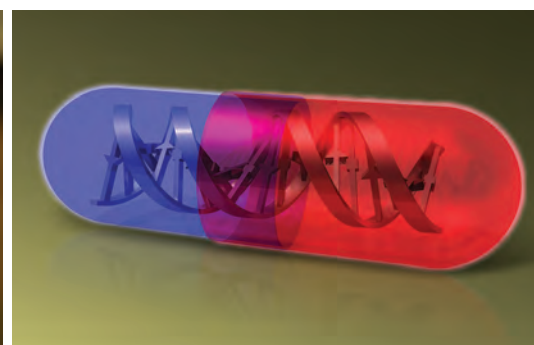
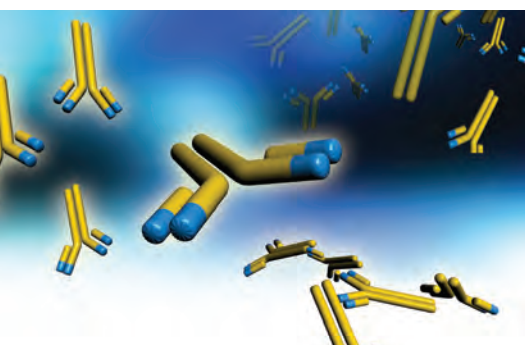


# Biopharmaceutical Chemistry Research & Development: New Studies of Biologic Drugs, Therapies, and Biomedical Analysis



*A white paper examining the latest research on using biologics to develop new drugs, vaccines, and gene therapies in the quest to realize the promise of personalized medicine.*



**ACS**  
Chemistry for Life™

# Biopharmaceutical Chemistry Research & Development: New Studies of Biologic Drugs, Therapies, and Biomedical Analysis

## TABLE OF CONTENTS

<b>I. INTRODUCTION</b>	<b>2</b>
<b>II. ANTIBODY THERAPEUTICS</b>	<b>4</b>
<b>Antibody-Drug Conjugates</b>	<b>5</b>
<b>Bispecific Antibodies</b>	<b>8</b>
<b>III. NUCLEIC ACID THERAPEUTICS</b>	<b>10</b>
<b>Antisense RNA Therapeutics</b>	<b>11</b>
<b>The Next Generation</b>	<b>12</b>
<b>Targeting Non-Coding RNAs</b>	<b>13</b>
<b>RNA Interference</b>	<b>13</b>
<b>IV. GENE THERAPY &amp; GENOME EDITING</b>	<b>15</b>
<b>Gene Editing</b>	<b>17</b>
<b>V. STEM CELL-BASED THERAPEUTICS</b>	<b>19</b>
<b>Human Embryonic Stem Cell Therapies</b>	<b>20</b>
<b>Induced Pluripotent Stem Cells</b>	<b>21</b>
<b>VI. CONCLUSION</b>	<b>22</b>
<b>Regulatory and Safety Concerns</b>	<b>23</b>
<b>VII. REFERENCES</b>	<b>24</b>

## ABOUT THIS REPORT

This report is for exclusive use by members of the American Chemical Society. It is not intended for sale or distribution by any persons or entities. Nor is it intended to endorse any product, process, organization, or course of action. This report is for information purposes only.

© 2014 American Chemical Society

## ABOUT THE AUTHOR

Jeffrey Perkel has been a scientific writer and editor since 2000. He holds a PhD in Cell and Molecular Biology from the University of Pennsylvania, and did postdoctoral work at the University of Pennsylvania and at Harvard Medical School.

## I. INTRODUCTION

---

Not so long ago, drug developers dealt largely with relatively small organic molecules that agonized receptors, triggered cascades, and blocked enzyme activity. Today, a considerable fraction of drug development concerns “biologics,” therapeutics extracted from or produced in living cells, such as enzymes, antibodies, and even the cells themselves. (Though not technically biopharmaceuticals per se, as they usually are chemically synthesized, we also include nucleic acid therapeutics in this category for the purposes of this report.)

Leveraging the exquisite selectivity of an antibody for its antigen, or a nucleic acid for its sequence complement, biologics promise a molecular specificity that small organics cannot match. With molecular specificity come reduced side effects and the potential for more targeted and “personalized” medicine, tantalizing benefits as the average cost of developing a successful drug soars past the billion-dollar mark.

But developing such therapeutic agents isn’t easy. Whereas organic molecules are relatively small entities created by chemical synthesis, biologics are larger and far more chemically complicated. They generally are produced in either bacterial or eukaryotic cells, and often are chemically or post-translationally modified, complicating both production and quality control.

Still, the biologics market is enormous, and growing. According to a 2014 report in *Nature Biotechnology*, “In 2012, the US biotech sector grew at a high double-digit rate,” with total sales of \$63.6 billion, “an 18.2% increase over 2011 sales;” by comparison, the pharmaceutical sector overall expanded just 2.5% that same year.<sup>1</sup>

By far, the lion’s share of the biologics market belongs to therapeutic antibodies – molecules like Herceptin (trastuzumab), a breast cancer therapeutic, and Remicade (infliximab), an anti-inflammatory molecule that targets tumor necrosis factor-alpha. According to a 2013 report from the Pharmaceutical Research and Manufacturers of America (PhRMA), an industry group, “America’s biopharmaceutical research companies are using biological processes to develop 907 medicines and vaccines targeting more than 100 diseases,” the vast majority either monoclonal antibodies (338) or vaccines (250).<sup>2</sup>

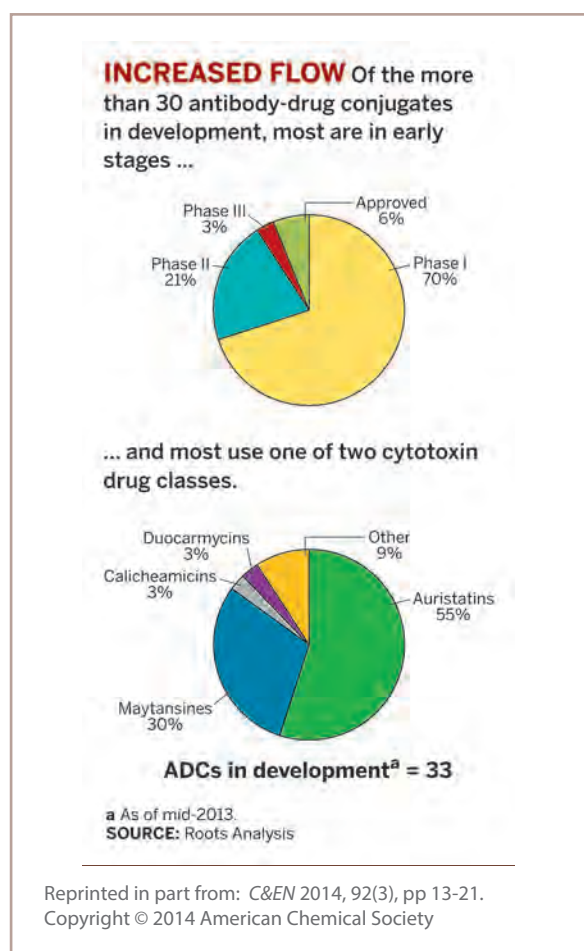
A report from 2012 predicted that antibody therapeutic sales would nearly double, from 22 billion euros (\$32 billion) in 2008 to nearly 40 billion euros (\$58 billion) in 2014.<sup>3</sup> In 2012, monoclonal antibody therapeutics were “the highest selling class of biologics,” with U.S. sales of approximately \$24.6 billion, about 38.5% of the total biologics market.<sup>1</sup> Hormones were the next-best sellers, with 2012 sales of \$16.1 billion, followed by growth factors, (\$8.1 billion), fusion proteins (\$5.8 billion), and cytokines (\$4.9 billion).<sup>1</sup>

Traditionally, monoclonal antibody (mAb) therapeutics operated by relatively straightforward mechanisms, impacting biological processes by agonizing or antagonizing cell surface receptors, tagging cells for immune targeting, or neutralizing growth factors. Today the market is diversifying, with traditional mAbs now competing with more sophisticated designs, including antibody-drug conjugates (ADCs) and bispecific antibodies. The latter produces a chimeric molecule capable of targeting two distinct molecules, for instance to link an immune cell to a cancer cell. The former couples potent toxins to antibodies, producing what often is referred to as a “guided missile.” Though only two have been approved by the U.S. Food and Drug Administration (FDA) – Seattle Genetics’ Adcetris (for lymphoma) and Roche’s Kadcyla (for breast cancer) – the market is growing, and fast. There were some 30 ADCs in clinical trials as of 2013, “accounting for around 15% of the clinical-stage anticancer antibody-based pipeline and outnumbering other modified mAbs such as bispecifics and fragments,” according to one report.<sup>4</sup>

And new drugs, targets, and conjugation strategies continue to be developed. Backed by substantial investments in infrastructure and R&D – \$200 million from Roche to build a dedicated R&D facility, \$440 million from AstraZeneca to acquire Spirogen, and additional moves by Bayer HealthCare, Bristol-Myers Squibb, Novartis, and Pfizer<sup>5</sup> – ADC sales could top \$5 billion by 2018.<sup>6</sup> And the pipeline is rich, notes an industry analysis by *Chemical & Engineering News (C&EN)*, with “between 100 and 150” ADCs in preclinical testing.<sup>6</sup>

The biologics market isn’t limited to proteins, however. According to one 2013 report, “Two [antisense/RNA interference]-targeted therapeutics have received FDA approval, and there were more than 127 projects in the clinical research pipeline using this approach, including 12 projects in Phase 3 or later, with many more in preclinical research.”<sup>7</sup>

Some companies hope to capitalize on the pathway called RNA interference (RNAi), while others develop antisense nucleic acids. Genzyme and Isis Pharmaceuticals’ Kynamro (mipomersen sodium), for instance, is a “first-in-class” antisense oligonucleotide targeting the mRNA encoding apolipoprotein B, approved by FDA for use in patients with homozygous familial hypercholesterolemia.<sup>8</sup> Other companies target the class of regulatory non-coding RNAs called microRNAs. Regulus Therapeutics’ RG-101, for instance, which targets the abundant hepatocellular microRNA, miR-122, currently is in Phase 1 testing for treatment of hepatitis C virus infection.<sup>9</sup>



Another biologics strategy uses cells themselves as therapeutic entities; according to PhRMA, some 69 such therapies are currently in the pipeline.<sup>2</sup> Geron was first out of the gate, with human embryonic stem cell (hESC)-derived oligodendrocyte progenitor cells for spinal cord injuries, but has since abandoned its efforts.<sup>10</sup> But Advanced Cell Technology remains committed to the approach, with Phase I trials ongoing for dry age-related macular degeneration and juvenile macular degeneration (Stargardt disease), using hESC-derived retinal pigment epithelial cells. “Adult” stem cell therapies are also in development, including StemCells Inc.’s HuCNS-SC human neural stem cell preparation for spinal cord injury. And now Japanese researchers have kicked off the world’s first clinical trial based on induced pluripotent stem (iPS) cells, also targeting age-related macular degeneration.<sup>11</sup>

But perhaps some of the biggest excitement in the biologics space these days involves genome editing, a gene therapy (and even cell therapy) application that promises to repair damaged genes rather than replace them. Editas Medicine, founded by some of the key innovators of CRISPR/Cas and TALEN technology, launched in November with \$43 million in venture capital funding.<sup>12</sup> Another startup, CRISPR Therapeutics, announced \$25 million in funding this past April.<sup>13</sup> Sangamo BioSciences, a company developing therapeutics based on an alternative approach called zinc-finger nucleases (ZFNs), already has some of its designs in clinical trials; in early March, the company reported in the *New England Journal of Medicine* the use of ZFNs in a small cohort of HIV+ patients.<sup>14</sup>

Each of these approaches has its strengths and weaknesses. Genome editing seems an elegant and simple approach to personalized medicine, but the possibility of off-target effects – of unintentionally breaking one gene even as another is repaired – lingers. Embryonic stem cells can generate any cell type desired, but the mechanics of differentiating those cells into the desired cell types, not to mention moral and ethical considerations, shadow their use. iPS cells are largely free of such issues, but the molecular manipulations required to generate them – usually involving the introduction and expression of oncogenes – are likewise problematic. And despite their growing popularity, ADCs are exceptionally complex to manufacture. Still, the fact remains that in research, funding, and excitement, it’s a golden age for biologics drug development.

