This year marks the 80th Anniversary of the National Cancer Institute, established by President Franklin D. Roosevelt to support research on the causes, diagnosis, and treatment of cancer. Since the 1940s, cancer researchers have produced nothing short of astonishing science.

The development of antibody drug conjugates (ADCs) ranks among one of the most important advancements in cancer treatments in recent history. The ability to precisely target abnormal cells throughout the body and deliver highly toxic drugs to the center of tumors significantly improves upon the negative side effects of traditional chemotherapies that employ a total war approach to defeating cancer.

Anticancer drug development has not come without challenges for pharmaceutical companies that manufacture ADCs. The potency and effectiveness of ADCs are dependent upon engineered nanoparticles (ENPs) — the cytotoxic payload that destroys cancer cells — but little is known about the environmental and human health hazards posed by ENPs. Yet, the promise ENPs hold for patients is why we continue to wield them in the quest for a cure even without a full understanding of their key physical characteristics, chemical properties, and associated hazards.

The National Institute of Occupational Safety and Health (NIOSH) has been a primary champion of safe nanotechnology. Their research suggests that nanoparticle exposure can happen through skin contact or ingestion, but the risk is greatest when the material is airborne and potentially inhaled. As a result, NIOSH recommends that laboratories use high-efficiency particulate (HEPA) filters along with a well-designed exhaust ventilation system to reduce the risk of exposure.

A BRIEF HISTORY of ANTICANCER TREATMENTS

Traditional chemotherapies have always posed serious side effects for patients because they cannot specifically target cancer cells. In the 1960s, “poison” was the general term used for chemical anticancer therapies. The label reflected scientists’ skepticism of the “chemical cure” hypothesis first imagined by Paul Ehrlich at the turn of the century.

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The advancement of cancer therapies has benefitted greatly from early pioneers like Ehrlich. The development of cancer drug screening models by Murray Shear was the first to test an array of compounds for their effectiveness in treating specific cancers. The discovery of hormone therapy in the 1930s by Charles Huggins also expanded treatment options that are still used today in combination with other therapies.

These early contributions to cancer treatments were largely individual accomplishments because there was no general public support for research. That changed in the 1950s with the inauguration of the Cancer Chemotherapy National Service Center (CCNSC). Widely recognized as a turning point in anticancer drug development, the CCNSC was the precursor to the multi-billion dollar cancer pharmaceutical industry. Up until the 1990s, all new cancer therapies were developed by the CCNSC.

It wasn’t until the 1960s that scientists began to conceptualize a cure for cancer, which greatly advanced after Howard Skipper introduced the “Cell Kill” hypothesis. Skipper hypothesized that a given dose of medicine would only kill a consistent fraction of cancer cells, which encouraged a more aggressive use of chemotherapy and dramatically increased remission rates. Through the 1970s and 80s, scientists made even more incredible advancements in the fight against cancer and were officially recognized in 1973 with the establishment of medical oncology.

It is a testament to the dedication of oncologists that, starting in 1990, cancer mortality rates have consistently declined. In 2007, the decline doubled largely as a result of prevention, diagnosis, and advances in cancer treatment.
Chemotherapy is an imperfect treatment that has historically been combined with surgery and radiotherapy along with immune-, hormone, and biological therapies to achieve remission in patients. Of all the available cancer treatments, chemotherapy is the most toxic to cancerous and healthy cells alike causing acute side effects for patients and limited therapeutic results. The limitation of chemotherapy as a treatment option is directly related to the systemic nature of disease.

Cancer spreads throughout the body based on changes in the molecular biology of tumor cells. While advances in research have allowed us to track and anticipate the spread of cancer, traditional chemotherapy cannot precisely target systemic cancers. The chemical composition and size of chemotherapy drugs also make them insoluble and incapable of overcoming biological barriers to reach cancer cells in sufficient concentrations. As a result, chemotherapies can damage a patient’s immune system and other organs, which is compounded by the fact that many patients also experience drug resistance, resulting in reduced dosage and low survival rates.

The history of anticancer drug development has only recently included nanomaterials, but they have quickly shown promise for combating some of the most serious side effects of chemotherapy treatments. This new class of highly potent biopharmaceutical drugs are gaining weight with oncologists in the fight to defeat cancer for their demonstrated ability to target cancer cells, bypass biological barriers, and combat drug resistance.

