovative products such as cancer-fighting drugs and gene therapies.

**TERM OF THE WEEK: MARKET ACCESS**
Market access is the process of proving a product’s value to ensure reimbursement with commercial payors, government payors and integrated delivery systems. These payor stakeholders are often referred to as “organized customer groups.”

According to Linda Lander, President of Inside Out Market Access, “market access aligns incentives between payors, pharmaceutical companies, providers, and patients to lead to cost-effective models.”

**DRUG APPROVAL DOESN’T EQUAL REIMBURSEMENT**
Launching a new drug, especially a high-cost one, requires proving its value early in the process. Just because a drug is approved doesn’t mean it will be reimbursed for coverage. Without reimbursement patients will not benefit, nor will the drug’s sponsor recover the significant resources it invested in development.

Organized customer groups are now demanding evidence of value. Market access involves a discussion and negotiation between the payor and the drug sponsor to define and demonstrate value. This is increasingly achieved via outcome-based reimbursement and real world evidence, in which patient health and outcomes data gathered outside of clinical trials drives downstream reimbursement.

Outcomes-based contracts include some sort of refund if the new drug does not perform as advertised. New England-based insurer Harvard Pilgrim’s contract with Amgen (Thousand Oaks, CA) provides a “pay-for-performance” rebate to Harvard Pilgrim if Amgen’s new cholesterol drug Repatha, a PCSK9-inhibitor drug, doesn’t perform as well as in clinical trials. The benefit to Amgen is preferred positioning on the Harvard Pilgrim formulary as well as access to real world and post-marketing data.

With other novel high-impact, high-cost drugs such as CAR-T treatments and gene therapies, payors are questioning how to best afford these medicines. For example, Spark Therapeutics (Philadelphia, PA) recently received FDA approval for Luxturna, a gene therapy treatment for hereditary blindness. The treatment is remarkable, in that it promises a lifetime cure with one administration - at a cost of $425,000 per eye. Spark Therapeutics is working on an outcome-based payment scheme in collaboration with Harvard Pilgrim Health and with Centers for Medicare and Medicaid Services (CMS). If Luxturna fails to meet the intended outcomes at certain intervals post treatment, Spark would refund some or all the treatment’s cost.

These types of agreements allow patients the chance to benefit from a new therapy that a payor might otherwise be reluctant to take a chance on.

**AN INDEPENDENT VOICE**
Organized customer groups are increasingly using independent analysis to guide reimbursement decisions. These independent agencies, which include the California Technology Association (CTA), the National Comprehensive Care Network (NCCN), and Institute of Clinical and Economic Review (ICER), evaluate and determine the “reasonable price/value” of drugs in the context of an overall healthcare budget.

According to Steve Pearson, Executive Director of ICER, the organization asks four key questions:

- How well does the drug work?
- How much better is it than what we already have?
- How much could it save us?
- How much would it cost to treat everyone who needs it?
In conducting its analysis, ICER reaches out to patients, doctors, and drug makers. The end goal is to calculate a value-based price benchmark that reflects the drug’s value to patients and to the healthcare system. According to the American Managed Care Pharmacy’s annual survey of payors, more than 50 percent now employ ICER evaluations in their reimbursement reviews.

As a result, biopharma companies increasingly work with ICER and other independent organizations to gain payor acceptance for their initial drug prices; for example, Spark Therapeutics met with ICER prior to the Luxturna launch to help guide pricing strategy.

As new technologies create highly innovative treatments, the issue of pricing must be addressed. By deftly navigating market access, companies can ensure more patients have access to these advances.
FROM THE LAB TO THE PATIENT

In this issue of the Biotech Primer WEEKLY we will recap the past seven issues that highlight the journey a molecule takes from the lab to the patient.

Beginning in the 1980’s, scientists took a new tack in developing drugs. They adopted an approach known as rational drug discovery. Using this methodology, researchers first seek to understand a disease at the cellular level to identify its mechanism. Once the cellular mechanism becomes clear, scientists can identify a drug target: the molecule involved in the illness that the ‘as yet undeveloped drug’ will hopefully act on.

Fast forward many years and hundreds of millions of dollars and drum roll please... the ‘as yet undeveloped drug’ may just become the drug that cures your once incurable illness.

DRUG DISCOVERY: IDENTIFY AND VERIFY

Researchers identify their molecular targets by answering one incredibly complex question: How does diseased tissue differ from healthy tissue?

Researchers verify the suitability of target molecules by answering two important questions: does the target play a key role in the disease? and will targeting the molecule likely be safe and effective?

Identifying and validating drug targets are only the first steps on the long road to safe, effective new medicines.

Drug Discovery 101, published December 2017

DRUG DISCOVERY: HIT TO LEAD

To find the best drug candidate, scientists need to design easy-to-perform, large-scale, fast, and accurate assays. Think automation. Think high throughput screening.