## ANTIBODY THERAPEUTICS

---

Nearly a century ago, Paul Ehrlich articulated the so-called “magic bullet” theory of cancer treatment,<sup>15</sup> an idea that has come to be embodied in today’s biotherapeutics arena. But it would be decades before monoclonal antibodies would be invented, and it was not until 1986 that muromonab-CD3, an immunosuppressant intended for transplant rejection, would win FDA approval, the first such drug to do so.

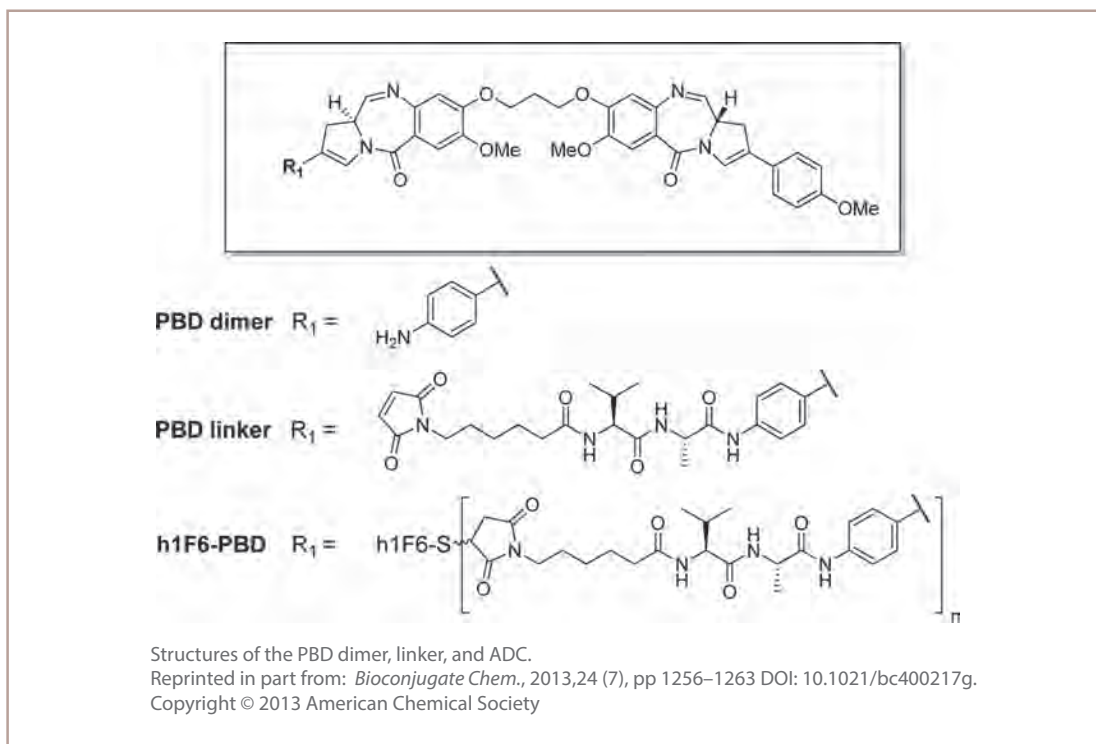
In the nearly three decades since, some 40 mAb therapeutics have come to market in the US.<sup>1</sup> Of those, Humira (adalimumab, a tumor necrosis factor-alpha [TNF $\alpha$ ]-targeted antibody for such autoimmune conditions as rheumatoid arthritis and psoriatic arthritis), Remicade (infliximab, also targeting TNF $\alpha$ ), and Rituxan (rituximab, which targets the B-cell receptor CD20 for rheumatoid arthritis, non-Hodgkin lymphoma, and chronic lymphocytic leukemia [CLL], among other conditions) are the biggest sellers, with 2012 sales of \$4.6 billion, \$3.6 billion, and \$3.5 billion, respectively.<sup>1</sup> These molecules tend to work in one of three ways. They either block or induce signaling through a cell-surface receptor – adalimumab and infliximab, for instance, bind up TNF $\alpha$  – or they decorate the cell to induce immune cell targeting. But when it comes to cancer, the goal is to kill targeted cells, and the problem is, what these molecules possess in specificity is rarely matched by cytotoxicity. In other words, the molecules can find their targets, but killing them is a tougher nut to crack.

Newly engineered molecules, though, may be more effective. One strategy is to alter the post-translation modifications – specifically, the glycosylation pattern – of therapeutic antibodies. “The presence of oligosaccharides attached at a single site on each of the immunoglobulin G (IgG) heavy chains is essential for the antibody’s effector functions, and efficacy can vary depending on the particular oligosaccharide that is attached,” writes Roy Jefferis of the University of Birmingham in a 2009 review on the topic.<sup>16</sup> Genentech’s “glyco-engineered” Gazyva (obinutuzumab), a successor to Rituxan for CLL, received FDA approval in November 2013.<sup>17</sup>

## ANTIBODY-DRUG CONJUGATES

Another approach is to couple the antibody to a potent drug molecule that can kill or poison the bound cell – that is, to arm the antibody “guided missile” with a “warhead.” There were some 30 so-called “antibody-drug conjugates” (ADCs) in clinical trials as of 2013, “accounting for around 15% of the clinical-stage anticancer antibody-based pipeline and outnumbering other modified mAbs such as bispecifics and fragments,” according to one report.<sup>4</sup> Two have been approved by the FDA – Seattle Genetics’ Adcetris (brentuximab vedotin, targeting CD30 for lymphoma) and Roche’s Kadcyla, (a drug-conjugated version of the breast cancer antibody therapeutic, trastuzumab).

An ADC is basically the union of three molecules in one – an antibody, a toxin, and the linker that joins the two – and ADC development involves tweaking all three sides of that triangle. Take, for instance, the toxin itself. Kadcyla uses a maytansinoid compound, and Adcetris employs an auristatin, both of which inhibit cytoskeletal components called microtubules. But not every cell is sensitive to every compound, so many companies are exploring other toxin types, as well. One recent report in *Bioconjugate Chemistry*, coauthored by researchers at both Seattle Genetics and Spirogen, describes an anti-CD70 ADC conjugated to a pyrrolobenzodiazepine dimer (PDB), a DNA crosslinking agent.<sup>18</sup>



Spirogen develops PDBs, and licensed the technology to Seattle Genetics; AstraZeneca recently acquired the company via its MedImmune division.<sup>5</sup> Other in-development toxins include Synthron's DNA-binding duocarmycins;  $\alpha$ -amanitin, the toxin found in death cap mushrooms, which inhibits RNA polymerases, advanced by Heidelberg Pharma; and deBouganin, from Viventia Bio, which targets protein synthesis.<sup>6</sup>

A related, alternative strategy, called radioimmunotherapy, links the antibody to a powerful radioisotope, as is the case with yttrium-90 Zevalin [ibritumomab tiuxetan], a combination used in part to treat non-Hodgkin lymphoma. GlaxoSmithKline's Bexxar [iodine-131 tositumomab], also for non-Hodgkin lymphoma, was pulled from the shelves earlier in 2014 due to poor sales.<sup>19</sup>

Linkers, too, are critical and variable. Linkers must be stable enough to hold the toxin as the ADC moves throughout the body, thereby reducing systemic toxicity, but flexible enough to release it when needed. Some linkages, for instance, are labile to lysosomes; others respond to reducing conditions or enzymatic activity. Some remain locked in the cells to which they are delivered, others can cross a cell's membrane to poison its neighbors.<sup>20</sup>

Both Adcetris and Kadcylla use maleimide linkages, but these "aren't always desirable because they can break apart and recombine with reactive groups in other biomolecules in the body."<sup>21</sup> One recent study by researchers at the Scripps Research Institute describes a new linkage called a methylsulfonylphenyloxadiazole, which "complement[s] maleimides by producing a more stable protein-drug linkage."<sup>21</sup>

And then there's the question of just where the toxins conjugate to the antibody, and in what stoichiometry (a value termed "drug-antibody ratio," or DAR). The choice of binding location is

not trivial – the site must not interfere with antigen-antibody interactions, for instance, but must remain accessible for regulated cleavage. One study by researchers at Genentech demonstrates the importance of the conjugation site; the authors observed profound differences in *in vivo* efficacy depending on just how solvent-accessible the conjugation site happened to be.<sup>22</sup>

Seattle Genetics' chemistry tethers toxins to cysteine residues, while ImmunoGen targets lysines, but there are multiple such residues in an antibody molecule, meaning a conjugation reaction can produce a heterogeneous mix of products with different DARs. Yet it is critical for quality control and regulatory purposes that drug developers know, and be able to control, just what the DAR is, and to avoid both too few toxins and too many.

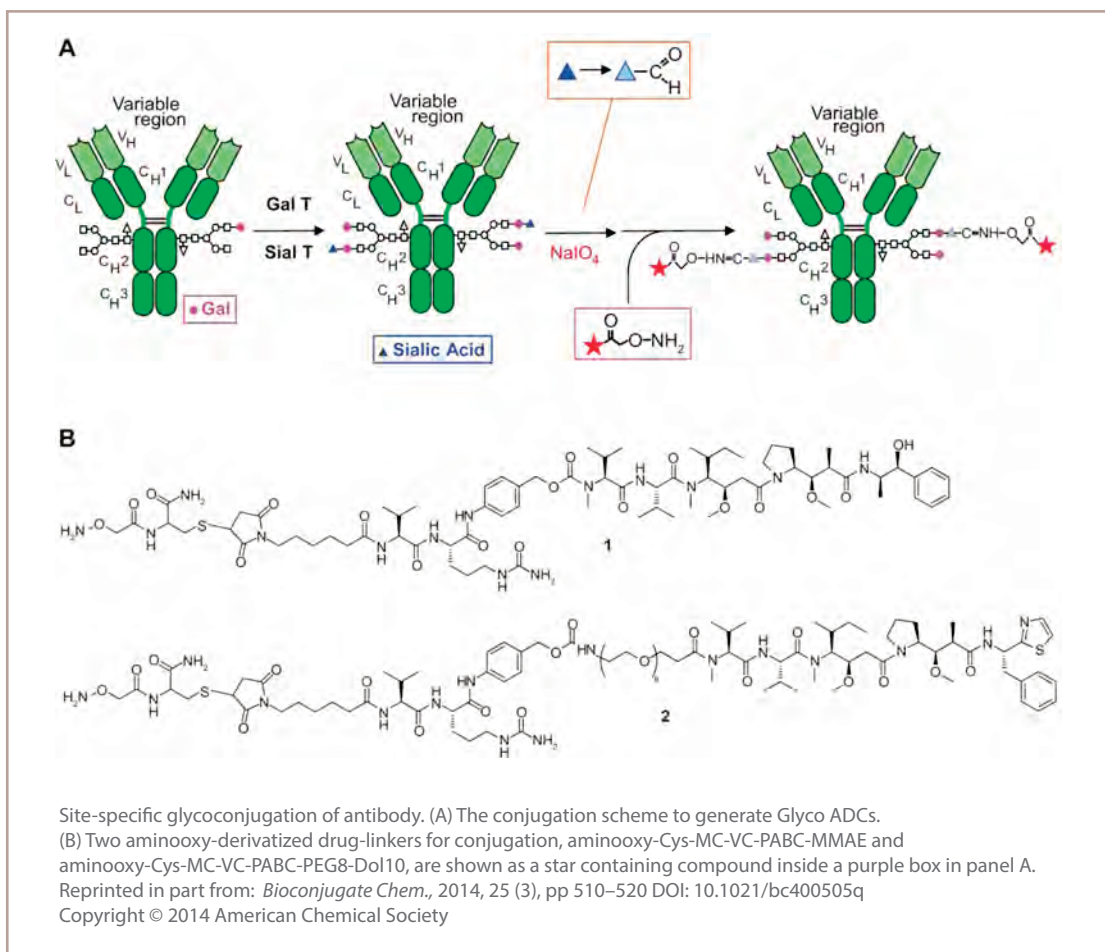
One alternative approach, advanced by Ambrx (founded by Peter Schultz of the Scripps Research Institute), leverages Schultz's work with incorporation of non-natural amino acids (such as *para*-acetylphenylalanine) in living cells to precisely control the site of incorporation.<sup>23</sup> These non-natural residues serve as precisely positioned chemical handles to which drug molecules can be linked. (Sutro Biopharma also pursues a non-natural amino acid-based approach, but in cell-free extracts.<sup>90</sup>)