The success of ADCs is a function of their unique structure that combines the selectivity of immunotherapy with the potency of chemotherapy to create a novel class of anticancer treatments. ADCs are made by connecting an antibody to a cytotoxic agent through a linker that controls the pharmacokinetics, therapeutic index, and efficacy of the drug. Without these three elements, ADCs would not be able to target and kill specific cancer cells. While it may sound simple, manufacturing ADCs is a tricky science. Cytotoxins and antibodies have to be combined in exact ratios, and linkers have to release drugs at precise times in order to achieve their desired results.

Developing targeted anticancer therapies that overcome the characteristic downfall of traditional chemotherapies has been a main goal of pharmaceutical and biopharmaceutical manufacturers since the discovery of monoclonal antibody technologies in the 1970s. Though these ADCs have experienced their own clinical hurdles—low delivery efficiency, the omnipresence of target antigens, and tumor antigen heterogeneity—they largely hold more promise for eventually realizing Ehrlich’s goal of a chemical cure, which wouldn’t be possible without the advent of nanotechnology.

**FIGURE 1**

Antibody–drug conjugate (ADC) timeline. Abbreviations: mAbs, monoclonal antibodies; MDR, multidrug resistance; MTX, methotrexate.

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**REVIEWS**

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NEW TECH on a NANO SCALE

Nanotechnology is the science of small. Nanoparticles are defined as materials that have at least one dimension measuring between 1 and 100 nanometers. The size, shape, and surface area of nanoparticles distinguish them from their macro-cousins and contribute to their high potency. The size of nanoparticles also influences their chemical properties. When combined, the size, toxicity, and solubility of nanoparticles represent the evolution of anticancer drug development.

Nanoscience research combines advancements in engineering and medicine to produce targeted therapies that can more effectively deliver drugs to patients suffering from intractable forms of cancer. Three ADCs have received market approval from the U.S. Food and Drug Administration (FDA). The first ADC to be approved in 2000, Mylotarg® was withdrawn from the market in 2010 after clinical studies had shown that it did not outperform traditional therapies. The initial setback in the development of Mylotarg® may have been more of a premature judgment than proven analysis. More recent studies have shown that using Mylotarg® in combination with other anticancer therapies significantly improves event-free and relapse-free survival in adults suffering from acute myeloid leukemia.

Adcentris® and Kadcyla® have also been approved by the FDA for treatment of two forms of blood cancer and HER-2+ metastatic breast cancer, respectively. Both drugs have demonstrated ability to positively affect survival and remission rates in cancer patients, leading oncologists and drug manufacturers alike to boast a new era of cancer treatment. In addition to targeted ADCs, scientists have also recently advanced bioaffinity nanoparticle probes for imaging and even nanodevices for early detection and screening.

EXPOSURE RISK

Nanoparticles are more effective at fighting tumors primarily because of their toxicity. The smaller-sized particles have more surface area than their larger, macro-cousins. When these potent cytotoxins are introduced into cancer cells, they are capable of delivering higher dose amounts because toxicity is inversely proportional to particle size. It is precisely their size that makes them capable of fighting formerly untreatable cancers, but their size alters more than their potency.

Nanoparticles also act differently than larger molecules with similar chemical compositions, which further expands the range of uncertainty and increases occupational exposure risks.

Nanotechnology is an emerging field. The side effects of exposure to nanoparticles has only been measured in animal...
studies; while these studies are not directly applicable to cases of human occupational exposure, they have proven that nanoparticles are more potent than their macro-cousins. We still do not know, and therefore cannot fully anticipate, all the risks associated with the production of ENPs like those used in ADC manufacturing. However, we do know that exposure to hazardous materials is calculated based on dose size, which is expressed as particle surface area. This means that an equal weight of nanoparticles is potentially more harmful than larger, chemically similar molecules due to the increased surface area exposure alone. Taking into account that the size of nanoparticles alters their chemical characteristics, the near-atomic size of the particles could also pose more adverse health risks.

The small size of ENPs makes inhalation exposure the biggest threat to scientists and technicians who work with and develop ADCs. In addition to their heightened toxicity, nanomaterials can agglomerate into larger particles or longer fiber chains, affecting their properties, behavior, and the exposure risk for humans. Skin can also be exposed to nanoparticles. Our outer layer of skin is only 10 μm thick. While it is difficult for particles and compounds to pass through the outer layer of skin, contact with anthropomorphic substances during nanomaterial manufacturing is a risk that is not fully understood and, therefore, should be managed.