High throughput screening ideally results in several promising drug candidates or “hits.” To become “leads” that merit animal testing, hits must pass rigorous in vitro testing. On this leg of the drug discovery voyage, researchers ask three questions:

1. Is the hit safe?
2. Is the hit specific to the target?
3. Does the hit show promise to treat the disease?

Biopharma companies typically put lead compounds through several rounds of optimization before they can become drug candidates for preclinical testing. Lead optimization is the process by which a drug candidate is designed via iterative rounds of synthesis.

Drug Discovery 201, published December 2017

DRUG DISCOVERY: SAFETY AND SUBMISSION

In vivo testing (“in a living thing”) comes next. This area of preclinical testing assesses a drug candidate’s toxicity in at least two different species of animals, such as mice and guinea pigs. The animals receive more of the drug for longer than would human volunteers. Meanwhile, lab technicians watch the animals for adverse effects.

Preclinical testing must follow the FDA’s Good Laboratory Practice (GLP) guidelines. These regulations help ensure scientific integrity and humane treatment of laboratory animals.

Once researchers amass enough safety data to ensure that a drug candidate will be safe for people, they submit an Investigational New Drug (IND) application to the FDA. If the FDA blesses the application, the drug candidate may enter Phase 1 clinical testing.

Drug Discovery 301, published January 2018

PHASE I/II: MAY THE ODDS BE IN YOUR FAVOR

Every drug in clinical use today, from the latest CAR-T treatment to older cholesterol-lowering statins, share one thing in common: they have all successfully navigated the rigorous clinical trials process. This is no small feat, as only ~10% of the drugs that enter Phase I testing successfully emerge as marketed products.

Those few drugs that show remarkable success in early clinical trials make headlines, and deservedly so.

Phase I/II, published January 2018

PHASE III: CHOICES, CHOICES, CHOICES

Phase III clinical trials continue to test a treatment’s efficacy and safety, but in still larger groups of patients. Bigger groups mean more statistically significant results. As in Phase II, the trials have traditionally been
randomized, double-blind studies. If the investigational drug appears to work, patients are allowed to continue taking the medication after the trial ends, before regulatory approval. This is an example of what doctors and other medical professionals call compassionate use.

Choices, choices, choices... In addition to randomized, double-blind trials, researchers have other study designs at their disposal, which we will explore below.

Phase III, published February 2018

PHASE IV: THAT SOMETHING SPECIAL
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Phase IV, published February 2018

GETTING MEDICATIONS TO PATIENTS: MARKET ACCESS

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Market Access, published February 2018

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Who’s Your Daddy? The Science of 23andMe

Hey—Check Out Those Genes!
There’s an old saying, “If you don’t know where you’ve come from, you can’t know where you’re going.” We used to rely on paper birth certificates, marriage licenses and memory to help discover where we’ve come from; but paper gets damaged, people are fallible, and memories fade. Leave it to biotech to come up with a better way. New heritage-hunting techniques come courtesy of one our greatest scientific achievements: the sequencing of the human genome.

We now have tools to genetically examine ourselves and our past. The ability to decode genetics at modest expense allowed the DNA testing industry to develop. Companies such as 23andMe, Ancestry.com, and National Geographic sell genetic tests online. These kits, consisting of a plastic tube, a baggy, and a postage-paid mailer, promise biological insight into the past. But what about the science? This Biotech Primer Weekly overviews commercial DNA testing and how it works.

A Dash of Difference
Every human being alive shares an astounding 99.9% of their DNA! Only one tenth of one percent of genetic “stuff” accounts for a planetful of differences. That’s because our genome consists of an astonishing three billion-plus building blocks—the famous As, Cs, Ts and Gs.

Areas of variation in genes are called genetic markers. Geneticists and others use genetic markers to assess the probability that two people share a common ancestor. The more genetic markers two people share, the more likely they are related. The only people with the same exact genetic markers are identical twins.

Alphabet Soup: SNPs and STRs
The two major types of genetic markers are SNPs and STRs. In the alphabet soup of genetics, “SNP” is shorthand for “single nucleotide polymorphism.” It simply means that one of the three billion building blocks that make up a person’s DNA has been switched. In other words, an A has been swapped with a G.

“STR” stands for “short tandem repeat.” These are short sequences of DNA that repeat from five to fifty times. The number of times a particular STR repeats varies from person to person.

This DNA Is Not That DNA
SNPs and STRs provide different types of information depending on their DNA origin. We humans have different types of DNA:

- The sex chromosomes are the X and the Y chromosomes. Men have one Y chromosome and one X chromosome; women have two Xs only. Thus, the Y chromosome contains DNA information about paternal ancestry.
- Mitochondrial DNA (mtDNA) is found in tiny compartments called mitochondria that convert sugar to energy in cells. Only woman pass on mtDNA because during fertilization the DNA in sperm mitochondria is quickly destroyed. Mitochondrial DNA can help trace maternal ancestry.
- Autosomal DNA is all the genetic material that is not found in the sex chromosomes and in mitochondrial DNA. Autosomal DNA is found in the pairs of autosomal chromosomes numbered one through 22. These 22 pairs come from both mom and dad – one copy from each. Autosomal DNA offers generic information about both the maternal and paternal lineages.