Another strategy inserts an enzyme-reactive site into the protein sequence, as is the case with Redwood Bioscience's SMARTag technology, which uses a five-amino acid sequence recognized by a "formylglycine-generating enzyme," and Innate Pharma's method, which uses a "bacterial transglutaminase enzyme."<sup>24</sup> In both cases, enzyme activity creates a reactive group that can be exploited for controlled linker conjugation. Mersana Therapeutics is pushing another strategy, called "Fleximers." A Fleximer is a biodegradable polymer conjugated to multiple drug molecules to "dramatically improve solubility, improve pharmacokinetics, reduce immunogenicity and optimize drug load," according to the company's web site.<sup>25</sup>

And there are still other strategies, including a recently described "glycoengineering" approach from Sanofi-Genzyme that introduces chemically targeted sialic acid moieties to the protein,<sup>26</sup> and a novel approach from Meditope Biosciences that leverages a unique peptide, called a "meditope," which binds a recognition sequence that can be genetically inserted into the angle between the arms of the antibody "Y" structure.<sup>24</sup>

With so many moving parts, ADC manufacturing poses significant hurdles. As reported in *C&EN*, "ADCs are still a niche area of manufacturing, and few [contract manufacturing organizations] offer all of the drugs' pieces, which makes for a complex supply chain. ADC assembly itself is challenging, requiring providers to simultaneously handle biologic materials in sterile conditions and manipulate highly potent small molecules under containment. Demand for experienced CMOs is strong."<sup>6</sup>





Some companies, though, can handle the process, including Lonza, which “is investing \$15 million to add a second commercial-scale ADC facility in Visp, Switzerland, later this year,” according to the *C&EN* report. Also, Roche (which includes antibody therapeutics developer Genentech under its umbrella) is investing \$200 million of its own funds in an ADC production facility in Basel.<sup>6</sup>

## BISPECIFIC ANTIBODIES

Another emerging antibody therapeutic strategy involves so-called bispecific antibodies. As reported in *Nature Reviews Drug Discovery*, (in a 2011 article entitled, “Buy buy bispecific antibodies”), corporate interest in bispecific antibodies is growing.<sup>27</sup> A bispecific-focused antibody firm called Zymeworks struck a deal in 2011 with Merck & Co. for up to \$187 million, “and “bispecific deals worth over \$7.5 billion biodollars have been struck since 2009 by the likes of Bayer Schering, Sanofi, Boehringer Ingelheim and Amgen, to name but a few.”<sup>27</sup>

Bispecifics can come in several formats, but two are most common. The first, called an “asymmetrical” molecule, doubles the number of targets a single antibody can bind to by having each antigen-binding site target a different molecule; the molecule is thus “asymmetric,” because the two arms bind different antigens. The second, called “symmetric,” is designed such that each antigen-binding site can bind both targets.<sup>27</sup> These constructs generally can be used in either of

two ways. One is to bind two separate molecules, effectively consolidating two antibodies into one and simplifying R&D. The second application, called “cell retargeting,” uses the two antibody variable regions to link two cells –, an immune effector cell and its cancerous target, for example.<sup>27</sup>

In 2012, researchers at Chugai Pharmaceutical in Japan reported a different application for bispecific antibodies. The researchers described a bispecific antibody that targets both blood clotting factors IXa and X to substitute for factor VIII, which is absent in patients with hemophilia A.<sup>28</sup> The result could theoretically function as a therapeutic alternative to factor VIII itself, toward which patients can develop immune reactions.

According to a report by Roots Analysis, bispecifics could be worth more than \$4 billion by 2023.<sup>29</sup> But to date, only one bispecific antibody has gained regulatory approval: Removab (catumaxomab), which targets both CD3 (on T cells) and EpCAM (on tumor cells), was approved in 2009 in Europe to treat malignant ascites.

One difficulty for the field is that normal antibodies are not meant to be bispecific. Immunoglobulin G (IgG) antibodies (the kind typically used for therapeutics) are heterotetramers comprising two molecules each of a heavy and light chain, linked by disulfide bonds. The heavy and light chains are expressed from two different genes, both of which must be expressed to produce a viable antibody. The antibody’s final form is like a “Y”, a divalent structure in which each arm is capable of binding the antibody’s target antigen. The stalk of the “Y” provides effector functions to engage other immune components, among other functions.

Creating a bispecific antibody requires interrupting or modifying the standard antibody assembly process. One approach is to skip the IgG format altogether. For instance, Amgen in 2012 paid \$1.2 billion to purchase bispecific developer Micromet, whose “bispecific T-cell engagement” (BiTE) platform links two single-chain variable fragment (scFv) domains in a single polypeptide chain.<sup>30</sup> A scFv joins the two halves of an antibody’s antigen-binding site, the heavy and light chain variable fragments, in a single molecule.

Micromet’s most advanced therapeutic candidate, blinatumomab (which links T cells to CD19+ B cells), currently is in Phase 2 testing for acute lymphoblastic leukemia and non-Hodgkin lymphoma.<sup>30</sup> As reported in *C&EN*, Amgen in 2012 “reported impressive results from a Phase II study of blinatumomab to treat an aggressive blood cancer called acute lymphoblastic leukemia. Of the patients given the bispecific antibody, 69% saw their disease go into complete remission.”<sup>30</sup>

In 2012, a research team led by Peter Schultz at the Scripps Research Institute described an alternative synthetic approach based on the unnatural amino acid, para-acetylphenylalanine.<sup>31</sup> By incorporating that residue in the trastuzumab (Herceptin) light chain and a heavy chain lysine from an anti-CD3 antibody, two Fab fragments (the “arms” of the antibody “Y” shape) could be linked by “flexible bifunctional cross-linkers” to create a synthetic bispecific molecule.

Other strategies seek to create true bispecific antibody molecules. One approach, described in 2013 by researchers at Genentech, relies on “co-culture of two bacterial strains, each expressing a half-antibody.”<sup>32</sup> The authors used their approach to produce 28 distinct bispecific antibodies, including one that can co-target the MET and EGFR proteins, which are implicated in non-small-cell lung carcinoma. A more recent approach, published earlier this year by researchers at the University of North Carolina at Chapel Hill and Eli Lilly, ports the same idea to eukaryotic cells.<sup>33</sup>

## NUCLEIC ACID THERAPEUTICS

---

Where antibodies target proteins that have been synthesized already, nucleic acid therapeutics seek to block or modify gene expression itself. There are several strategies researchers and companies can use. Single-stranded antisense oligonucleotides bind to complementary RNA transcripts to prevent their translation or their functional activity (e.g., in the case of non-coding RNAs), to induce degradation, or to block access by cellular machinery, such as spliceosomes or ribosomes.<sup>34</sup> Others hope to leverage RNA interference (RNAi) to achieve therapeutic aims. In this case, a short double-stranded RNA (often called a short interfering RNA or small interfering RNA [siRNA]) is delivered to cells, where one strand, complementary to the targeted transcript, is loaded into a protein complex called RISC and used to guide enzymatic cleavage of that mRNA, effectively silencing (or at least dampening) the gene.

These technologies have numerous potential applications; a recent story in *C&EN* reports that agricultural companies such as Monsanto and Syngenta are pursuing RNAi to develop genetic pesticides.<sup>35</sup> But it is in biotherapeutics where these technologies hold perhaps the greatest promise. The PhRMA 2013 Biologics report counts 30 antisense biologics in development and

### TWO DECADES OF RNAi-BASED AGRICULTURAL PRODUCTS

**1992** The Food & Drug Administration declares nucleic acids “generally recognized as safe.”

**1994** FDA approves the genetically modified Flavr Savr tomato, which uses antisense RNA—a similar biological process to RNA interference (RNAi)—to regulate expression of an enzyme responsible for ripening fruit. The product is discontinued

because it isn’t profitable. **1997** EPA approves virus-resistant papaya, squash, and potato, all of which use antisense RNA technology.

**1998** RNAi phenomenon is first described in literature.

**2010** EPA approves virus-resistant plum that uses posttranscriptional gene silencing.

**2010 & 2011** USDA ap-

proves two high-oleic soybean traits for cultivation in the U.S. The technology uses RNAi to block expression of certain soybean enzymes, leading to increased monounsaturated fat and reduced saturated fat soybean oil.

**2013** EPA grants Monsanto a permit to field-test an RNAi-based pesticide that targets western corn rootworm.

Reprinted in part from: *C&EN* 2014, 92(7), pp 29-31.  
Copyright © 2014 American Chemical Society

15 RNAi-based ones.<sup>2</sup> Another report notes, “Two RNAi-targeted therapeutics have received FDA approval, and there were more than 127 projects in the clinical research pipeline using this approach, including 12 projects in Phase III or later, with many more in preclinical research.”<sup>7</sup>

## ANTISENSE RNA THERAPEUTICS

One of the most prominent players in antisense therapeutics is Isis Pharmaceuticals, with some two dozen antisense-based products in development. In January 2013, the FDA approved Kynamro (mipomersen sodium), Genzyme and Isis Pharmaceuticals’ “first-in-class” antisense oligonucleotide for patients with homozygous familial hypercholesterolemia, an extremely rare condition.<sup>8</sup>

Targeting the mRNA encoding apolipoprotein B (apoB), mipomersen is a 20-mer “synthetic phosphorothioate oligonucleotide salt,” heavily modified with 2'-O-(2-methoxyethyl) nucleotides. As described in its prescribing information, “Mipomersen inhibits synthesis of apoB by sequence-specific binding to its messenger ribonucleic acid (mRNA) resulting in degradation of the mRNA through enzyme-mediated pathways or disruption of mRNA function through binding alone.”<sup>36</sup>

Kynamro’s approval marks just the second for the antisense approach, the other being fomivirsen (for cytomegalovirus retinitis in AIDS patients), which was approved in 1998 but ultimately discontinued “owing to reduced need of the therapeutic.”<sup>37</sup> Mipomersen’s approval, according to a news report in *Nature Reviews Drug Discovery*, “was highly anticipated as a potential validation of the scientific and commercial promise of antisense as a platform technology.”<sup>37</sup> Isis has several additional antisense molecules in Phase 3 testing, including molecules targeting transthyretin (TTR, with GlaxoSmithKline) and clusterin (with Teva/OncoGenex).