As the production of ENPs continues to grow in response to their successful use in cancer treatments, they will continue to pose hazards for the people who make them. It is acutely ironic that the characteristics of ENPs for which they are so useful—small dimension, large surface area, and high toxicity—also increase the occupational risks associated with their development. As researchers continue to learn more about the risks of occupational exposure to ENPs, we will be able to fine-tune our risk-based assessment guidelines and regulatory decision-making. In the meantime, we can still minimize risks by applying the precautionary principle.

More research is needed to determine the key physical and chemical characteristics of nanoparticles and their associated hazards, but this lack of information is precisely why taking measures to minimize worker exposure is prudent. At the very least, when working with nanoparticles, employers must establish workplace-engineering controls and include effective source ventilation and capture protocol to minimize exposure risk. NIOSH recommends the use of local exhaust ventilation systems and high-efficiency particulate (HEPA) filtration for any workplace task that would increase risk of exposure to nanoparticles.
ADC production requires a laboratory that can provide both product and personnel protection during the initial familiarization phase as well as conjugation, verification, purification, and scale-up. Flow Sciences has designed a comprehensive containment solution that covers the entire scope of ADC development and simplifies laboratory setup.

The Glovebox Workstation was designed specifically for ADC development with a HEPA filtration inlet and Bag-In/Bag-Out technology offering both product and personnel protection for antibody-drug development and conjugation. Our engineers have analyzed all phases of the manufacturing process and designed the Glovebox Workstation to specifically address all of the containment and exposure risks. They have also submitted the Glovebox Workstation to rigorous engineering and performance testing to ensure effective containment.

Manufacturing ADCs requires specialized equipment and careful handling. One of the largest challenges pharmaceutical companies face is the need to balance conflicting requirements for handling antibodies alongside highly potent active pharmaceutical ingredients—HPAPIs or cytotoxins. Maintaining a clean environment is absolutely necessary for successful antibody-drug conjugation just as reducing occupational risk is necessary for a successful laboratory. The Glovebox Workstation guarantees product protection by applying isolator design principles to prevent contamination. Personnel protection is also vital while weighing cytotoxins because they are designed to disrupt cell reproduction and damage DNA, posing significant risks to operators. The Glovebox Workstation provides personnel protection for working with HPAPIs by operating under negative pressure.

In order to meet the requirements for product and personnel protection while accommodating the unique process of ADC development, laboratories typically have to invest in both positive- and negative-pressure enclosures. The cost of equipping a laboratory for ADC production is oftentimes cost-prohibitive, leading some laboratories to shop out ADC production and cede process control to contract manufacturers. Instead of bearing the cost of purchasing multiple enclosures to encompass the complex process of ADC production, the Glovebox Workstation can be used for weighing HPAPIs as well as conjugation, purification, and filling.

Successful ADC manufacturing depends upon thorough control and tracking of molecular-level characteristics, including: drug-to-antibody ratio (DAR), monomer content, drug distribution, and cell killing activity or antigen recognition. It also depends upon designing a process that controls for successful experimental parameters within selected ranges so that the manufacturing of ADCs can be scaled up to grams. Purification techniques that are crucial in the manufacture of ADCs can only be performed on process solution volumes at the gram scale. As production continues to be scaled up for early clinical phases, the manufacturing process ultimately depends upon careful analysis and control during the earlier experimental phases. Turning over this process to contract manufacturers forces pharmaceutical companies to turn over control. The Glovebox Workstation allows companies to save money and keep ADC processing in house.

GLOVEBOX WORKSTATION

The Glovebox Workstation provides negative-pressure containment for toxic applications using HPAPIs requiring isolation that meets or exceeds ISO 5 clean processing. The Glovebox Workstation comes standard with a HEPA inlet that creates a clean environment ensuring product protection; it also uses horizontal laminar flow to reduce turbulent airflow and reproduce consistent, performance-based results. Laminar, or unidirectional, airflow systems direct filtered air in a constant stream, reducing turbulence. Consistent airflow is necessary for limiting exposure risk and ensuring reproducibility.
The Glovebox Workstation is designed to offer both product and personnel protection for an all-in-one approach to safe ADC manufacturing. More features include:

- **HEPA INLET** exceeds ISO 5 requirements for cleanroom classification.
- **BAG-IN/BAG-OUT HEPA** exhaust ensures safe recirculation of air in the room.
- **LAMINAR AIRFLOW** reduces turbulence and allows for consistent, performance-based results.
- **BALANCE STABILITY** to the 7th decimal place makes the Glovebox Workstation ideal for weighing HPAPs like those used in ADC manufacturing.