Deep Ancestry
Not only are types of DNA inherited differently – some from mom, some from dad, some from both – but the rate at which their DNA sequence changes from generation to generation also varies. This is because mtDNA and Y chromosomal DNA change only through random mutation of their As, Cs, Ts and Gs. Change can take centuries before a noticeable difference appears. By comparing genetic markers from mtDNA and Y chromosomal DNA with those of indigenous peoples from various parts of the world, it’s possible to estimate where your ancestors hailed from way back when.
MAKING FAMILY CONNECTIONS

Each person shares half their genetic markers with siblings, parents, and any children they have. We share about a quarter with grandparents or grandchildren, aunts and uncles, nieces and nephews, and half-siblings. The amount of DNA we have in common with relatives diminishes by half in each successive generation. By seven generations back, the amount of DNA shared among relatives is less than one percent.

So, autosomal DNA is useful in trying to make connections between living relatives or determining how far back you share an ancestor with someone. However, it doesn’t shed light on “deep ancestry,” or the region of the world our ancestors came from centuries or even millennia ago.

COCKTAIL FODDER: SAME FAMILY, DIFFERENT ANCESTOR?

Can siblings’ ancestral origins be different? The surprising answer is yes! Imagine that a small percentage, say 10 percent, of a woman’s DNA contained genetic markers indicating Mongolian ancestry. Because each egg a woman produces only carries half her DNA only some will carry Mongolian markers. Therefore, one sibling could show Mongolian ancestry, while another doesn’t.

OUR POSSIBLY HAIRY PAST

What’s one of the most intriguing secrets genetic testing can reveal? Just how much of a Neanderthal you really are! Our smaller, sturdier cousins’ genome was published in 2010, based on some very well-preserved DNA taken from bones found in a Croatian cave. The evidence suggests that early humans mated with Homo neanderthalensis. Thanks to DNA testing, we can find out just how cozy our distant relatives were with Neanderthals. The average person is about five percent Neanderthal.

Today’s technology makes it possible for the curious to shed light on their DNA. The databases of genetic markers are growing apace, making it easier to uncover long-lost relatives, for better or for worse. So how about it? Are you ready to find out who you really are, biologically speaking?
MEATLESS MEAT: BIOTECH BURGER AIN’T NO BEAN PATTY

MISSION IMPOSSIBLE: MEATLESS MEAT?
Imagine biting into a juicy cheeseburger: the flavor, the texture, the smell. Now, imagine the cheeseburger meatless. Impossible? No. Impossible Foods, a Redwood City, CA-based company has used biotechnology to create a plant-based burger amazingly similar to the bovine original. In this edition of the Biotech Primer Weekly, we examine how they did it.

UNCOVERING THE MYSTERY OF MEAT
Impossible Foods founder Patrick Brown knew he needed to understand America’s favorite sandwich at its most basic level. Discovering the specific molecules that imbue hamburgers with their unique deliciousness, he reasoned, would enable him to build a kinder, more planet-friendly burger.

So how to get at what makes a burger a burger?
Step one: acquire hamburger meat.
Step two: cook. Researchers at Impossible Foods heated ground beef, essentially vaporizing it into a usable sample. These gaseous burger components include some of the same molecules responsible for those mouth-watering smells that waft through neighborhoods and parks in warm weather.

Step three of the molecular dissection is a little more complicated than meat and heat. It requires a gas chromatogram mass spectrometer, which is really two gadgets in one. Not standard kitchen equipment.

Chromatography is the process of separating components of a mixture. Burger “gas,” like the earth’s atmosphere, is composed of molecules with different physical and chemical properties. Think different sizes, shapes, and charges. Scientists pass the burger fumes through a long, narrow tube that contains a material that various molecules “stick to” to a greater or lesser degree, based on their makeup. The assorted molecules leave the tube at different rates, separating.

The exiting molecules then move on to the spectrometer which “ionizes” or gives them an electric charge. Negatively charged molecules then pass through a magnetic field toward a negatively charged panel. Molecules with different masses travel at different rates, creating a graph or mass spectrum. Researchers compare their speeds to a baseline spectrum, which allows them to detect the molecules in their sample burger.

THE HAMBURGER MOLECULE
One key molecule Impossible Foods scientists identified as responsible for a hamburger’s meaty flavor is heme. This iron-carrying molecule is associated with one of the major muscle proteins, myoglobin. Since meat is pretty much muscle, it’s no surprise that it’s pretty much heme that accounts for meat’s taste.