Prosensa uses antisense technology to achieve a different biological aim. Rather than using antisense oligonucleotides to block translation or induce degradation, the Dutch biopharmaceutical company uses it to induce “exon skipping” – a strategy that enables the translation of mutant genes containing a frame-shift or nonsense mutation. In June Prosensa announced plans to submit a new drug application (NDA) to the FDA in 2014 for its lead antisense therapeutic, drisapersen.<sup>38</sup> Intended for treatment of Duchenne Muscular Dystrophy (DMD), drisapersen uses exon skipping to eliminate exon 51 of the dystrophin gene, an approach that can potentially aid some 13% of all DMD patients.<sup>39</sup> And the company has additional therapeutics in development to skip mutations in exons 44, 45, and 53, as well. At least seven antisense therapeutics were in Phase 3 testing as of 2013,<sup>37</sup> including drisapersen, which failed in a placebo-controlled study in 186 DMD patients to show “a statistically significant or clinically meaningful treatment difference” in either its primary endpoint or “the majority of secondary endpoints,” the company reports.<sup>39</sup> However, “further clinical data analyses of drisapersen across all studies ... suggest that treating earlier in the disease and treating longer shows a delay in the progression of the disease,” the company adds.<sup>39</sup>

Gene Signal's aganirsen (GS-101), an eye drop formulation that targets the angiogenic gene IRS-1 to combat corneal neovascularization, has also been subjected to a Phase 3 clinical trial, the 69-patient I-CAN study. Though no statistical difference was observed in visual acuity between dosed and placebo groups (the study's primary endpoint), the study did detect a 26.2% decrease in "the relative area of corneal neovascularization after 90 days and this improvement persisted after 180 days."<sup>40</sup> (The drug is also in Phase 2a testing for treatment of a different condition, psoriasis.)

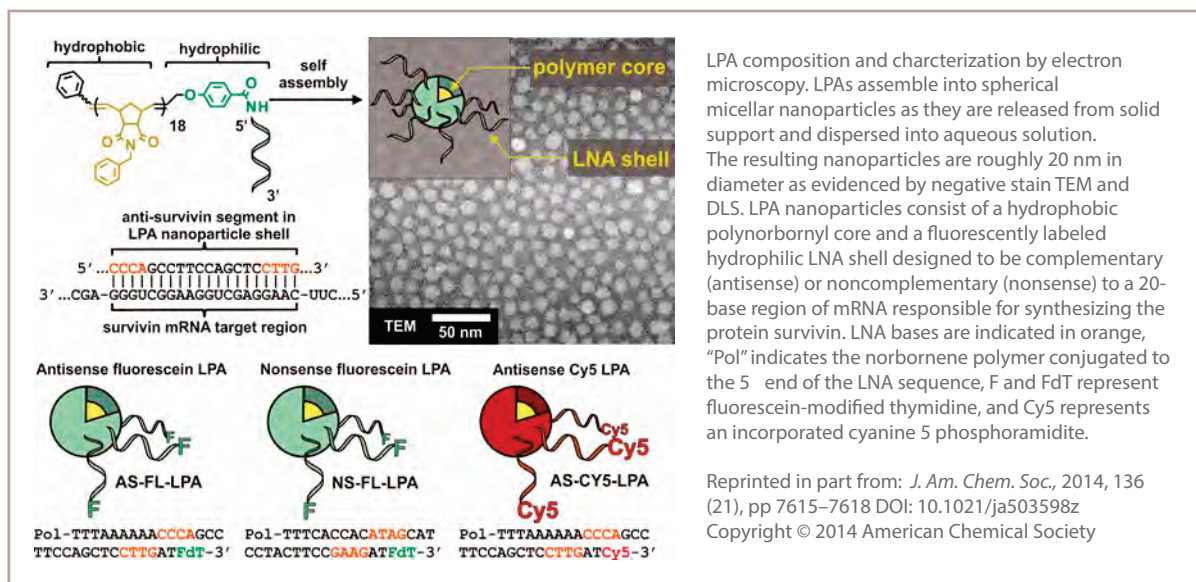
## THE NEXT GENERATION

One of the fundamental difficulties in developing oligonucleotide-based therapies is that nucleic acids, and especially RNAs, are relatively unstable and subject to enzymatic degradation in vivo. Also, nucleic acids on their own cannot cross cell membranes. As a result, companies tend to chemically modify the nucleic acids and/or encapsulate or package them in some way.

With its 2'-O-(2-methoxymethyl) modifications, for instance, mipomersen is a so-called "second-generation" antisense oligonucleotide. First-generation molecules contain substitutions in the phosphate group oxygen atoms; second-generation oligos modify the 2'-position of the nucleotide ribose ring; and third-generation oligos contain more dramatic structural modifications, in some cases replacing the ribose sugar altogether, for instance, with "locked nucleic acids" (LNAs).<sup>41</sup>

Santaris Pharma is one company pursuing third-generation antisense molecules. Santaris' miravirsen is an LNA-containing antisense oligo targeting the abundant hepatocellular microRNA miR-122 for treatment of hepatitis C virus, currently in Phase 2 testing.<sup>42</sup>

One recent study by researchers at the University of California, San Diego, describes self-assembling "spherical nucleic acids" called LNA-polymer amphiphile (LPA) nanoparticles.



Essentially core-shell nanoparticles coated with antisense molecules, these were taken up within about 10 minutes by human cells, 10 times more efficiently than antisense molecules alone, effecting intracellular dampening of the target mRNA – in this case, a transcript called survivin.<sup>43</sup>

Sarepta Therapeutics's exon-skipping therapeutic for DMD, eteplirsen, currently in Phase 2 testing, is also a third-generation design. Eteplirsen is a “phosphorodiamidate morpholino oligomer,” or PMO. In a PMO, the ribose sugar of RNA is replaced with a six-membered morpholino ring, and a “phosphorodiamidate linkage” is swapped for the usual phosphodiester backbone.<sup>44</sup> In April 2014 the company indicated its intention to file an NDA to use eteplirsen to treat DMD “by the end of 2014.”<sup>45</sup>

## TARGETING NON-CODING RNAS

Implicated in development and disease, microRNAs are increasingly popular targets for nucleic acid therapeutics, especially for cancer.<sup>46</sup> Non-coding RNAs can be attacked with any number of methods, among them LNA anti-miRs and antagomirs, which induce degradation of the targeted transcript; miR “sponges,” which absorb unwanted miRNAs; and enzymatic ribozymes. Companies can supply missing microRNAs using microRNA mimics, expression vectors, or small molecules.<sup>46</sup>

Particularly attractive, says one 2011 review, are antisense oligonucleotide (ASO) therapeutics. “ASOs readily inhibit miRNAs, far more reliably than they do mRNAs, and the unique properties of Argonaute proteins permits the use of remarkably short ASOs: 15 nucleotide long ASOs are now in clinical trials and 8 nucleotide long versions show promise in non-human primates.”<sup>47</sup> Regulus Therapeutics, a microRNA-focused company spun out of Isis Pharmaceuticals and RNAi company Alnylam Pharmaceuticals, is targeting some six miRNAs for hepatocellular carcinoma, glioblastoma, and kidney fibrosis, among other applications. Its RG-101, which targets miR-122, currently is in Phase 1 testing for HCV infection.<sup>9</sup>

And at least two companies, RaNA Therapeutics and Opko-CuRNA, are targeting another class of non-coding transcripts, long non-coding RNAs.<sup>48</sup> These regulatory molecules are involved in epigenetic control of gene expression via multiple mechanisms, including recruitment of the chromatin-modifying complex PRC2, which dampens gene expression. RaNA Therapeutics is developing antisense oligonucleotides to block that interaction in spinal muscular atrophy, Friedreich's ataxia, and other conditions, boosting expression of nearby genes. Opko-CuRNA targets so-called “natural antisense transcripts” (NATs), which modulate expression of complementary mRNAs, with antisense-antisense molecules it calls “antagoNATs.”<sup>49</sup>

## RNA INTERFERENCE

Meanwhile, following waning investor and corporate interest and disappointing clinical performance in 2008–2010, RNAi therapeutics is back in vogue in 2014.<sup>50</sup> In the mid-2000s, bullish companies invested billions in RNAi technology. Then the bottom fell out, fueled by questions surrounding delivery efficacy, time to market, and other concerns.<sup>51</sup>

Alnylam Pharmaceuticals, one of the leaders in this space, struck deals with Novartis and Roche, among others. But both partners ended their relationships with Alnylam in 2010, forcing the RNAi developer to trim its staff.<sup>52,53</sup> Still, by 2011 Alnylam was estimating that it could have five genetic medicines in clinical testing by 2015 – a plan it called “Alnylam 5x15”. Earlier this year, the company released new, more optimistic guidance, announcing it “expects to end 2015 with six to seven genetic medicine programs in the clinic, including at least two programs in Phase 3 and five to six programs that will have achieved human proof-of-concept results supporting further development.”<sup>54</sup> Shortly thereafter, Sanofi/Genzyme invested some \$700 million in Alnylam.<sup>50</sup> Focusing specifically on delivery to the liver – “nobody has consistently shown that RNAi drugs can be effectively delivered anywhere but the liver,” writes Luke Timmerman in *Xconomy.com*<sup>55</sup> – Alnylam, which encapsulates siRNAs in lipid nanoparticles, currently has at least 10 compounds in the pipeline, including one in Phase 3. That drug is patisiran (ALN-TTR02), for treatment of transthyretin (TTR) amyloidosis.

Phase 1 data on patisiran, published in August 2013 in the *New England Journal of Medicine*, are highly encouraging. The company observed a decrease in serum TTR levels of up to 93.8% after a single dose, and reductions averaging between 56.6% and 76.8% at 28 days.<sup>56</sup> Phase 2 data, announced in November, coincident with the launch of the company’s Phase 3 APOLLO study, “showed that multiple doses of patisiran led to robust and statistically significant knockdown of serum TTR protein levels of up to 96%, with mean levels of TTR knockdown exceeding 85%.”<sup>57</sup> Alnylam is also developing a GalNAc-conjugated subcutaneous version of this molecule, called ALN-TTRsc, currently in Phase 2.

