BioProcess
SPECIAL REPORT

Cancer Immunotherapies

Fulfilling the Promise of Protein and Cell Therapies

by Angelo DePalma

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With few exceptions, both small-molecule and biological cancer treatments have contributed only incrementally towards achieving long-term responses or outright cures. In this regard, emerging cell- and protein-based cancer immunotherapies represent game-changing strategies for treating even refractory cancer. With long-term responses now possible, medical science may be on the verge of delivering on the long-unfulfilled promise of making cancer a manageable disease.

But impediments to commercializing cancer immunotherapies are substantial. Producing cell-based treatments entails substantial hands-on manipulation and perfecting the logistics of harvesting and expanding therapeutic cells and delivering them to patients. Given the handling requirements and high cost of goods (CoG) for cell-based immunotherapies, reimbursement considerations will force developers to demonstrate indisputable value. Those developing immunotherapies based on monoclonal antibodies (MAbs) will experience fewer such issues thanks to platform manufacturing technologies, but even they are likely to be priced to perfection.

Issues in Protein Immunotherapy

Immunotherapy Squared: Bavituximab, a monoclonal antibody from Peregrine Pharmaceuticals (Tustin, CA), is a classic protein immunotherapy targeting phosphatidylserine (PS), a novel immune system checkpoint. PS exists on the inside membrane layer of every cell, but it externalizes when cells die. "In circulation, PS signals the immune system to engulf dying cells," explains Steve King, Peregrine's chief executive officer (CEO). PS also limits the immune response. As tumors proliferate, they often outgrow their blood supply so that many cells die, sending more PS into circulation. Tumors also release microparticles containing PS, ultimately suppressing immune response to the tumor by keeping the host's immune system busy fighting particles and dead cells.

Peregrine's collaboration with AstraZeneca for clinical development could be described as "immunotherapy squared." Bavituximab's presumed mode of action is to block immunosuppression while activating a tumor-killing T-cell immune response. AstraZeneca's investigational anti-PD-L1 immune checkpoint inhibitor, durvalumab, targets the programmed cell death ligand PD-L1, which helps tumors go undetected by the immune system. Both companies believe that combining the enhanced T-cell–mediated antitumor activity with a checkpoint inhibitor will extend the ability of tumor-specific T-cells to attack cancerous cells.

Like many small biopharmaceutical companies with a promising pipeline product, Peregrine chooses to emphasize clinical development over manufacturing or process development, confident that if bavituximab succeeds in the clinic, then CoG issues will resolve themselves. "Our process flexibility assures that we could duplicate the entire facility and all its infrastructure in an open warehouse space almost anywhere," King affirms. "We built the current facility with the idea of supporting production lots early in commercialization. At that point you have substantial revenue, so all your manufacturing avenues open up. And the risk of sticking with the same systems, at the same scale, from a comparability standpoint is negligible."

Downstream operations could very well become a bottleneck. Peregrine has learned through its contract manufacturing business, Avid Bioservices, that high yields — even from 1,000-L or 2,000-L bioreactors — impose operational and financial pressures on downstream processing and purification. Protein A affinity chromatography columns, for example, begin at about $1 million for resin alone and go up from there. "That's a big investment for a small-to-midsized company," King admits. Peregrine is handling such challenges through a hybrid approach of maintaining a revenue-generating manufacturing business that mitigates the cost of preparing for commercialization of its own products. "Not many companies have that flexibility."

Blocking the Immunity Blockers: In November 2015, Faron Pharmaceuticals (Turku, Finland) entered into an agreement with Swiss company Selexis through which Faron will access the Selexis SUREtechnology platform system and SURE CHO-M cell line expression technology. Faron will use them to develop high-expressing and stable clonal cell lines for production of its Clevegen cancer immunotherapy antibody. The Selexis technologies rapidly generate high antibody-expressing clonal cells with predictable titers and genetic stability.

Immune defenses are often suppressed in cancer patients. Faron's product targets the cell-surface receptor Clever-1 on the surfaces of tumors' vascular endothelial cells and tumor-associated macrophages (TAMs). Binding of the drug to Clever-1 prevents TAM accumulation around tumors and decreases their antiinflammatory function, allowing a patient's natural tumor-fighting immunity to take over. Thus, the Clevegen

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antibody cleverly and indirectly stimulates a patient's immune system to fight his or her tumor.

According to Faron, the Clevegen antibody is well differentiated from competing products by its ability to specifically target TAMs of the M2 variety (which facilitate tumor growth) while sparing M1 macrophages that support antitumor immune activation and desirable immunity in general. Chief executive officer Markku Jalkanen likens immune-suppressive mechanisms with a shrink-wrap that keeps beneficial immune cells at bay. "These mechanisms block immune recognition cells from entering. If these suppressants are not removed, even activated T cells cannot reach the tumor."