Heme isn’t merely some muscle-bound molecule. It’s also found in the roots of soy plants, attached to the protein leghemoglobin. Soy contains far less heme than cow muscle. That’s why soy-based veggie burgers lack that signature meaty taste. Soy contains so little vegetarian heme that using the plant to meatify veggie burgers just won’t work.

There’s more than one way to skin a soybean though—those savvy Impossible Foods people genetically engineered yeast cells to contain the soy leghemoglobin gene. Voila! A sustainable, high-volume production platform for leghemoglobin is suddenly possible. Heme is harvested from the yeast to become a major ingredient the secret Impossible Burger recipe. The recipe also includes wheat and potato proteins for texture, and coconut oil as a stand in for fat.

ENGINEERING THAT BLOODY TASTE
Impossible Foods set out to recreate not only the taste, but other parts of the burger-eating experience. That leads to the question: does a medium rare Impossible Burger ooze a little “blood?”

Of course. The faux blood looks strikingly similar to the real thing. That’s because heme not only makes meat taste meaty, it makes blood red. Heme binds iron, which in turn binds oxygen. The chemical bond between iron and oxygen reflects light so that it appears crimson.

While the editorial staff of the Weekly has yet to sample the Impossible Burger (hint hint, Dr. Brown), eater-on-the-street reports are favorable. How does it stack up nutritionally? Protein, fat, and iron content are comparable to a lean ground beef patty – with the added benefit of zero cholesterol. The Impossible Burger
is currently available in restaurants at select locations around the country.

**BEYOND IMPOSSIBLE: LAB-GROWN MEAT**

But wait, there’s more! Another type of biotech burger is on the horizon: lab-grown meat. Basically, researchers extract stem cells from cow muscle and grow them in the lab.

This approach to making “cultured” or “clean” meat still has a way to go before it lands on your dinner plate. Growing cells in the lab isn’t cheap. Similar to animals, cultured meat cells need care and feeding. The health, safety and technological requirements of culturing meat result in great expense. One pound of lab-grown beef runs thousands of dollars. Miraculously, Kobe beef seems almost affordable.

Several companies are working to make lab-grown beef cheaper and easier to produce. These include **SuperMeat** (Tel Aviv, Israel); **Mosameat** (Maastricht, The Netherlands); and **Memphis Meats** (San Francisco, CA). Memphis Meats expects to have a supermarket-ready product by 2021; Mosameat estimates a similar timeline.

Going vegetarian can be tough, but many find it a pathway to better health. Moreover, beef production uses significantly more natural resources than the equivalent amount of veggie or cultured meat. And that’s not to mention all that veggie methane. Global livestock production accounts for about 15% of this greenhouse gas, which contributes to global warming. For those who want to cut back or eliminate meat consumption, but still crave the taste of a good old-fashioned burger, biotechnology just might have the answer.
Alzheimer’s Disease: A Tough Nut To Crack

AFFECTING 5.1 MILLION

Alzheimer’s disease (AD) ranks as one of the toughest nuts to crack within drug discovery and development. Current treatments merely manage symptoms, so finding a better solution becomes more and more urgent as the aging population grows.

Approximately 70 percent of dementia cases are caused by AD. It is a neurodegenerative disorder—neurons progressively lose structure and function. As the disease continues and more neurons are damaged and die, symptoms get worse. Neurons in the hippocampal region of the brain associated with memory formation are among the first affected. By 2025, the number of people age 65 and older with AD is projected to reach 7.1 million—a 40 percent increase from the 5.1 million affected in 2015 (Alzheimer’s Association).

A number of different companies are working to develop treatments, with a few already in clinical trials. In this two-part series, we’ll first explain what is known about the molecular causes of AD, then explore Alzheimer’s drugs that are in development.

PLAQUES AND TANGLES

PLAQUES

Alzheimer’s disease is associated with the build-up of amyloid-beta (Aβ) plaques in patients’ brains. But what, exactly, are Aβ plaques? Aβ plaques derive from the cleavage of a protein called the amyloid precursor protein, which is thought to play a role in the formation of synapses. Individual Aβ molecules clump together to form the plaques associated with Alzheimer’s.

Until recently, the mechanism by which Aβ plaques might cause Alzheimer’s was not known. Recent research from Stanford University suggests the plaques bind to a receptor on nerve cells, disrupting their function. However, there is no absolute consensus on whether these clumps of protein are the origins of AD or a symptom of the underlying cause.

TANGLES

Another brain protein associated with Alzheimer’s is tau, which is concentrated in the neurons and is primarily understood as a component in stabilizing nerve cell structure. Abnormal aggregates of tau form “tangles” within nerve cells. These tangles are correlated with the onset of Alzheimer’s along with the characteristic plaque formations.