Other players in the RNAi marketplace include Dicerna Pharmaceuticals, which netted some \$93 million in its February 2014 initial public offering,<sup>50</sup> and Arrowhead Research, which is developing “dynamic polyconjugates” (DPCs) and “homing peptides” for siRNA targeting in vivo.<sup>55</sup> “DPCs are small nanoparticles, 5-20 nanometers (nm) in size, composed of an amphipathic polymer to which shielding agents such as polyethylene glycol, as well as targeting ligands are reversibly attached,” the company explains on its web site.<sup>58</sup> Arrowhead’s lead compound, ARC-520, targets hepatitis B virus in the liver, apparently knocking down viral proteins to enhance immune cell action.<sup>55</sup> According to the company, a study in a single chimpanzee with chronic HBV infection detected “a 95% reduction in circulating viral DNA, and approximately 90% reductions in hepatitis e-antigen (HBeAg) and s-antigen (HBsAg), which are thought to be important in establishing a functional cure.”<sup>59</sup> (Those findings mirrored earlier results in rodent models.) Following completion of a Phase 1 safety trial in normal volunteers, the company in March 2014 initiated a Phase 2a trial in HBV-infected patients.<sup>60</sup>

## GENE THERAPY & GENOME EDITING

---

It has been, as Aaron Krol notes in *Bio-IT World*, nearly 25 years since the first gene therapy trial was launched in the U.S., and the field has failed to generate “a single commercial product to this day.”<sup>61</sup> In the U.S., that is; in November 2012 the European Medicines Agency (EMA) approved uniQure BV’s Glybera (alipogene tiparvovec) “for the treatment of the ultra-rare inherited disorder, lipoprotein lipase (LPL) deficiency.”<sup>62</sup>

There are many ways to effect gene therapy, but the basic idea is to deliver a nucleic acid, generally via a recombinant virus, which supplies a missing genetic element – say, a functional copy of a gene. As reported in *Nature Biotechnology*, Glybera is “a recombinant adeno-associated viral (AAV) vector expressing the Ser447X variant of human LPL,” administered “in a single sitting by means of several injections into the leg muscles, resulting in a long-term and clinically important reduction in the occurrence of acute pancreatitis, the most insidious symptom of LPL deficiency.”<sup>63</sup>

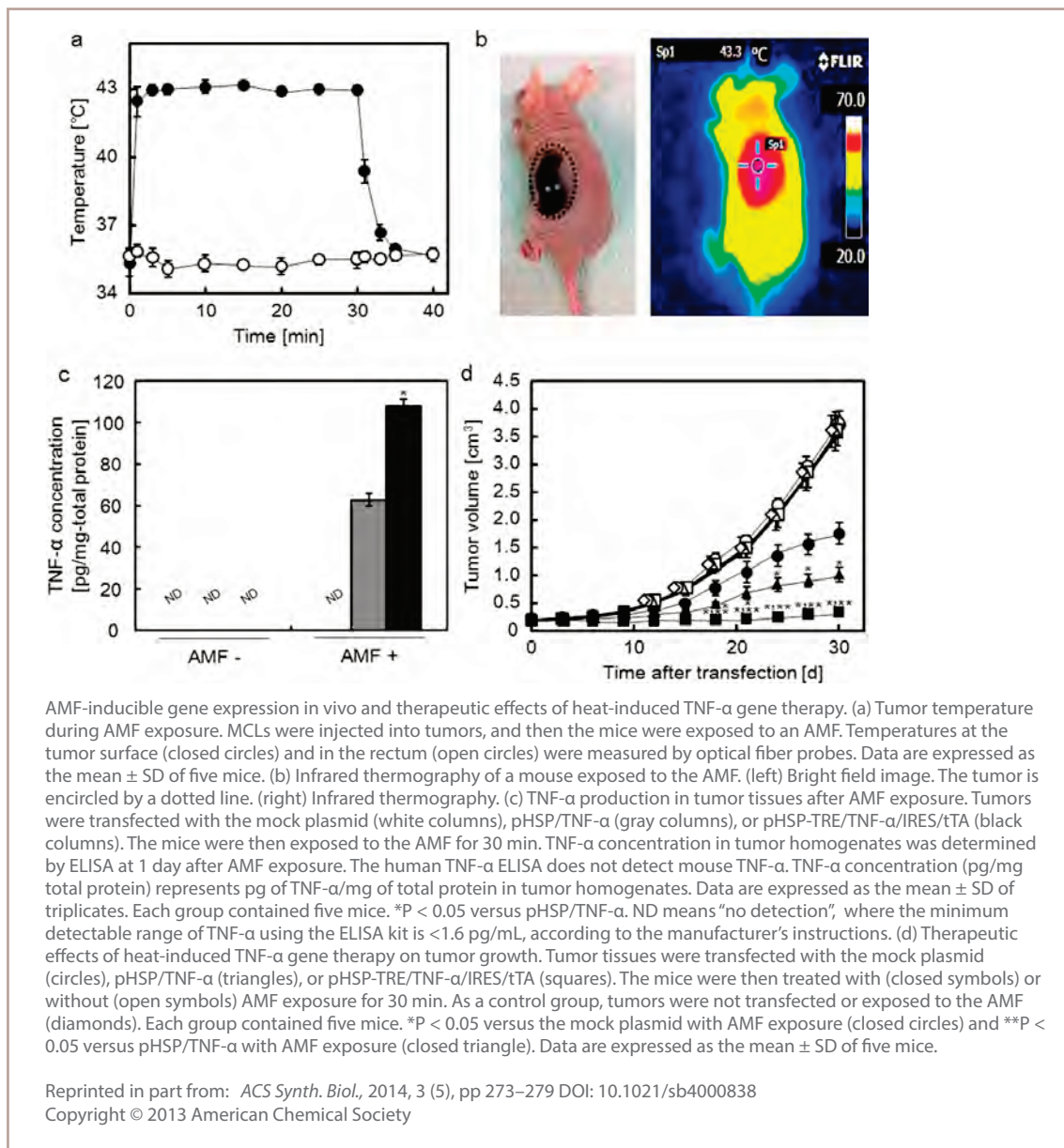
*Nature Biotechnology* counted some 14 gene therapies in clinical development in September 2012. The PhRMA report, *Medicines in Development: Biologics*, counted 46 as of 2013,<sup>2</sup> and *Innovation in the Biopharmaceutical Pipeline* counted 99, including 12 in Phase 3.<sup>7</sup> One of those in Phase 3 was Vical’s Allovectin (velimogene aliplasmid), a DNA plasmid encoding the two components of a human major histocompatibility type I molecule; injection of this into melanoma, it was hoped, would induce an immune response against the tumor.<sup>64</sup> But underscoring the difficulty of successfully advancing a biologic to market, the Phase 3 data were disappointing: “The 390-subject trial failed to demonstrate a statistically significant improvement vs. first-line chemotherapy for either the primary endpoint of objective response rate at 24 weeks or more after randomization or the secondary endpoint of overall survival,” Vical reported – and the company opted to terminate the program.<sup>65</sup>

Still, several gene therapy-focused firms have launched recently, according to *Nature Biotechnology*, including Spark Therapeutics, GenSight, Voyager Therapeutics, and NightstaRx.<sup>66</sup> The latter is an Oxford University spin-off commercializing an AAV-based therapeutic for choroideremia, an X-linked form of blindness stemming from a mutant REP1 gene.<sup>67</sup> *The Lancet* published the results of a six-patient Phase 1 and 2 test earlier in 2014: “The initial results of this retinal gene therapy trial are consistent with improved rod and cone function that overcome any negative effects of retinal detachment,” the authors conclude.<sup>68</sup>

This year has also seen the launch in the UK of a “landmark” gene therapy-based “HIV vaccine” trial, which uses AAV to deliver an anti-HIV neutralizing antibody.<sup>69</sup> And other researchers have conducted small human trials based on lentiviral vectors for such rare genetic conditions as metachromatic leukodystrophy and Wiskott-Aldrich syndrome.<sup>70</sup>



There is considerable work going on at the preclinical stage, too. A promising study in mice, for example, demonstrates the potential of an AAV-based vector to treat Friedreich's ataxia (FRDA), a mitochondrial disease that impacts the heart, among other tissues, and is caused by a faulty frataxin gene. The researchers used a form of AAV that can zero in on the tissues typically impacted in this disease and inserted into its genome a functional copy of the human frataxin gene. They then injected those viruses into a mouse model of FRDA. "A single intravenous dose of this vector at 3 weeks of age completely protected *Mck* mice from the onset of cardiomyopathy, and molecular, cellular and physiological analysis at 35 weeks revealed no differences between cardiomyocytes of the treated *Mck* mice and wild-type mice."<sup>71</sup>



Another study describes an interesting approach to limiting gene therapy's impact beyond targeted cells. Researchers in Japan in 2013 connected heat-responsive promoters to cell death-inducing genes (such as TNF $\alpha$ ) for cancer therapy. By injecting these constructs into mouse tumors, along with magnetic nanoparticles that heat up in an alternating magnetic field, they induced tumor death. "After 30 days, mice placed in the magnetic field had tumors that were just 8% of the volume of those in unexposed mice," according to a news report on the study in *C&EN*.<sup>72</sup>

## GENE EDITING

There's also excitement over genome editing, a gene therapy application that promises to repair damaged genes rather than replace them. There are three competing approaches to gene editing, but all exploit the same basic biological machinery.<sup>73</sup> In each case, genomic DNA is cut at a specific nucleotide sequence, e.g., a gene or regulatory region. In attempting to repair that cut, the cell can either render that sequence nonfunctional by nonhomologous end joining (NHEJ), or replace it with another, researcher-supplied sequence by homologous recombination. Thus, researchers can use gene-editing approaches to knock a gene out, create targeted mutations (e.g., to study the impact of known genetic lesions in stem cells), or repair a mutation.

How the genetic cut is made is what differentiates genome-editing strategies. One approach uses a custom transcription factor-nuclease fusion protein called a zinc finger nuclease (ZFN). ZFNs interact with DNA via their zinc finger domains, with each domain specifying a specific three or four-base sequence. Researchers can therefore program these enzymes' sequence specificity by altering and rearranging the specific zinc finger modules used. TALENs, or TALE nucleases, operate on a similar principle, but their "code" uses one module per base in the recognition sequence, providing greater flexibility in protein design but correspondingly larger proteins.<sup>73</sup>

The newest approach relies on the so-called CRISPR/Cas system. CRISPR/Cas is a form of bacterial immunity that allows bacteria to recall viral infections and fight them off later. In 2012, Jennifer Doudna at the University of California, Berkeley, and colleagues figured out that the system relies on a pair of short RNAs, one of which specifies the sequence to be targeted, plus the generic nuclease, Cas. By supplying their own guide RNAs, researchers can direct Cas to any sequence they desire, and can, in fact, target multiple sites simultaneously.<sup>73</sup> These approaches, and especially CRISPR/Cas because of its simplicity and efficiency, promise tremendous opportunities in the area of gene therapy, as it suddenly becomes possible to extract a patient's cells, "fix" them in culture, and return them to the patient.

At least two companies have been founded to leverage CRISPR/Cas. Editas Medicine, founded by some of the key innovators of CRISPR/Cas and TALEN technology, including Doudna, launched in November 2013 with \$43 million in venture capital funding.<sup>12</sup> Another startup, CRISPR Therapeutics, announced \$25 million in funding in April 2014.<sup>13</sup> "Editas is betting that recent advances in the technology of genetic engineering have leapfrogged previous generations of

therapies, opening a huge market niche for a company at the cutting edge of gene editing if it's game to try its hand at treating genetic diseases," wrote Aaron Krol in a corporate profile in *Bio-IT World* earlier this year.<sup>61</sup>

Yet some worry about specificity – that is, that the Cas nuclease will target other sequences than the one desired – because the CRISPR guide RNA is much shorter (and thus less specific) than the binding sites for ZFNs and TALENs. Recently, two papers in *Nature Biotechnology* describe one approach to that problem. J. Keith Joung of Massachusetts General Hospital and David Liu of Harvard University – both cofounders of Editas Medicine – independently fused an inactive Cas9 to the FokI nuclease, which is used in both ZFNs and TALENs and functions as a dimer.<sup>74,75</sup> As a result, cleavage requires two separate binding events, increasing specificity more than 140 times, according to Liu's report.<sup>75</sup> Joung's team has also reported separately that, paradoxically, use of slightly shortened guide RNAs (17 or 18 nucleotides of complementarity instead of 20) with a wild-type Cas9 protein can boost specificity "by 5,000-fold or more," as well.<sup>94</sup>

One recent study demonstrated an alternative strategy to minimize off-target effects. William Skarnes of the Wellcome Trust Sanger Institute, and colleagues used a "paired nickase" approach, in which mutant Cas9 proteins that can produce only a single-strand cut are used with paired guide RNAs targeting a single locus, to generate specific mutations in both mouse embryos and human cells with "minimal off-target effects."<sup>95</sup> ZFN nickases, also for boosting specificity, have also been described.<sup>97</sup>