Depending on the jurisdictions in which it is licensed, Clevegen probably will be approved as a combination or salvage therapy. But the company cites a strong scientific justification for it as a first-line treatment. "The reason we are interested in standalone therapy is we can recognize Clever-1–positive monocytic cells in circulation in cancer patients," Jalkanen explains. "So we may already have a surrogate end-marker for both disease and efficacy of treatment, levels of which we can determine through flow cytometry." With a test available, Faron could identify patients who have higher levels of protumor macrophages and thus are most likely to benefit from Clevegen therapy.

Cell-Based Immunotherapies

An Existential Challenge: Cell-based immunotherapies fall into two categories. Autologous treatments, by far the more common in the development pipeline, are based on manipulating an individual's own (immune) cells, usually expanding them ex vivo, and reinjecting them into the same patient. Allogeneic treatments use banked cells from a common source.

Manufacturing is the critical — some might say existential — challenge for autologous cell-based immunotherapies. "These therapies emerged from academic centers, so production needs to catch up with the equivalent innovation that has occurred during discovery," explains Jim Faulkner, head of manufacturing at Autolus (London, UK) and editorial advisor to *BioProcess International*. Three key enablers for success will be automation, single-use technologies, and sophisticated logistics. "It really is a very different paradigm from the conventional supply chain model that has dominated the pharmaceutical business to date. New service providers are emerging to offer creative solutions to the manufacturing challenges that T-cell therapies present."

Faulkner notes that human capital is key to solving logistical and technical problems related to treatment delivery. "Ultimately, it is all about smart scientists who have a deep understanding of biology and who can come up with creative ways to translate that knowledge into commercial bioprocesses. That's what will determine whether you succeed or fail in this business."

Allogeneic cell-based treatments could be a game-changer because of their potential mass-production and distribution, which could make them off-the-shelf therapeutic cells. In December 2015, Cellectis (Paris, France) submitted a clinical trial application to the United Kingdom's Medicines and Healthcare Products Regulatory Agency to begin first-in-human studies of UCART19, an allogenic, gene-edited T-cell treatment for CD19-positive leukemias. This treatment would outcompete labor-intensive autologous cell-based therapies from a production standpoint. It offers the potential for being equally effective as well — provided developers can eliminate host-vs.-graft reactions that often occur in patients receiving allogenic treatments.

Another ingenious (if not downright fearless) application of allogeneic therapies is embodied in Mologen's (Berlin, Germany) MGN1601, which operates based on cross-immunity. The active agents are genetically modified allogeneic cancer cells. When MGN1601 is injected, patients' immune systems react based on the presense of "nonself" antigens, then generalize that response to attack their own cancers. To induce that effect as strongly as possible, the modified allogeneic tumor cells are combined with an adjuvant. This product could also be referred to as a "cancer vaccine."

Building on Existing Biomanufacturing: Sartorius Stedim Biotech (Göttingen, Germany) entered the regenerative medicine market through acquisition of TAP Biosystems (Royston, UK) in 2013. Before that merger, TAP had positioned its automated cell culture and microbioreactor systems toward regenerative medicine. Those efforts continue as the company encourages use of those products in development of cancer immunotherapies.

Kim Bure, director for regenerative medicine at Sartorius Stedim Biotech, hopes the next few years will see the transfer of cell-based immunotherapies into single-use bioreactors of different sizes and technologies, from rockers to stirred-tanks. "There may be a bridge between today's innovations in

large-scale biologic production and smaller, single-use scales in the future for more personalized therapies," she suggests. Cost remains a primary driver in the push toward allogeneic therapeutic cells, Bure says, because "limited available automation for autologous treatments results in significantly more expensive production."

Bure says that gene editing is a likely approach to minimizing immunogenicity of allogeneic cells. For example, Cellectis and Pfizer (Groton, CT) have been collaborating since 2014 on a cell-based therapy that uses Cellectis' chimeric antigen receptor T-cell (CAR-T) platform technology to engineer T-cells from a single donor for use in multiple patients. And in 2015, Juno Therapeutics (Seattle, WA), which specializes in delivery of cell-based CAR-T immunotherapies, teamed up with Editas Medicine (Cambridge, MA), whose strength is genome editing.

Two principal cell-based immunotherapies are based on CAR-T and T-cell receptors (TCRs). In CAR-T therapy, T cells are removed from a patient, then engineered through viral-vector transduction to recognize tumor antigens by presenting tumor-specific antibodies. The modified cells are expanded and reintroduced into the same patient. Those antibodies help them locate and destroy that patient's tumor.