GENETICS PLAYS A ROLE

About 70% of Alzheimer’s cases are thought to have at least some genetic association, with different genes being implicated depending on the type of Alzheimer’s.

A gene found on chromosome 19 called the apolipoprotein E gene (APOE) influences the development of late-onset Alzheimer’s. Individuals with different variants of the APOE gene have different risk profiles:

- Variant ε2 (APOE2) is rare and possibly lessens or delays Alzheimer’s onset
- Variant ε3 (APOE3) is neutral
- Variant ε4 (APOE4) is associated with a significantly increased risk of Alzheimer’s

The APOE proteins plays a role in clearing Aβ from the brain, with APOE4 carrying out this function less efficiently than the other variants. There is also some evidence that APOE4 contributes to the breakdown of the blood-brain barrier seen in patients, resulting in increased brain inflammation—another marker of Alzheimer’s. A better understanding of APOE4’s role in Alzheimer’s onset may lead to the development of a whole new class of drug.

IF AT FIRST YOU DON’T SUCCEED...

A number of drug developers have attempted to use monoclonal antibodies (mAbs) to disrupt the formation of the AD-associated Aβ plaques. Unfortunately, this approach has yet to experience clinical success. However, this doesn’t mean that the approach is not viable – different mAbs attach to different parts of Aβ, meaning that the outcome of one mAb trial does not necessarily predict the outcome of another. Biogen (Cambridge, MA) and Roche (Basel, Switzerland) both
have mAb therapies targeting Aβ plaques in Phase III clinical development. In earlier phase studies, these mAbs showed the most promise in patients with less advanced forms of the disease.

The prevention of Aβ plaques is also the focus of growing interest in creating an AD vaccine. Leading this effort is Novartis (Basel, Switzerland), whose CAD106 vaccine contains fragments of the Aβ protein and has been shown to be safe in Phase I trials. The goal of the vaccine is to activate an immune response against Aβ, thereby reducing its accumulation and potential to form plaques in the brain. CAD106 is currently in Phase II/III studies of efficacy, in which the vaccine is being tested in cognitively normal individuals between the ages of 60 and 70 who are at high risk of developing the disease based on their APOE4 status.

UNTANGLING TAU

AbbVie (North Chicago, IL) is targeting tau, the other major protein associated with AD. The company currently has an anti-tau mAb therapy in Phase II clinical testing, and last month announced a partnership with Voyager Therapeutics (Cambridge, MA) to develop a gene therapy treatment that targets tau. The treatment will deliver a gene encoding an anti-tau antibody directly to cells in patients’ brains – enabling those cells to make the antibody. If successful, this would bypass altogether the blood-brain barrier that makes it difficult for some drugs to even enter the brain.

THE FUTURE?

Amyloid-beta and tau remain tantalizing targets for AD drug development because of their close association with the disease. However, new approaches are springing up as frustration over the lack of progress in treating this devastating disease grows. Stay tuned for the next Weekly, where we will describe some of these new approaches.
Exploring Different Strategies to Fight Alzheimer’s

TAKE THAT, ALZHEIMER’S

Alzheimer’s pernicious amyloid-beta plaques and tau tangles, discussed last week, remain important targets for the biotech industry. In the past few years, however, companies have begun to search more broadly for new treatments. This Weekly looks at products in development that use different strategies to fight this heartbreaking illness.

REVIVING THE BRAIN?

Loss of neurons is Alzheimer’s signature, devastating effect. What if we could jump start the development of new brain cells? Two companies are trying to do just that.

Neurotrope Biosciences (New York, NY) is developing bryostatin, a drug that activates protein kinase C epsilon (PKCε). This protein plays a key role in forming memories. In animal models of stroke, traumatic brain injury, and Alzheimer’s disease, bryostatin appears to restore deficits in synapses (connections between brain cells) and decrease cell death. These results suggest that bryostatin could help to prevent the loss of neurons and restore and generate new synapses.

Phase II clinical studies of late-stage Alzheimer’s patients demonstrated improved cognitive function as measured by the Severe Impairment Battery Scale (SIB), a standard tool for evaluating treatment response in advanced Alzheimer’s. Their improvement was greater than that seen in patients given the placebo, but the difference was not statistically significant. A Neurotrope Biosciences spokesperson says that it considers the Phase II study exploratory, designed to determine correct dosing. The company is planning a larger confirmatory trial in hopes of demonstrating statistically significant efficacy.

Neuronascent (Clarksville, MD) also aims to develop small molecule activators of neurogenesis. By screening large chemical libraries, the company has identified compounds that show promise of sparking neurogenesis from adult neural stem cells in both tissue culture and mouse models.

The company’s lead compound, NNI-362, promoted the growth of new hippocampal neurons in mice. The new cells migrated to the correct location and differentiated. Moreover, they survived long enough to reverse previously observed cognitive declines. The hippocampus is one of the first regions of the brain to show damage in AD and is thought to play a role in memory formation and spatial navigation. Neuronascent is preparing for Phase I trials of NNI-362.