To date, however, only one genome editing strategy has reached clinical trials, and that is the use of ZFNs. In March 2014, researchers reported in the *New England Journal of Medicine* the results of an "open-label, nonrandomized, uncontrolled study of a single dose of ZFN-modified CD4 T cells" in patients with "chronic aviremic HIV infection."<sup>76</sup> The ZFN, Sangamo Biosciences' SB-728, targets the CCR5 gene, which is an HIV co-receptor, and disrupts it via NHEJ. Introducing SB-728 into CD4+ lymphocytes taken from 12 individuals with HIV elevated those patients' T-cell counts for extended periods.<sup>76</sup> Specifically, median total lymphocyte counts increased from 1.27x10<sup>3</sup> per cubic millimeter prior to treatment to 2.33x10<sup>3</sup> per cubic millimeter one week later, before stabilizing at 1.70x10<sup>3</sup> per cubic millimeter at six weeks. CD4+ T cell counts tripled from 448 to 1517 per cubic millimeter in one week before stabilizing at 615 cells per cubic millimeter. At least one additional ZFN trial is also in the clinic, according to *C&EN*, a Phase 1 trial at City of Hope of a nuclease "that knocked out glucocorticoid receptors in engineered T cells to treat malignant glioblastoma."<sup>97</sup>

Of course, that's a genetic knockout. But researchers would prefer also to be able to repair genes, and work is progressing on that front, too. For instance, researchers at the University of Minnesota reported in 2013 the ability to repair the type VII collagen gene in skin cells from a patient with recessive dystrophic epidermolysis bullosa (RDEB), a genetic condition that causes severe skin blistering and an elevated skin cancer risk, using TALENs and an oligonucleotide encoding the "correct" gene sequence.<sup>77</sup> Transfection of those elements into cultured primary skin

fibroblasts from RDEB patients corrected the error, and dedifferentiation into induced pluripotent stem (iPS) cells and subsequent differentiation in an in vivo teratoma model produced “skin-like structures that when further analyzed showed the presence of type VII collagen protein at the [dermal-epidermal junction], which confirmed the ability of TALENs to correct the primary genetic defect and restore functional protein production at the proper ultrastructural location in vivo.”<sup>77</sup>

## STEM CELL-BASED THERAPEUTICS

---

Perhaps the ultimate “biologic” is a cell-based therapeutic. In this case, as the saying goes, the cell isn’t a tool to make a therapy, the cell *is* the therapy. Most of the excitement surrounding cell-based therapeutic strategies involves stem cells, whether from bone marrow, cord blood, or human embryonic stem cells, but other strategies have been and are, being pursued. Fibrocell Science’s laViv (azfcil-T), for instance, a “first-in-class personalized cell therapy for eliminating fine wrinkles or nasolabial folds around the nose and mouth,” was approved in 2011 by the FDA.<sup>78</sup> As reported in *Nature Biotechnology*, treatment with laViv “involves harvesting a person’s own fibroblasts from behind the ear, culturing them for 90 days, and then reinjecting the expanded cells into dermal layers in the patient’s face, during a series of treatments, given three to six weeks apart.”<sup>78</sup> At the time, laViv was the third cell therapy to win regulatory approval in the U.S., behind Genzyme’s Carticel, autologous cultured chondrocytes for cartilage repair, and Dendreon’s Provenge, cultured and biochemically manipulated autologous dendritic cells for prostate cancer.<sup>78</sup> Cellectis, a French biopharmaceutical company, is blending cell therapeutics and TALEN-based genome-editing technology to create immune cells specifically targeted towards cancer. The company has said it plans to initiate a Phase 1 trial of its UCART19 T-cell formulation for CD19-expressing leukemias in 2015.<sup>96</sup>

Another cell therapy strategy is “adoptive T cell therapy,” an immune-boosting approach for cancer treatment. In early June, researchers from the National Cancer Institute announced at the American College of Clinical Oncology national meeting that extracting T cells from women with advanced cervical cancer, expanding them in the lab, and then returning them to the patients could shrink their tumors. Three of nine women tested responded to the treatment, the researchers reported, with two complete remissions and one tumor shrinking by 40%.<sup>79</sup>

In the stem cell realm, the nature of the cell therapy depends on the condition and type of cell, of course, but the idea generally is either to leverage the developmental plasticity of stem cells to restore a missing or diseased cell type, or to secrete soluble factors that promote cell recruitment, differentiation, or protection. For example, bone marrow transplantation is essentially a stem cell therapy, in this case to reconstitute the blood system.

In some cases, the therapy is allogeneic – that is, the implanted cells are genetically distinct from the recipient. Other stem cell therapies are autologous, or derived from the patients themselves.

Multiple companies are pursuing both lines of research, and numerous stem cell-based clinical trials are ongoing.<sup>80</sup> Indeed, the 2013 report, *Innovation in the Biopharmaceutical Pipeline*, counted 245 projects using cell therapy.<sup>7</sup> Several involve adult stem cells. StemCells Inc., for example, has developed a formulation of purified allogeneic human neural stem cells called HuCNS-SC, which the company is testing for spinal cord injury, Pelizaeus-Merzbacher Disease, and dry age-related macular degeneration. Neuralstem's NSI-566 is a formulation of human spinal cord stem cells that it is using in a Phase 2 trial for amyotrophic lateral sclerosis (Lou Gehrig's disease). Also pursuing adult stem cells is Mesoblast, which acquired Osiris Therapeutics' mesenchymal stem cell-based therapeutics portfolio, including its lead product, Prochymal, in October 2013.<sup>81</sup> Prochymal is in Phase 3 testing for acute graft versus host disease and Crohn's disease.

GlaxoSmithKline's GSK 2696273 combines hematopoietic stem cell therapy with gene therapy. As described in the ClinicalTrials.gov record for its ongoing Phase 2 trial for severe combined immunodeficiency caused by adenosine deaminase (ADA) deficiency, "The drug product studied in this protocol consists of autologous CD34+ hematopoietic stem/progenitor cells engineered ex vivo with a retroviral vector encoding the therapeutic gene ADA."<sup>82</sup>

## HUMAN EMBRYONIC STEM CELL THERAPIES

Perhaps the greatest potential, and excitement, in cell therapeutics revolves around pluripotent stem cells – human embryonic stem (hES) cells and induced pluripotent stem (iPS) cells. These cells have the theoretical capacity to differentiate into any cell type in the body, provided they are grown under the appropriate conditions – though determining just what those conditions are is rarely easy. Researchers can use those cells for drug development and to determine how genetic mutations lead to disease, among other applications. But they also can use them to produce transplantable cells and cell products.

Geron Corp. was the first company to advance an hES cell-based therapy into the clinic. In 2009, it launched a Phase 1 trial of GRNOPC1, an hES cell-derived oligodendrocyte progenitor cell product, in spinal-cord injury patients. Four patients ultimately were injected. But the company, citing "capital scarcity and uncertain economic conditions," halted the trial in late 2011.<sup>83</sup> It subsequently sold its stem-cell program to BioTime.<sup>84</sup>

Advanced Cell Technology (ACT) is also pursuing hES cell therapies. The company has hES cell-derived retinal pigment epithelial (RPE) cells in Phase 1 and 2 trials for both Stargardt's macular dystrophy and dry age-related macular degeneration, publishing preliminary data on two patients (who received 50,000 cells each) in *The Lancet* in 2012.<sup>93</sup>

Another firm, ViaCyte, uses hES cells to produce pancreatic endoderm cells (PEC-01), which can serve as a therapeutic for type 1 diabetes. But rather than injecting those cells directly, ViaCyte encapsulates the cells in a semi-permeable container, called Encaptra, which is porous to glucose,

oxygen, and other nutrients and signals, but not to immune cells. The combined product is called VC-01, and the company has said it plans to file an Investigational New Drug (IND) application with the FDA in 2014.<sup>85</sup>

## INDUCED PLURIPOTENT STEM CELLS

Others also are pursuing hES cell therapies. But the problem with all such therapies is they are – by definition – allogeneic. It simply isn't possible to obtain hES cells after birth. Besides the moral and ethical problems, that also raises the possibility of immune rejection of transplanted cells. iPS cells, though, circumvent that shortcoming. Derived from a patient's own cells, iPS cells are created by introducing into adult somatic cells (e.g., skin cells) a cocktail of genes encoding four DNA-binding transcription factors. This effectively "rewinds" the cellular clock, producing cells with the growth and differentiation properties of hES cells.

Once created, iPS cells can be differentiated into any cell type desired, for instance to create new neurons for implantation in individuals with certain neurodegenerative diseases. In the event of problematic genetic lesions, those can be repaired by gene editing prior to iPS cell generation to effect a cellular therapy, much as was done in culture in the case of recessive dystrophic epidermolysis bullosa using TALENs.<sup>77</sup> iPS cells have problems of their own, however. Traditionally, they are generated by genetically modifying cells with oncogenic factors, which raises the possibility of uncontrolled growth. But researchers have developed workarounds for that, such as replacing some of the transcription factor genes with small molecules.<sup>86</sup>

In May 2014, researchers at NIH demonstrated the potential of iPS cell therapy in nonhuman primates (rhesus monkeys) using a reprogramming strategy that excises the reprogramming factors after iPS cell generation.<sup>87</sup> The authors generated iPS cells from several tissues – bone marrow stromal cells, skin cells, and CD34+ cells – and differentiated them into bone marrow stromal cells in culture. When implanted beneath the skin of autologous rhesus monkeys in combination with hydroxyl apatite/tricalcium phosphate, the implants "showed robust bone formation," the authors report.<sup>87</sup>

More recently, a research team led by Yuet Wai Kan of the University of California, San Francisco, demonstrated in June "seamless" targeting of the CCR5 gene, an HIV coreceptor, in human iPS cells using either CRISPR/Cas or TALEN genome editing combined with the piggyBac transposon. Immune cells derived from those iPS cells were resistant to HIV infection, while unedited cells were not.<sup>91</sup>

The world's first clinical study using iPS-derived cells in humans actually kicked off in 2013.<sup>11</sup> Japanese researcher Masayo Takahashi plans to implant sheets of iPS-derived RPE cells into one eye each in six patients with wet-type age-related macular degeneration. Patient enrollment for the trial began in August 2013,<sup>92</sup> but given the time required to produce iPS cells and monitor the patients after transplantation, results are not likely any time soon.

## CONCLUSIONS: THE PROMISE OF PERSONALIZED MEDICINE

---

In the world of 21<sup>st</sup> century healthcare, “personalized medicine” is a buzzword used with ever-greater frequency. The idea, of course, is that each individual is genetically unique, and therapies that work well for one may not be quite so efficacious for another. By delivering to patients the therapies best tailored to their particular molecular makeups, physicians can maximize efficacy, increase efficiency, and minimize side effects. Similarly, by paying attention to genetic and molecular phenotypes, drug developers can more accurately target their therapeutics to the patients most likely to benefit from them, and administer them at the appropriate doses, thereby increasing their chance of success in clinical trials and subsequent commercialization.

To a large degree, it is biologics that enable personalized medicine. Cancers, for instance, are lumped together based on the tissue from which they originated, but, using breast cancer as an example, they are not all alike. The anticancer antibody therapeutic Herceptin is intended specifically for those individuals with HER2+ breast cancer, a phenotype that includes, according to Genentech, about one-quarter of breast cancer patients.<sup>88</sup> The three-quarters of patients who do not have HER2+ tumors derive no benefit from the drug, so why give it to them? Not only is that a waste of money, it also wastes precious time that could be spent on other therapeutic strategies.

Similarly, though they have mutations in the same gene, not all patients with Duchenne muscular dystrophy have the same genetic lesion. Prosensa’s drisapersen specifically helps those patients with mutations in the region of exon 51 of the dystrophin gene – a group the company says includes 13% of DMD patients.<sup>39</sup> This represents the largest patient subpopulation in DMD, according to the company, but still excludes 87% of the possible patient pool. As a result, Prosensa has initiated trials of exon-skipping strategies for exons 44, 45, and 53, as well, which could potentially benefit 6%, 8%, and 8% of the DMD population, respectively, according to the company.