Companies such as Adaptimmune (Abingdon, UK) use engineered, increased-affinity T cell receptors (TCRs). Adaptimmune technology engineers the natural TCR affinity to cancer protein epitopes on one patient's cells to target and then destroy cancer cells in multiple patients.

Bure calls gene editing "the third arm" of immunotherapy — and it's used in both CAR-T and TCR approaches. "About 80% of clinical trials in cell-based immunotherapy follow those two approaches," she asserts, "and of those, about 60% are CAR-T. The remaining research approaches aim to invoke direct gene editing for oncologic and other indications, where editing constructs are injected systemically or directly into tumors, creating a type of human antibody bioreactor."

Making CAR-T Cell Immunotherapies Work

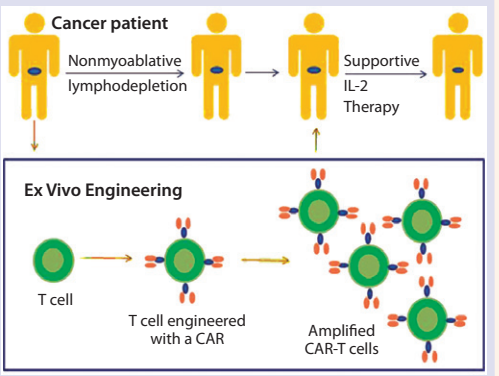
by Mazen W. Karaman

Experimental and clinical evidence has shown that the human immune system can specifically identify and destroy cancer cells, leading to substantial enthusiasm in medicine regarding the promise of immunotherapies for cancer treatment (**1**). T cells are widely distributed in tissues and tumor microenvironments, and they play a central role in cell-mediated immunity. Adoptive cell therapy (ACT) is one approach that involves genetically engineering a patient's own T cells to recognize and attack their tumors using chimeric antigen receptors (CARs) (**2**, **3**).

The first clinical trial using CAR-T cell therapy was conducted in ovarian-cancer patients (**4**). However, that and other trials have showed limited efficacy. Subsequent improvements in molecular biology and immunology have led to significant successes (**3**). Because of those promising results and significant funding support in the pharmaceutical industry to develop CAR-T cells for cancer therapy, optimization of the commercial CAR-T cell manufacturing process is now required (**5**).

The seemingly straightforward method for generating CAR-T cells outlined in Figure 1 has several complex steps. As such, ACT using CAR-T cells has been carried out only by a limited number of investigators who have developed manufacturing processes for small-scale clinical trials (**5**). Producing personalized therapies is a multifaceted biological process coupled with a potentially high failure rate. Clearly, the manufacturing process needs to be optimized for commercial production.

Figure 1: Adoptive cell therapy with CAR-T cells (**2**); T cells are collected from a cancer patient's blood and genetically engineered to produce receptors called CARs, which recognize a predefined target on cancer cells. Billions of CAR-T cells are grown at laboratory scale, then transferred to the same patient by transfusion. CAR-T cells then migrate to the tumor lesion, where they induce a proinflammatory reaction and eliminate cancer cells, resulting in lasting tumor regression.



To be viable, a CAR-T manufacturing process must meet certain technical, regulatory, and economic requirements. Automation is key. A number of devices can perform certain parts of the development process, but each device must work seamlessly with the next. Personnel must be trained, instrument servicing must be scheduled, and operational and performance qualifications must be in place. The CliniMACS Prodigy system (Photo 1) from Miltenyi Biotec is a platform that automatically performs

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Manufacturing — A Unique Onus: Having the spotlight for cell-based immunotherapy treatments clearly focused on manufacturing to lower CoG places a unique burden on product and process developers. That's "unlike elsewhere in biopharmaceutical manufacturing, where processes have matured into industrialized scales and robust platforms," notes Uwe Gottschalk, chief technology officer for pharma/biotech at Lonza (Basel, Switzerland). "That mammalian cells are not just the expression systems, but the active principal creates novel challenges."

Autologous treatments require true patient-scale processes that generate multiple doses in parallel. Such manufacturing approaches typically take up to three weeks and involve significant manual product handling. Gottschalk identifies the negatives: "unfavorable cost structures with no volume benefits, complications from human error, and complex logistics to the point of care."

As a result of difficulties in producing individual doses cost effectively, the whole autologous market is unsustainable in its current form, he adds. "This is bad news, especially for patients who could benefit from new breakthrough therapies. In essence, new manufacturing technology is required to tackle these shortfalls. This is a make-or-break issue."