The Glovebox Workstation has been evaluated by third-party testing facilities that have confirmed containment levels at or below 50 ng/m3 with balance stability to the 7th decimal place. This makes the Glovebox Workstation ideal for antibody-drug conjugation that requires accurate methods and precise measuring.

**ENGINEERING and ADMINISTRATIVE CONTROLS**

The foundation of any effective workplace safety program is establishing risk management programs that include the use of good work practices and appropriate personal protective equipment (PPE). Flow Sciences has partnered with pharmaceutical companies and laboratories that manufacture ENPs and ADCs to design task-specific containment enclosures that minimize product loss and exposure to nanoparticles.

In addition to the applying the patented engineering controls developed by Flow Sciences engineers, both national and international experts on the risks of occupational nanoparticle exposure agree that laboratories can limit exposure levels by implementing a thorough risk management program. By using good work practices and appropriate personal protective equipment, laboratories can limit exposure to nanoparticles and reduce the risks associated with these hazardous materials.

Flow Sciences is committed to partnering with our customers to ensure that they have access to the most effective occupational risk assessments, education and training, and PPE.

There are no specific limits for airborne exposure to ENPs. While occupational exposure limits (OELs) have been set for micro- and macroparticles of similar chemical composition, these limits may be insufficient for recommending protection against exposure to nanoparticles. Applying the precautionary principle, there are a number of additional measures that employers and workers can take to reduce potential exposure to nanoparticles: good work practices like cleaning using HEPA vacuums and wet wiping method; preventing food consumption in the workplace; setting up hand-washing facilities for showering and changing clothes; and proper PPE.

**THE FLOW SCIENCES ADVANTAGE**

We are now closer than ever before to realizing a chemical cure for cancer, with engineered nanoparticles and targeted delivery systems that do not cause the same side effects as traditional chemotherapies. The advancements in anticancer drug research enabled by nanoscience raise exciting opportunities for personalized oncology. Scientists are already beginning to imagine using biomarkers to diagnose patients and develop individualized treatments. As researchers continue to bridge the data gap, it’s important to stay abreast of risk assessments and incorporate those into environmental and occupational health and safety plans.

Nanomaterials present new options for cancer patients who once had little hope, but they also bring with them new challenges that need to be addressed in order to fully realize their potential. Partnering with a company that understands the process and risks of ADC development is crucial for drug developers who are leveraging new technologies and need to manage exposure risks. Responsible development of any new material requires that laboratories manage risks to health and the environment. The engineers at Flow Sciences are experts in containment technology and have worked closely with companies that produce ADCs. You can rest assured that we understand the manufacturing process and the risks involved. We can help you overcome any challenge that stands in the way of developing new life-saving technology.
Flow Sciences’ team of industrial engineers design workstations and enclosures that reduce product contamination and maximize protection for professionals who work with toxic substances and uncertain risks. All of our products are engineered and manufactured at our corporate headquarters in Leland, NC and are backed by our sophisticated design process and award-winning excellence in engineering, including 11 U.S. Government patents. We have worked with pharmaceutical companies, research and development laboratories, manufacturing, and production facilities for 30 years. Our task-specific designs are dynamic solutions that are adaptable to our clients’ workflow and specific needs.

Flow Sciences was one of the first companies in the U.S. to use computational fluid dynamics (CFD) in drafting our enclosures to ensure optimum airflow. Our engineers use CFD algorithms to simulate fluid flows and interactions within contained spaces. This enables us to predict and control airflow through design, which we then test in our state-of-the-art laboratory. Working closely with our clients to mimic real-world applications, we develop testing protocols based on the intended use of our enclosures and measure them against industry-accepted standards to ensure proper containment. We have designed, manufactured, and tested over 13,000 enclosures, generating a wealth of data on situational flow dynamics, which allows us to control for consistency, safety, efficacy, and overall quality.