NEUROINFLAMMATION

Neuroinflammation is one of the drivers of neurodegeneration in Alzheimer’s disease, multiple sclerosis and other brain disorders. Research conducted at Stanford University (Palo Alto, CA) suggests that the protein c1q is present at higher levels in people with Alzheimer’s disease. C1q accumulates at neuronal synapses, the key points of communication between brain cells. This protein also signals other immune cells, such as macrophages—which then chomp up cellular debris present in affected brains. The accumulation of c1q could account for the loss of synapses and accompanying mental decline.

South San Francisco-based Annexon is working on a promising therapy that centers on controlling inflammation in the brain. ANX005, now in preclinical development, is a monoclonal antibody that mops up excess c1q.

Another company homing in on neuroinflammation is vTv Therapeutics (High Point, NC). Their drug, Azeliragon, now in Phase III clinical development, is a small molecule inhibitor of the receptor for advanced glycation endproducts (RAGE). RAGE is present on many neurological cell types. Its activation may promote amyloid-beta production and transport, tau aggregation, and chronic inflammation. Preventing any of these developments could improve Alzheimer’s symptoms.

AND THAT AND THAT AND THAT!

Rather than target Aβ plaques directly, Yumanity Therapeutics (Cambridge, MA) is trying to identify the problems they cause. Yumanity scientists have engineered yeast cells to overproduce the Aβ protein and monitor its detrimental effects, such as disrupting the action of other important cellular proteins.

Surprisingly, yeast share many molecular pathways with humans. This similarity means researchers can use the little fungi to screen for potential drugs that address
protein disruption. Promising candidates are then tested in Alzheimer’s patient-derived cells. By tackling a completely different disease mechanism, the new compounds may achieve greater success than seen so far with drugs that act directly on amyloid beta or tau. Yumanity is currently in the lead-optimization phase of pre-clinical development.

In partnership with Biogen (Cambridge, MA), Cambridge-based Proteostasis Therapeutics is targeting AD-associated protein aggregates by activating proteasomes. These cellular components get rid of damaged proteins and dysfunctional protein aggregates by dismantling their chemical bonds. The protein USP14 inhibits proteasomes. Proteostasis is working on the preclinical development of a USP14 inhibitor that allows proteasomes to fully activate in AD patients. This makes it more likely that the proteasomes will recognize and destroy amyloid plaques and tau tangles.

Oryzon Genomics (Barcelona, Spain) is taking an epigenetic approach to Alzheimer’s. Epigenetic modifications are chemical changes to gene sequences that don’t change the information content but instead affect how much that content is used – in other words, the amount of a particular protein that the body makes.

Oryzon researchers identified an enzyme, lysine-specific histone demethylase 1 (LSD1), which makes epigenetic modifications to genes that results in “turning them down” so they produce less of the corresponding protein. LSD1 makes these changes to genes that support neuronal survival. Oryzon scientists have designed a drug, ORY-2001, that inhibits LSD1. Inhibiting LSD1 could mean that more neurons survive in AD patients, leading to improved cognitive function. ORY-2001 recently entered Phase II clinical trials.

A TWIST ON TAU

Finally, the elusive AD treatment may lie in pursuing a well-established target after all—but at a new angle. That’s where Ionis Pharmaceuticals (Carlsbad, CA) is headed in the Phase I/IIA clinical studies of their drug, IONIS-MAPT. This antisense drug targets the source of the tangles associated with AD. Like other antisense drugs, IONIS-MAPT destroys tau mRNA, thereby diminishing tau protein production.

It’s encouraging to know how many therapies are in the Alzheimer’s treatment pipeline. With more hard work and investment, perhaps one of the many introduced above will lead to a cure.
THE LATEST IN CANCER DIAGNOSTICS

Hearing the words “it might be cancer” paired with your doctor’s perplexed look is enough to send shock waves through your body. Getting to the heart of a diagnosis usually requires a surgical biopsy—removal and examination of the suspected tissue for visible signs of cancer.

Less invasive diagnostic tests—called liquid biopsies—might just bring more choices to doctors and patients. They are becoming today’s reality thanks to our ability to isolate molecules from body fluids. These diagnostic innovations pair technology with the latest in biomarkers, and are rapidly gaining acceptance as a reliable way to screen for cancer and to monitor disease progression and response to treatment. This week we’ll examine the different types of liquid biopsies and how they work.

TERM OF THE WEEK: LIQUID BIOPSY

A liquid biopsy is a test that is able to detect the presence of cancer using blood, urine, saliva, or other bodily fluid as the sample rather than tissue from a specific organ. The technique is possible because cancerous tissues shed cells, DNA, and tiny lipid-encased compartments called exosomes. Liquid biopsies detect the presence of these cancer-associated biomarkers.