Automation is an obvious means of addressing high production costs, but it is only one part of the solution. Developers must address root causes such as cleanroom space allocation per patient and the currently limited integration of unit operations. "That makes footprint reduction at commercial scale the most important design feature for engineering solutions," Gottschalk says.

Meanwhile, contained systems have emerged that support automated cell expansion and purification in a closed system instead of requiring dedicated classified space. Producing multiple doses in parallel will help as well. For example, Lonza is evaluating Cocoon technology from Octane Biotech. Under monitored and controlled conditions in a single-use cassette, it enables integrated production of a final autologous product from a starting cell population donated by a single patient.

Making CAR-T Cell Immunotherapies Work (continued)

by Mazen W. Karaman

all steps from cell preparation to final formulation and sampling in a closed, sterile single-use tubing set (6). Currently used in clinical applications for stem-cell enrichment and virus-reactive T-cell preparation, the system also has been developed as a platform for CAR-T cell production. It simplifies and improves the robustness of these manufacturing processes, reducing associated personnel and facility costs, and frees up resources for other purposes.

The feasibility of T-cell ACT was first reported over 20 years ago, and we are now poised for significant clinical advances (7). But researchers must tackle associated challenges, including those associated with the manufacturing process, for CAR-T cell therapy to become routine treatment. Platforms enabling automated manufacture are highly beneficial and should help companies make these exciting personalized cellular therapies available to patients who need them. To learn more about the CliniMACS Prodigy system, go online to http://bit.ly/CliniMACS.

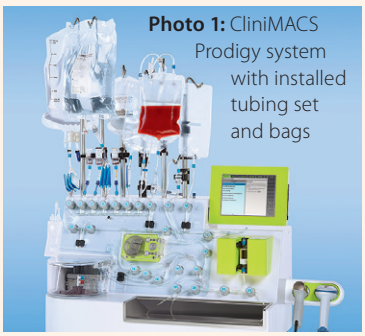

Photo 1: CliniMACS Prodigy system with installed tubing set and bags

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Successful establishment of single-use bioreactor-based manufacturing of allogeneic manufacturing has in addition demonstrated the potential for implementing modern, competitive cell-manufacturing platforms. "Although recent studies show that larger quantities of certain immunotherapy treatments ultimately may be possible, the trend towards personalized medicine and decentralized manufacturing will not go away," says Gottschalk. "Commercial and therapeutic success for patient-scale manufacturing will come from robust and game-changing technologies, some of which are on the verge of implementation."

Bacterial Cell Immunotherapies

Cell-based oncology immunotherapy is not limited to the manipulation of human cells. Axalimogene filolisbac (AF), the lead immunotherapy candidate from Advaxis (Princeton, NJ) for treating cancers associated with human papilloma virus (HPV), is in clinical trials for invasive cervical cancer, head and neck cancer, and anal cancer. AF has received orphan drug designation from the US Food and Drug Administration for each indication. The company's core technology platform uses bioengineered *Listeria monocytogenes* bacteria to generate cancer-fighting T cells. The AF treatment provides two of the most common immunotherapeutic effects: direct activity against a cancer antigen and neutralization of factors that protect tumors from immunologic attack.

In Phase 2 trials, AF treatment resulted in prolonged survival, objective tumor response, and a manageable safety profile in patients with recurrent/refractory cervical cancer. In a Phase 2 study of metastatic cervical cancer, 38.5% of patients survived for 12 months, which constituted a significant improvement over the current standard of care.

Aduro Biotech is also working with live, attenuated *Listeria* for cancer immunotherapies, mainly metastatic pancreatic cancer. The company's live-attenuated, double-deleted *L. monocytogenes* (LADD) technology renders *Listeria* administration safe by eliminating two genes responsible for the bacteria's virulence. Once tamed, *L. monocytogenes* is modified genetically to express tumor-specific antigens. After administration, the engineered bacteria are absorbed by a patient's antigen-presenting cells, including dendritic cells (which are primary initiators of both innate and adaptive immune responses).

Immunotherapies based on bacteria present unique challenges for manufacturing and distribution, says Daniel Platt, executive director of medical strategy at Advaxis. "The critical production issue is maintaining a monoseptic (single-species) environment throughout manufacturing, from inoculation through fill–finish." Monoseptic conditions and *L. monocytogenes* viability must persist through storage and distribution as well. According to Platt, the major barrier to the success of bacteria-based immunotherapy treatments will be educating the public and physicians to overcome the "ickiness" factor associated with bacteria in general. He says his company will achieve that by carefully validating its attenuation methods and otherwise ensuring safety.