Personalized therapies like drisapersen and Herceptin have other benefits, too. Because of their molecular targeting, they generally produce far fewer side effects than do traditional therapies, as they (ideally) affect only the targeted cells. And in some cases – gene editing, for instance – they enable therapeutic possibilities that simply cannot be achieved any other way.

## REGULATORY AND SAFETY CONCERNS

Yet for all their potential benefits, biologics also pose significant hurdles for their developers. Because of their size and complexity, they are harder to create and characterize than small molecules, increasing cost and complexity. They offer fewer routes of administration (most biologics are delivered intravenously, and few are available orally), and because they are manufactured in cells, require sophisticated production facilities, efficient purification regimens, and extensive quality control procedures. Biologic cell cultures can become contaminated, for instance, so quality control measures must be in place to ensure the resulting products are free of pathogens. “The challenge in manufacturing biologic medicines is to control variability in this process to ensure compliance with quality standards so that every patient can be treated with a medicine of consistent quality, every time,” states Amgen in company literature on biologics and biosimilars.<sup>89</sup>

Obviously, some biologics are more difficult than others. Antibody-drug conjugates are particularly complicated, with three elements that must be optimized, produced, and linked – and must pass regulatory muster. First, there are questions of physical structure, such as antibody-drug ratio and post-translational modification. But there also are more functional questions, such as how the drug compound behaves in vivo, how the ADC functions relative to the unconjugated antibody, how conjugation impacts bioavailability and pharmacokinetics, and so on.

With some biologics, drug specificity can be harder to track. The only way to know if a TALEN is cutting where it isn't supposed to, for instance, is to sequence the cellular genome and look at what changed. Similarly, a perennial concern with pluripotent cell-based therapies is the possibility of offsite tumors, which could necessitate expensive screening.

All of these issues only multiply as new therapeutic classes emerge, as drug developers and regulatory agencies work out just what evidence is required for approval. In a *Nature Biotechnology* news article on Geron's decision to terminate its stem cell therapeutics program, company cofounder Michael West is quoted on the steps the company went through in preparing its clinical trial for GRNOPC1. “Nearly 2,000 rodents with spinal-cord injuries” were tested, West said, and in the end, it was “the largest ever investigational new drug filing in the FDA's history – over 22,000 pages – and the problems were apparently not going away.”<sup>10</sup>

Despite these considerations, the pharmaceutical industry obviously is bullish on biologics, and with good reason. Given the robust pipeline, generous funding, and exciting data coming from all corners of the biologics marketplace, there's no reason to believe the situation will look any different in the years to come.



## REFERENCES

---

- [1] Aggarwal, S. "What's fueling the biotech engine – 2012 to 2013." *Nat. Biotechnol.*, **2014**, 32, 32-39.
- [2] Pharmaceutical Research and Manufacturers of America (PhRMA). "Medicines in Development: Biologics" (report). **2013**; available online at <http://www.phrma.org/sites/default/files/pdf/biologicsoverview2013.pdf>.
- [3] Morrow, K. J., Jr. "The New Generation of Antibody Therapeutics: Current Status and Future Prospects." *Insight Pharma Reports* (Cambridge Health Institute), **2012**; available online at [http://www.insightpharmareports.com/reports\\_report.aspx?id=115904&r=9771](http://www.insightpharmareports.com/reports_report.aspx?id=115904&r=9771)
- [4] Mullard, A. "Maturing antibody-drug conjugate pipeline hits 30." *Nat. Rev. Drug Disc.*, **2013**, 12, 329–332.
- [5] Thayer, A. M. "Drug Conjugates Lure Investment." *Chem. Eng. News*, **2013**, 91(42), 7.
- [6] Thayer, A. M. "Building Antibody-Drug Conjugates." *Chem. Eng. News*, **2014**, 92(3), 13-21.
- [7] Long, G.; Works, J. "Innovation in the Biopharmaceutical Pipeline: A Multidimensional View." Report for PhRMA by Analysis Group, Inc., Boston. **2013**; available online at <http://www.phrma.org/sites/default/files/pdf/2013innovationinthebiopharmaceuticalpipeline-analysisgroupfinal.pdf>.
- [8] Bigelow, B. V. "FDA Ok's First-in-Class Drug From Isis for Rare Cholesterol Disorder." *Xconomy.com*, **2013**; available online at <http://www.xconomy.com/san-diego/2013/01/29/fda-oks-first-in-class-drug-from-isis-for-rare-cholesterol-disorder/>.
- [9] Regulus Therapeutics. "Regulus Commences Dosing RG-101 in Healthy Volunteers in Phase I Study" (report). **2014**; available online at <http://www.regulusrx.com/regulus-commences-dosing-rg-101-in-healthy-volunteers-in-phase-i-study/>.
- [10] Frantz, S. "Embryonic stem cell pioneer Geron exits field, cuts losses." *Nat. Biotechnol.* **2012**, 30, 12–13.
- [11] "Japan to start stem-cell study on humans." *Nature News Blog*, **2013**; available online at <http://blogs.nature.com/news/2013/07/japan-to-start-stem-cell-study-on-humans.html>.
- [12] Shen, H. "CRISPR technology leaps from lab to industry." *Nature News*, **2013**; available online at <http://www.nature.com/news/crispr-technology-leaps-from-lab-to-industry-1.14299>.
- [13] "CRISPR Therapeutics raises \$25 million in Series A financing." *Startupticker.ch*, **2014**; available online at <http://www.startupticker.ch/en/news/april-2014/crispr-therapeutics-raises-25-million-in-series-a-financing>.
- [14] Reardon, S. "Gene-editing method tackles HIV in first clinical test." *Nature News*, **2014**; available online at <http://www.nature.com/news/gene-editing-method-tackles-hiv-in-first-clinical-test-1.14813>.
- [15] Strebhardt, K.; Ullrich, A. "Paul Ehrlich's magic bullet concept: 100 years of progress." *Nat. Rev. Cancer*, **2008**, 8(6), 473–480.
- [16] Jefferis, R. "Glycosylation as a strategy to improve antibody-based therapeutics." *Nat. Rev. Drug Disc.*, **2009**, 8(3), 226–234.
- [17] Ratner, M. "Genentech's glyco-engineered antibody to succeed Rituxan." *Nat. Biotechnol.*, **2014**, 32(1), 6–7.
- [18] Jeffrey, S. C., et al. "A potent anti-CD70 antibody-drug conjugate combining a dimeric pyrrolobenzodiazepene drug with site-specific conjugation technology." *Bioconjug. Chem.*, **2013**, 24(7), 1256–1263.
- [19] Timmerman, L. "Why Good Drugs Sometimes Fail: The Bexxar Story." *Xconomy.com*, **2013**; available online at <http://www.xconomy.com/national/2013/08/26/why-good-drugs-sometimes-fail-in-the-market-the-bexxar-story/>.
- [20] Jarvis, L. M. "Rethinking Antibody-Drug Conjugates." *Chem. Eng. News*, **2012**, 90(25), 12–18.
- [21] Drahl, C. "New Linkers Might Provide Highly Stable Protein-Drug Conjugates." *Chem. Eng. News*, **2013**, 91(41), 29–30.

- [22] Shen, B. Q., et al. "Conjugation site modulates the in vivo stability and therapeutic activity of antibody-drug conjugates." *Nat. Biotechnol.*, **2012**, 30(2), 184–189.
- [23] Tian, F., et al. "A general approach to site-specific antibody drug conjugates." *Proc. Natl. Acad. Sci.*, **2014**, 111(5), 1766–1771.
- [24] Thayer, A. M. "Altered Antibodies Offer Improved Conjugates." *Chem. Eng. News*, **2014**, 92(3), 13–21.
- [25] Mersana Therapeutics. "R&D Approach" (corporate statement). Collected June 2, **2014**; available online at <http://www.mersana.com/our-technology/r-and-d-approach.php>.
- [26] Zhou, Q., et al. "Site-Specific Antibody-Drug Conjugation through Glycoengineering." *Bioconjug. Chem.*, **2014**, 25(3), 510–520.
- [27] Holmes, D. "Buy buy bispecific antibodies." *Nat. Rev. Drug Disc.*, **2011**, 10(11), 798–800.
- [28] Kitazawa, T., et al. "A bispecific antibody to factors IXa and X restores factor VIII hemostatic activity in a hemophilia A model." *Nat. Med.*, **2012**, 18(10), 1570–1577.
- [29] Roots Analysis. "Bispecific Antibody Therapeutics Market, 2013–2023" (report). **2013**; available online at [http://www.rootsanalysis.com/reports/view\\_document.html?report\\_id=34](http://www.rootsanalysis.com/reports/view_document.html?report_id=34).
- [30] Jarvis, L. M. "Bispecific Antibodies Deliver Two for the Price of One." *Chem. Eng. News*, **2012**, 90(51), 20–22.
- [31] Kim, C. H., et al. "Synthesis of Bispecific Antibodies using Genetically Encoded Unnatural Amino Acids." *J. Am. Chem. Soc.*, **2012**, 134(24), 9918–9921.
- [32] Spiess, C., et al. "Bispecific antibodies with natural architecture produced by co-culture of bacteria expressing two distinct half-antibodies." *Nat. Biotechnol.*, **2013**, 31, 753–758.
- [33] Lewis, S. M., et al. "Generation of bispecific IgG antibodies by structure-based design of an orthogonal Fab interface." *Nat. Biotechnol.*, **2014**, 32, 191–198.
- [34] Kole, R., et al. "RNA therapeutics: beyond RNA interference and antisense oligonucleotides." *Nat. Rev. Drug Disc.*, **2012**, 11(2), 125–140.
- [35] Erickson, B. E. "Gene-silencing pesticides." *Chem. Eng. News*, **2014**, 92(7), 29–31.
- [36] Genzyme Corp. Highlights of prescribing information for Kynamro (information sheet). **2013**; available online at <http://www.genzyme.com/Products/~-/media/B866EE59A7C14916BFBFB4ECA1C7087.pdf>.
- [37] Tse, M. T. "Regulatory watch: Antisense approval provides boost to the field." *Nat. Rev. Drug Disc.*, **2013**, 12, 179.
- [38] Prosensa, press release (June 3, 2014). "Prosensa announces regulatory path forward for drisapersen as a potential treatment for DMD." Available online at <http://secure.marketwatch.com/story/prosensa-announces-regulatory-path-forward-for-drisapersen-as-a-potential-treatment-for-dmd-2014-06-03>.
- [39] Prosensa, product update. "Drisapersen (PRO051)." Available online at <http://www.prosensa.eu/technology-and-products/pipeline/drisapersen-pro051>.
- [40] Gene Signal, press release (May 12, 2014). "Gene Signal announces positive data from Phase III trial of aganirsen eye drops for corneal neovascularisation due to inflammation." Available online at [http://www.genesignal.com/fileadmin/user\\_upload/documents/Gene\\_Signal\\_I-CAN\\_Press\\_Release\\_ENGLISH\\_12\\_May\\_2014.pdf](http://www.genesignal.com/fileadmin/user_upload/documents/Gene_Signal_I-CAN_Press_Release_ENGLISH_12_May_2014.pdf).
- [41] Integrated DNA Technologies. "Antisense technologies" (corporate review). **2011**; available online at <https://www.idtdna.com/pages/docs/educational-resources/antisense-technologies.pdf>.
- [42] Santaris Pharma A/S, press release (Aug. 27, 2013). "Santaris Pharma A/S Completes Enrollment in Phase 2 Clinical Trial of Miravirsen." Available online at <http://www.santaris.com/news/2013/08/27/santaris-pharma-completes-enrollment-phase-2-clinical-trial-miravirsen>.
- [43] Rush, A. M., et al. "Intracellular mRNA Regulation with Self-Assembled Locked Nucleic Acid Polymer Nanoparticles." *J. Am. Chem. Soc.*, **2014**, 136(21), :7615–7618.
- [44] Sarepta Therapeutics. "PMOs: The basics" (corporate review). Available online at <http://www.sarepta.com/technology/technology-platform>.