DISCOVERY BY CELL-FREE DNA

When cells in the body die, they release cell-free DNA (cfDNA)—this includes dying tumor cells. cfDNA-based tests are a type of liquid biopsy because they seek out a biomarker—in this case, tumor DNA—in body fluids and then identify cancer-specific mutations using PCR or next-generation sequencing analysis.

Troagene (San Diego, CA) analyzes cfDNA found in urine samples, which patients collect at home. Currently, Troagene has tests detecting mutations associated with melanoma, colon cancer, and non-small cell lung cancer, as well as the presence of viral DNA for the diagnosis of human papilloma virus. Exact Sciences Laboratories (Madison, WI) uses at-home collection in their colon cancer test, which analyzes cfDNA in stool samples for cancer-associated DNA.

Genomic Health (Redwood City, CA) currently markets tissue-based genomic tests for the detection and classification of breast and prostate cancer, and is developing cfDNA-based tests for breast (blood sample) and bladder (urine sample) cancers.

Qiagen (Hilden, Germany) is developing cfDNA liquid biopsy diagnostics in partnership with pharmaceutical companies such as AstraZeneca (London, U.K.), Tokai Pharmaceuticals (Boston, MA), Novartis (Basel, Switzerland), and Eli Lilly (Indianapolis, IN).

EXTRACTING EXOSOMES

Exosomes are lipid-encased vesicles that contain cellular protein, DNA, and RNA and typically have surface proteins specific to their native cell. These attributes, combined with the fact that they are found in many different body fluids, make exosomes a very attractive possibility for liquid biopsy. The idea is to capture exosomes based on tumor-specific surface markers or to collect exosomes and identify them as cancer-associated by examining the enclosed DNA or RNA.

Aptly named Exosome Diagnostics (Cambridge, MA) has an exosome-based urine and blood tests for prostate cancer on the market, and another in development for lung cancer. Qiagen has a partnership with Exosome Diagnostics to help develop additional exosome-based cancer diagnostics to complement their work with cfDNA.

CONSIDER CIRCULATING TUMOR CELLS

The final category of liquid biopsy is perhaps the most obvious—circulating tumor cells (CTCs), or cells splintered from a tumor and circulating in the bloodstream. The challenge lies in detecting CTCs: some estimates classify them as rare as one circulating tumor cell per billion normal cells!

Janssen Diagnostics (Raritan, NJ) currently markets CellSearch, the single FDA-approved test that allows physicians to identify early CTCs from blood samples. Monoclonal antibodies (mAbs) capable of recognizing proteins on the surface of migrating tumor cells are chemically linked to magnetic nanoparticles and then added to a patient’s blood sample. These tumor-specific mAbs grab hold of the CTCs, and a strong magnetic field...
is then applied to the sample, isolating the captured cells for identification and analysis. CellSearch is currently used to monitor the efficacy of treatments for breast, prostate, and colorectal cancer. A higher number of CTCs detected may indicate a higher incidence of metastasis, or a less than effective treatment route if used to quantify cancer therapy success.

Another way to identify CTCs may be cell size—CTCs tend to be significantly larger than other cells in the blood, and this size differential may be exploited in a microfluidics-based approach to cell separation. Researchers at National University in Singapore (Singapore) and MIT (Cambridge, MA) have developed a microfluidics chip that routes cells from a blood sample into different channels based upon cell size. Although still in the preclinical research phase, this approach shows promise for capturing a wide range of CTCs.

**Epic Sciences** (San Diego, CA) adopts a “no cell left behind” game plan thanks to technology developed by the Scripps Research Institute (La Jolla, CA). Automated fluorescence-microscopy identifies the CTCs in blood samples placed on microscope slides. A detailed analysis of three million cells per slide is performed, each blood sample yielding approximately twelve slides. This technology may potentially hone in on the presence of a single CTC. Epic Sciences’ CTC-detecting platform is used by Genomic Health in their Oncotype-Dx AR-V7 Nucleus Detect test, which determines whether or not prostate cancers patients’ tumors have developed mutations that make them resistant to common types of treatment.

**THE FUTURE**

Ultimately, the best liquid biopsies may contain a combination of all the above approaches. **Biocept** (San Diego) is leading the way by developing liquid biopsies that analyze both cfDNA and CTCs. Biocept currently markets liquid biopsy tests for the detection of lung cancer, breast, colorectal, gastric, prostate, and melanoma. Biocept also has its eye on combination liquid biopsies for both colon cancer and melanoma.

Today, liquid biopsies are mainly used for monitoring the progress of or response to treatment of already-diagnosed cancers rather than as initial diagnostic tests. A major goal in the field is to develop tests that can be used routinely to detect cancer in seemingly healthy people, which should translate to better treatment outcomes. Research published by a group at **Johns Hopkins University** (Baltimore, MD) in January 2018 suggests that a liquid biopsy test that detects both cancer-associated cfDNA and proteins known to be characteristic of certain types of cancer may be better at detecting cancer early on than those that look at just one biomarker. Dubbed Cancer-SEEK, the tool is capable of detecting a number of different cancers, including ovary and liver, and may soon begin testing as a screening tool.