Cell Therapy Costs

Over the past 30 years, improvements in manufacturing processes, culture and chromatography media, and expression systems have reduced the cost of manufacturing a gram of therapeutic MAb from ~$10,000 to ~$100. Further innovations in continuous processing, cell engineering, purification, and quality systems could provide a further many-fold reduction in production costs. CoG reductions of such magnitude are rarely seen outside the semiconductor industry.

Contrast the cost-per-dose of antibodies with those of cell therapy products. The autologous therapeutic CAR-T cells manufactured by Novartis, Kite Pharma, and Juno Therapeutics are estimated to have direct manufacturing cost on the order of $20,000–45,000/patient, according to Jason Carstens, vice president of manufacturing at Nohla Therapeutics (Seattle, WA). "Sales prices could be as high as $300,000–500,000."

Clearly, a one-batch–one-patient approach presents great challenges. "It is impossible to enjoy the same advantages of economy of scale as with antibodies, where tens of thousands of doses can be made in a single manufacturing lot," Carstens explains. To modify patients' T cells, manufacturers rely on viral vectors that are themselves challenging and expensive to manufacture, with the potential for very long lead times. "Over time I'm sure companies will eliminate the use of viruses and develop faster and less expensive methods of introducing the CAR constructs," he adds. Robust clinical responses have made cell-based immunotherapy an exciting field, and the possibility of real cures is proposed as justification for why insurers should provide reimbursement for such treatments. "Those hefty prices might be justified if we indeed are talking about a cure," Carstens says, "but the jury is still out on how durable individual responses will be."

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He likes an allogeneic, off-the-shelf cell therapy approach that should enable efficient manufacturing, although probably never on the same level as antibody manufacturing. Each manufacturing run would begin with a vial of cells from a master cell bank, then be used to manufacture many doses of the same product. "The strategy also eliminates the complicated step of having the patient undergo leukapheresis and then transporting the starting material to the manufacturing facility," says Carstens.

Because cell therapy is a nascent field, many biological unknowns related to manufacturing remain, an issue that is analogous to early protein biotherapeutic production. "Having a complex biology, cells are difficult to characterize fully," Carstens explains. "This creates the challenge of identifying markers that are predictive of clinical behavior." Cells are also unique in that they constitute a living product: Once administered, they can grow and divide. "As hard as it is to predict how a well-characterized antibody may behave in vivo when administered to a human," he concludes, "it is orders of magnitude more complex to predict in vivo cell behavior."

Value-Based Pricing, Reimbursement: Robert Preti, president of PCT (a Caladrius company), admits that costs will be higher for cell-based immunotherapies than for protein-based drugs, "but they won't be as high as people think." Pricing and reimbursement will come down to value. Current cancer drugs are mostly palliative, whereas immunotherapies are expected to provide long-term responses and perhaps even cures, which would lead to value-based reimbursements.

The main challenge with cellular immunotherapies will be how to industrialize production. Providing for sterility assurance, precision, repeatability, and robust manufacturing requires highly trained individuals who gown up in cleanroom suits, enter those manufacturing environments, and conduct multiple unit operations according to strict standard operating procedures (SOPs) while maintaining detailed records. "Additional workers, also well trained, must follow them during the process to verify that critical steps were executed properly and conduct manufacturing review," Preti adds. All the while, samples are moving into and out of those cleanrooms while data and test results come out so manufacturing can continue.

Scientific challenges exist as well. Mechanisms of action are not quite as clear with cell-based therapies as for small-molecule and protein drugs. "These heterogeneous products do more than one thing in a patient," Preti says. "In what other medical field can developers abdicate the responsibility of figuring out what their product does inside the body to the product itself?"

For example, scientists know that inserting antigens into dendritic cells, maturing those cells, and administering them to patients allows the cells to transfer those antigens to T cells, which then clone out and attack tumor cells. But many intermediate steps remain mysterious. "We're not really sure how it all happens," Preti admits. Cell therapy companies therefore lack the tools required to improve their products rationally. "All these moving parts make manufacturing quite difficult."

Preti believes that the value of cell-based immunotherapies eventually will help companies overcome both manufacturing and deliverability issues. "If the therapies continue to be transformative, then the rest will follow." But they must be transformative for reimbursement models to adapt to the new paradigm.

He points to another field plagued in its early days by high costs: "There was a time when experts believed biologics would not be deliverable because milligrams cost millions of dollars to produce. Engineers came in and figured out how to create larger lots." Hundred-fold improvements in expression titers followed. "That kind of scale-up is impossible with autologous cell therapies because one lot equals one treatment regimen for one patient," Preti cautions. "With that underlying challenge in the autologous space, the engineering approaches must be different. The objective is not so much to scale up, but to scale out." Under the current manufacturing model, scaling out means building larger cleanrooms and facilities, perhaps modularizing them, and hiring more skilled workers. "But that's a very expensive way to push product out and difficult way to obtain economics of scale."