- [45] Sarepta Therapeutics, press release (April 21, 2014). "Sarepta Therapeutics announces plans to submit new drug application to FDA for eteplirsen for the treatment of Duchenne Muscular Dystrophy by year end 2014." Available online at <http://investorrelations.sarepta.com/phoenix.zhtml?c=64231&p=irol-newsArticle&ID=1920025&highlight=>.
- [46] Ling, H., et al. "MicroRNAs and other non-coding RNAs as targets for anticancer drug development." *Nat. Rev. Drug Disc.*, **2013**, 12(11), 847–865.
- [47] Broderick, J. A.; Zamore, P. D. "MicroRNA therapeutics." *Gene Ther.* **2011**, 18(12), 1104–1110.
- [48] Sheridan, C. "RaNA Therapeutics." *Nat. Biotechnol.* **2012**, 30, 909.
- [49] Wahlestedt, C. "Targeting long non-coding RNA to therapeutically upregulate gene expression." *Nat. Rev. Drug Disc.*, **2013**, 12, 433–446.
- [50] Bouchie, A. "Markets, venture investors and big pharma interest in RNAi soars." *Nat. Biotechnol.*, **2014**, 32, 203–204.
- [51] Haussecker, D. "The business of RNAi therapeutics in 2012." *Mol. Ther. Nucleic Acids*, **2012**, 1, e8.
- [52] Timmerman, L. "Alnylam Cuts 25–30% of Workforce, as Novartis Alliance Ends." *Xconomy.com*, **2010**; available online at <http://www.xconomy.com/boston/2010/09/23/alnylam-cuts-25-30-of-workforce-as-novartis-alliance-ends/>.
- [53] Timmerman, L. "Roche Dumps RNAi, Sends Shock Waves Through Alnylam, Tekmira." *Xconomy.com*, **2010**; available online at <http://www.xconomy.com/national/2010/11/17/roche-dumps-rnai-sends-shock-waves-through-alnylam-tekmira/>.
- [54] Alnylam Pharmaceuticals, Inc., press release (Jan. 10, 2014). "Alnylam provides key 2014 goals for RNAi therapeutics pipeline." Available online at <http://investors.alnylam.com/releasedetail.cfm?ReleaseID=818582>.
- [55] Timmerman, L. "Arrowhead Picks Up Where Roche Left Off, Jumps Ahead in RNAi." *Xconomy.com*, **2013**; available online at <http://www.xconomy.com/wisconsin/2013/12/20/arrowhead-picks-roche-left-jumps-ahead-rnai/>.
- [56] Coelho, T., et al. "Safety and efficacy of RNAi therapy for transthyretin amyloidosis." *N. Engl. J. Med.*, **2013**, 369(9), 819–829.
- [57] Alnylam Pharmaceuticals, Inc., press release (Nov.10, 2013). "Alnylam Reports Positive Phase II Data for Patisiran (ALN-TTR02), an RNAi Therapeutic Targeting Transthyretin (TTR) for the Treatment of TTR-Mediated Amyloidosis (ATTR), and Initiates Phase III Trial." Available online at <http://investors.alnylam.com/releasedetail.cfm?ReleaseID=805999>.
- [58] Arrowhead Research Corporation. "Dynamic polyconjugates" (technology overview). Retrieved June 6, 2014; available online at <http://www.arrowheadresearch.com/technology/dynamic-polyconjugates>.
- [59] Arrowhead Research Corporation, press release (March 25, 2013). "Arrowhead Induces 90% Reduction in Hepatitis B Antigens in Chimpanzee with Chronic Infection: Data Follow Previous Report of Reduction in Viral DNA." Available online at <http://ir.arrowheadresearch.com/releasedetail.cfm?ReleaseID=841006>.
- [60] Arrowhead Research Corporation, press release (March 24, 2014). "Arrowhead Begins Dosing in Phase 2a Trial of RNAi Therapeutic ARC-520 in Chronic Hepatitis B Patients." Available online at <http://ir.arrowheadresearch.com/releasedetail.cfm?ReleaseID=840131>.
- [61] Krol, A. "Gene Therapy's Next Generation." *Bio-IT World*, **2014**; available online at <http://www.bio-itworld.com/2014/1/29/gene-therapys-next-generation.html>.
- [62] Moran, N. "First gene therapy approved." *Nat. Biotechnol.*, **2012**, 30, 1153.
- [63] Moran, N. "First gene therapy nears landmark European market authorization." *Nat. Biotechnol.*, **2012**, 30, 807–809.
- [64] Doukas, J.; Rolland, A. "Mechanisms of action underlying the immunotherapeutic activity of Allovectin in advanced melanoma." *Cancer Gene Ther.*, **2012**, 19, 811–817.

- [65] Vical Inc., press release (Aug. 12, 2013). "Vical Phase 3 trial of Allovectin fails to meet efficacy endpoints." Available online at <http://www.vical.com/investors/news-releases/News-Release-Details/2013/Vical-Phase-3-Trial-of-AllovectinR-Fails-to-Meet-Efficacy-Endpoints/default.aspx>.
- [66] Huggett, B. "Biotech's wellspring: The health of private biotech in 2013." *Nat. Biotechnol.*, **2014**, 32, 428–435.
- [67] Cassasus, B. "AAV gene therapy continues to woo investors." *Nat. Biotechnol.*, **2014**, 32, 302.
- [68] MacLaren, R. E., et al. "Retinal gene therapy in patients with choroideremia: Initial findings from a Phase 1/2 clinical trial." *Lancet*, **2014**, 383(9923), 1129–1137.
- [69] "HIV immunity goes direct" (editorial). *Nat. Biotechnol.*, **2014**, 32, 397.
- [70] Flight, M. H. "Trial watch: Clinical trial boost for lentiviral gene therapy." *Nat. Rev. Drug Disc.*, **2013**, 12, 654.
- [71] A. Flemming, "Gene therapy protects heart in Friedreich's ataxia," *Nat Rev Drug Disc*, 13:416–7, **2014**.
- [72] Fellet, M. "Heated Nanoparticles Throw Gene Therapy Switch." *Chem. Eng. News*, **2013**; available online at <http://cen.acs.org/articles/91/web/2013/11/Heated-Nanoparticles-Throw-Gene-Therapy.html>.
- [73] Perkel, J. M. "The New Genetic Engineering Toolbox." *BioTechniques*, **2013**, 54(4), 185–188.
- [74] Tsai, S. Q., et al. "Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing." *Nat. Biotechnol.*, **2014**, 32, 569–576.
- [75] Guilinger, J. P., et al. "Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification." *Nat. Biotechnol.*, **2014**, 32, 577–582.
- [76] Tebas, P., et al. "Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV." *N. Engl. J. Med.*, **2014**, 370, 901–910.
- [77] Osborn, M. J., et al. "TALEN-based gene correction for epidermolysis bullosa." *Mol. Ther.*, **2013**, 21(6), 1151–1159.
- [78] Schmidt, C. "FDA approves first cell therapy for wrinkle-free visage." *Nat. Biotechnol.*, **2011**, 29, 674–675.
- [79] Steenhuisen, J. "Personalized therapy helped women with advanced cervical cancer." Reuters, June 2, **2014**; available online at <http://www.reuters.com/article/2014/06/02/health-cancer-cervical-idUSL1N0OJ0SD20140602>.
- [80] National Institutes of Health, U.S. Department of Health & Human Services. "Stem Cells and Diseases" (information sheet), retrieved June 4, **2014**; available online at <http://stemcells.nih.gov/info/pages/health.aspx>.
- [81] Waltz, E. "Mesoblast acquires Osiris' stem cell business." *Nat. Biotechnol.*, **2013**, 31, 1061.
- [82] "ADA Gene Transfer into Hematopoietic Stem/Progenitor Cells for the Treatment of ADA-SCID (Gene-ADA)," ClinicalTrials.gov identifier NCT00598481, retrieved June 5, **2014**; available online at <http://clinicaltrials.gov/show/NCT00598481>.
- [83] Geron Corporation, press release (Nov. 14, 2011). "Geron to focus on its novel cancer programs." Available online at <http://ir.geron.com/phoenix.zhtml?c=67323&p=irol-newsArticle&iD=1635764&highlight=>.
- [84] Brower, V. "BioTime acquires Geron's stem cell program." *Nat. Biotechnol.*, **2013**, 31, 94.
- [85] ViaCyte, Inc., press release (Feb. 6, 2014). "JDRF to Provide Additional Support for Upcoming Clinical Trial of ViaCyte's Encapsulated Cell Therapy for Type 1 Diabetes." Available online at <http://viacyte.com/press-releases/jdrf-to-provide-additional-support-for-upcoming-clinical-trial-of-viacytes-encapsulated-cell-therapy-for-type-1-diabetes/>.
- [86] Hou, P., et al. "Pluripotent Stem Cells Induced from Mouse Somatic Cells by Small-Molecule Compounds." *Science*, **2013**, 341(6146), 651–654.
- [87] Hong, S. G., et al. "Path to the Clinic: Assessment of iPSC-Based Cell Therapies In Vivo in a Nonhuman Primate Model." *Cell Reports*, **2014**, 7(4), 1298–1309.

- [88] Genentech, Inc. "Fight HER2+ breast cancer with Herceptin" (web site), accessed June 5, 2014. Available online at <http://www.herceptin.com/breast/herceptin>.
- [89] Amgen, Inc. "Biologics and biosimilars: An overview." **2014**; available online at [http://www.amgen.com/pdfs/misc/Biologics\\_and\\_Biosimilars\\_Overview.pdf](http://www.amgen.com/pdfs/misc/Biologics_and_Biosimilars_Overview.pdf).
- [90] Mullin, R., "Cell-free approach to antibody-drug conjugates," *Chem. Eng. News*, **2013**, 91(44):Web exclusive. Available online at <http://cen.acs.org/articles/91/i44/Cell-Free-Approach-Antibody-Drug.html>.
- [91] Ye, L., et al., "Seamless modification of wild-type induced pluripotent stem cells to the natural CCR5 $\Delta$ 32 mutation confers resistance to HIV infection," PNAS, published online June 9, **2014**, doi:10.1073/pnas.1407473111.
- [92] Cyranoski, D., "iPS cells in humans," *Nat. Biotechnol.*, **2013**, 31(9):775.
- [93] Schwartz, S.D., et al., "Embryonic stem cell trials for macular degeneration: A preliminary report," *The Lancet*, **2012**, 379(9817):713–20.
- [94] Fu, Y., et al., "Improving CRISPR-Cas nuclease specificity using truncated guide RNAs," *Nat. Biotechnol.*, **2014**, 32(3):279–84.
- [95] Shen, B., et al., "Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects," *Nat. Methods*, **2014**, 11(4):399–402.
- [96] Cellectis. "Pipeline." Available online at <http://www.cellectis.com/en/content/therapeutic-products>.
- [97] Borman, S., "New way to modify DNA," *Chem Eng News*, **2012**, 90(22):52-53.



**ACS**  
Chemistry for Life<sup>®</sup>

**AMERICAN CHEMICAL SOCIETY**

1155 SIXTEENTH STREET, NW  
WASHINGTON, DC 20036

[www.acs.org](http://www.acs.org)