As the technologies to detect cfDNA, CTCs, and cancer-specific exosomes progresses, we can expect to see an increasing number liquid biopsies available, making the detection and treatment of a range of cancers less invasive and more manageable.
PROTEASOMES TO THE RESCUE

Many drugs work by stopping overactive proteins that cause disease. The leukemia drug Gleevec, for example, is a small-molecule inhibitor (antagonist) of the protein Bcr-Abl, whose over-activity promotes excessive cell division. Humira treats a range of autoimmune diseases by stopping TNF-alpha, a protein that activates inflammation.

Such antagonists can be powerful. However, it’s not always possible to develop a strong inhibitor of a disease-associated protein. And when scientists do develop them, resistance often emerges, rendering a drug ineffective.

What if, instead of merely inhibiting a protein, we could totally get rid of it? It turns out that our cells already have that ability. In this Biotech Primer WEEKLY, we look at a new class of drugs scientists are developing to take advantage of our bodies’ microscopic sanitation departments.

A CELLULAR GARBAGE DISPOSAL

If allowed to accumulate, proteins can interfere with normal cell function. Therefore, all cells contain proteasomes, compartments that break apart unneeded or damaged proteins. Proteasomal degradation also provides a way to recycle the amino acid building blocks of proteins. Once a protein is broken down, a cell can use the leftover amino acid “bits” to rebuild new proteins.

Proteins are targeted for degradation through the action of E3 ligase. This enzyme attaches another protein, ubiquitin, to the targeted protein. Ubiquitin then guides the target into a proteasome, where it’s broken down. If scientists figure out how to “tag” disease-associated proteins with ubiquitin, they can activate our cellular garbage disposal to fight illness. Several companies are working on clever ways to do just that.

SMALL MOLECULES, BIG RESULTS?

Researchers at Arvinas (New Haven, CT) are developing a platform to target disease-causing proteins based on ubiquitination/proteasome systems. Dubbed PROTAC (Proteolysis-Targeting Chimera), the platform consists of “bifunctional small molecules” – which simultaneously bind to two different proteins.

With PROTAC, one end binds to the target, the other to E3 ligase. This interaction transfers ubiquitin to the target protein for eventual disposal. PROTAC doesn’t necessarily have to recognize a specific part of the target, such as the active site of an enzyme. That allows researchers to focus on a wider range of proteins than possible with existing technologies, such as small molecule inhibitors, which must fit precisely in an enzyme’s active site to work.

Arvinas has released preclinical data suggesting that PROTAC successfully lowers levels of the protein BRD4 in lymphoma, multiple myeloma, and prostate cancer cells. BRD4 plays a role in cell division, and mutated versions are associated with various cancers. In the past year, Arvinas announced collaborations with Pfizer and Genentech, which should speed the progress of getting these molecules to the clinic.

C4 Therapeutics (Cambridge, MA) is developing a similar small molecule platform that connects disease-associated proteins with cellular ubiquitination enzymes. Dubbed “degronimids,” the molecules are still in preclinical development. They have already attracted the attention of Google-backed Calico, with whom C4 recently signed a five-year deal to work together to treat diseases of aging.

Kymera (Cambridge, MA) is also utilizing small molecules to activate target-specific proteasome degradation, focusing first on oncology and autoimmunity. In March, Celgene (Summit, NJ) announced a project with Vividion (La Jolla, CA) in the hopes of discovering ubiquitin-proteasome system interacting drugs.

NOW WAIT A SECOND…

It’s clear that many drug developers place great faith in tapping proteasome power to advance human health. In what could seem completely contradictory, other companies are taking the opposite approach: squelching the proteasome.

The process of apoptosis, or programmed cell death, occurs naturally in cells as a protective mechanism. For example, cells that sustain large amounts of damage to
DNA activate apoptosis to prevent them from seeding a tumor.

Many pharmaceutical companies are trying to co-opt apoptosis to treat cancer. One way to induce the process is to inhibit the action of proteasomes. The resulting buildup of damaged proteins signals the cell that something is seriously amiss, setting off cell death.

The FDA has approved Velcade, marketed by Millennium Pharmaceuticals (Cambridge, MA), to treat multiple myeloma. Krypolis, developed by Onyx (South San Francisco), is another proteasome inhibitor approved as a second-line treatment for multiple myeloma. Both are small molecule drugs.

A better understanding of how our cells process unwanted proteins has opened up an entirely new approach to treating diseases. Manipulating the world’s tiniest garbage disposals may hold the key to healing otherwise untreatable conditions.