Preti believes that stakeholders will bite the bullet during the induction phase of this emerging approach to cancer treatment, and that will attract the same kind of engineering talent that helped to create the largest therapeutic biotechnology companies. "Our engineers examine processes and break them down into unit operations, and one by one they fix or improve them." Eventually, working as they did with protein biotherapeutics, they will "build what is the closest we'll ever get to an automated manufacturing system."

When that manufacturing model arrives, it will not be as simple as putting cells into one end of a

machine and getting treatments out the other end. The transformation might require three or four systems, analogous to the culture, harvest, capture, and purification steps of MAb production. But their implementation could lead to the type of industrialization that this industry will require to achieve sustainability. "Manufacturing systems for cell-based immunotherapies eventually will be scalable and sustainable," Preti says, "but it has to move toward more automated processes versus manual."

The Role of Diagnostics

The FDA has approved a record number of new drugs in 2015, including several immunotherapy treatments for melanoma and lung cancer. That has put even more pressure on precision-medicine initiatives to treat the right patient with the right drug at the right time. In addition, with the high cost of cancer immunotherapies (especially combination therapies) and issues related to administering unnecessary or possibly harmful treatments, diagnostics to guide treatment have become a top priority.

For example, about 40% of advanced-stage melanoma patients and 20% of those with advanced lung cancer benefit from immunotherapy. Yet according to data presented at the 2015 Annual Meeting of the American Society of Clinical Oncology, tissue-based diagnostics have proved to be an "imperfect standard." The shortcomings of such diagnostics originate in the technology itself.

To be reliable, immunohistochemistry demands consistent, uniform tissue preparation. "But the techniques themselves are not uniform," notes Richard Hockett, chief medical officer at Biodesix (Boulder, CO). "Pathologists use as many as 10 different protocols to fix tissues, which affects how relevant epitopes are preserved and interact with antibodies." Technique variability thus almost guarantees inconsistent test results among laboratories. Other challenges include identifying the most appropriate cell types for analysis, defining cut-off values for positivity, assessing the consistency of results across fresh and archived tissue, identifying the potential effects of previous treatments, and turnaround times of up to several weeks. Additionally, repeated biopsies may be risky or impossible. "These challenges are not limited to immunotherapies, but apply across the board," Hockett adds. "The path forward is through standards, but these are not generally available for tissue-based diagnostics for immunotherapies."

Measuring specific disease-related biomarkers in blood is a well-established alternative to tissue-based diagnostic methods. Biodesix has developed an interesting twist: to look for rising or falling protein markers that correlate with clinical outcomes. "What we measure are host-response proteins to a tumor, not tumor-specific antigens or molecules," Hockett says. The approach is analyte-agnostic in the sense that any protein is fair game that appears in blood or serum and changes concentration following a treatment. Biodesix uses matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, with a gentle ionization method that preserves the molecular weight of analytes to enable precise measurements. "Our only limitation is the protein's concentration at that mass-to-charge ratio, and how that changes," Hockett explains. "If it doesn't rise or fall between the two clinical conditions we're assessing, then we ignore it."

Biodesix designs tests by comparing the differences in serum proteins between two clinically distinct groups (e.g., patients with long- and short-term survival). Those proteins that distinguish the two sets of patients then can be used to classify patients on the basis of their likely clinical outcomes. During validation, the performance of those classifiers is characterized using a new set of patient samples. The outcome is a multivariate blood test that stratifies patients into those who that are likely to have good or poor clinical outcomes.

At the 2015 annual meeting of the Society for Immunotherapy of Cancer, Biodesix presented initial results for a test in development that could help guide therapeutic choices in the context of immunotherapy. As part of a precision-medicine approach, the company has adapted MALDI-TOF analysis to a serum proteomic test called VeriStrat that is both prognostic and predictive for lung cancer. In addition, Biodesix has a target-mutation profiling blood test called GeneStrat, which uses a droplet digital polymerase chain reaction (PCR) platform to measure sensitizing and resistance mutations for several tumor types. Both tests provide results within 72 hours, enabling physicians to make treatment decisions relatively early in patient care.

Oncological diagnostic tests tend to be specific to particular cancers, but Biodesix believes that certain proteomic-response assays could have broader applicability. The appropriateness of a test for more than one condition depends on similarity of biology and host responses across tumor types. For example, would the mechanisms and biology of

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response to a checkpoint inhibitor in melanoma be the same as in lung cancer? "We can't answer that a priori," Hockett says. "You'd need to assess a test in both tumor types. I suspect that the checkpoint inhibitor (antiPD-1) test we developed will work across tumor types, but we still need to prove it."

Future approved immunotherapies are likely to be developed with companion diagnostics. US regulators and insurance companies have been keen on this idea. Hockett notes although both checkpoint inhibitors Keytruda (pembrolizumab) and Opdivo (nivolumab) have programmed-death ligand 1 (PD-L1) immunochemistry tests available, only the former has an indication (in nonsmall-cell lung cancer) that is limited by the results of such tests.

Formularies are particularly pushing for tests to accompany the most expensive treatments. Hockett notes that those two products are very similar in that they both target programmed cell-death protein 1 (PD-1). I suspect we're going to see formularies tell developers that, without a test to determine who will benefit from an expensive treatment, they will not carry it because it makes no sense to treat nonresponders."

Provenge: A Litany of Errors

If there's a prime example for business-school graduate studies of great science and bad business, then Dendreon's Provenge prostate cancer immunotherapy would be it. "The drug worked, but from a business standpoint it was horribly mismanaged," notes BPI editorial advisor Bryan Monroe of Primus Consulting (Kingston, WA). "It was overpriced, and Dendreon was underprepared for the production costs, delivery logistics, and reimbursement agreements from groups such as Medicare. You don't get a do-over for those things."

Early in 2015, Valeant Pharmaceuticals purchased Dendreon's oncology portfolio (including the Provenge technology) for $495 million. According to Monroe, Valeant would like to expand the label to include treating patients with less-advanced cancer. "That's what the scientists at Dendreon pushed for," says Monroe, who was part of the development team at the time. Dendreon dropped the idea in part because it required monitoring relatively healthy patients for longer periods. Also, cancer treatments tend to be approved cautiously, often for patients who have run out of options — or if not, then as an adjunct to the standard of care.

Regulatory burdens may also have contributed to this product's crash and burn. As the first approved immunotherapy, the Provenge treatment broke new regulatory ground. Final licensing was delayed by FDA concerns, even though the drug had nearly no side effects.

"Provenge might have succeeded had Dendreon teamed with a deep-pocket partner who could have shouldered some burden and assisted with the commercial logistics," Monroe says. "For good reasons, small companies typically don't develop such breakthrough products on their own."

Double Whammy: Dendreon suffered from the double curse of offering an immunotherapy and a personalized treatment. One of the greatest challenges in developing an immunotherapy is delivery logistics. Provenge treatment involved extraction, ex-vivo treatment of each patient's antigen-presenting cells, cell expansion, and reinfusion of those cells. So the treatment was available at only a limited number of centers. Local patient monitoring was possible, but only the study sites could process adverse events.

Additionally, the product was for its time (2010) phenomenally expensive. Dendreon at first planned to price it near the upper limit of targeted oncology drugs (~$65,000), but Provenge treatment hit the street at about $30,000 higher for a course of three infusions. "These treatments will never be cheap because they're more complex," Monroe explains, "not only in terms of the nature of the procedure itself and delivery logistics, but also in the number of touch points."

Some newer immunotherapies targeting PD-1 or PD-L1 targets are under development at Bristol-Myers Squibb, Astrazeneca, Genentech (Roche), and other companies. They target much broader patient groups than Dendreon did in terms of health status, but narrower groups in terms of susceptibility. Nevertheless, companion diagnostics will be critical to the economic and medical success of future immunotherapies.

That peripherally raises the point of perfection-pricing, known as perceived-value pricing in the United States, which to a large degree makes life-saving pharmaceuticals available in nations with less ability to spend freely. This is a topic for another article, perhaps. But developers of immunotherapies (particularly highly personalized ones like Provenge cell therapy) eventually will need to demonstrate unassailable value or adapt their pricing/development models toward greater business sustainability.

"The early immunotherapy market wasn't necessarily smart or expansive about competitiveness," says Bure. A high reimbursement

case for a treatment that adds a few months to a patient's life is difficult to make if an equally effective small molecule is available at much lower cost. Janssen validated this scenario with the release of Zytiga abiraterone acetate, which competes favorably with Provenge immunotherapy.

Moreover, the biotechnology industry must view the entire treatment market for a given treatment rather than approach product development solely based on the uniqueness or novelty of biologic treatment regimens. "Don't just sit in your silo," Bure adds. "Think about what competitors are using to treat your target indication and respond to the entire landscape."

Factors other than costs contributed to the Provenge failure. Bure says logistics were just as challenging and critical to the therapy's success or lack thereof. The leukapheresis, activation, and reinfusion processes required understanding resource networks and proximity. "Dendreon failed to think through their complete production route," she explains.

Criticizing the Provenge launch has become popular, but not every expert is riding that particular bandwagon. "Pointing to Dendreon as an example of a cell therapy failure is unfair," says Jason Carstens of Nohla Therapeutics. "Dendreon is sometimes considered the poster child of what happens when a manufacturing process is ill-designed. However, they were pioneers in the field and didn't have the luxury of a roadmap to follow."

A Success Story?

A kidney-cancer immunotherapy called AGS-003 from Argos Therapeutics (Durham, NC) is a variation on the dendritic-cell idea, but with a twist. The company collects monocytes from a patient and (through a proprietary process) isolates optimized dendritic cells from them. Then it programs those using ribonucleic acid (RNA) from the patient's tumor. After infusion, the resulting cells "instruct" that patient's T cells to attack only his or her own specific tumor. Because it is based on biopsy tissue, AGS-003 captures all the unique mutations or neoantigens found in that tumor. "The job of dendritic cells is immune system surveillance, to capture proteins or fragments, process them and express them on their surface, to educate T cells," says president and CEO Jeff Abbey. Dendritic-cell therapy has shown promise in cancer and viruses as well. Argos has a similar treatment for HIV currently in phase 2 studies.

AGS-003 is unique in that it generates patient-specific T-cell responses that incorporate, among other things, the rapid mutations observed in both cancer and HIV. After five doses, the company believes, disease-specific T-cell responses are quantifiable. "Those responses above baseline correlate with tumor regression and survival," Abbey says. He claims that Argos is the only immunotherapy company to demonstrate a direct link between its product's mechanism of action and clinical benefit. "Before this, it's been a big black box. If you can't show some correlation between your mechanism of action and clinical benefit, then you have a problem."

A strong intellectual-property position is mandatory in the immunotherapy business. For Argos, the RNA technology comes from a patent owned by Duke University (Durham, NC). Also patented are techniques for generating, optimizing, and culturing dendritic cells — all of which are key to obtaining memory T-cell responses that are specific to one patient and one tumor. Argos has developed a strategy for receiving the two components of dendritic-cell programming and for shipping product worldwide. For the RNA isolation, the tumor sample is collected and shipped at room temperature in a preservative that maintains stability for up to 10 days. White cells from which the dendritic cells are isolated are collected at a leukapheresis center and transported in a cold-pack shipper maintained at 4–9 °C. Cell storage life under such conditions is 96 hours. As testimony to the success of these logistics AGS-003, study participants have come from across the United States and Canada, Europe, and Israel.

From typical samples, Argos produces on average enough AGS-003 for 16 doses, covering three years of treatment. Frozen doses keep for five years at liquid-nitrogen temperatures and ship as needed in cryogenic shippers. Doses are thawed at room temperature and injected intradermally. "It's as simple as getting a flu shot," Abbey says. Injection occurs near a draining lymph node under the patient's arm, which is where dendritic cells are likely to encounter T cells. Side effects are similar to those of an influenza vaccine shot: injection site reactions, tender lymph nodes, and flu-like symptoms. "That's exactly what you want to see from an immune response."

Argos expects AGS-003 to be approved as a first-line therapy in combination with Sutent (sunitinib malate), Pfizer's tyrosine kinase inhibitor, which is the standard of care for kidney cancer. In a phase 2 trial, patients with advanced disease survived for 30 months on the combination; based on historical controls, they lived just 15 months with the small

molecule alone. This immunotherapy has an excellent chance of first-line approval because it works with the gamut of tyrosine-kinase inhibitors and thus could help patients throughout the course of their disease. "In a phase 3 trial," Abbey explains, "if a patient switches off Sutent therapy because of disease progression or toxicity, he or she can keep taking AGS-003 and move on to one of the other standard of care therapies."

One potential benefit of combination treatment is that despite its significant toxicity, Sutent treatment enhances immunity to a modest but significant degree by suppressing immunosuppressive regulatory T cells. Another benefit is that in many instances of advanced disease, it produces significant responses in which tumor progression slows, possibly providing an opportunistic environment for AGS-003 to do its work.

Receiving periodic boosters of AGS-003 is an intriguing possibility as a means of managing kidney cancer. At some time after treatment, memory T cells begin losing their ability to attack a tumor, but their potency is restored on subsequent treatments. Patients in deep remission might receive periodic booster treatments every few months to keep their disease at bay for years. Two Argos study patients are in fact alive and leading relatively normal lives about eight years after participating in a phase 2 study. Abbey believes that this strategy could allow cutting back on the small-molecule drug or even taking "holidays" from the toxic treatment.

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