INTRODUCTION

Oncologists have dreamed of harnessing the target specificity of monoclonal antibodies to deliver cytotoxic drugs to the site of tumors for as long as monoclonal antibodies have existed. This approach would reduce undesirable side effects associated with traditional systemic administration of chemotherapeutic agents which does not differentiate between healthy and diseased tissue and often limits the therapeutic efficacy and potency of small molecule drugs (1). In the intervening three decades since the concept was explored two ADC have been approved with the first approved ADC gentuzumab ozogamcin (Mylotarg®) being voluntarily withdrawn from the US market in 2010 due to post market evidence of lack of efficiency, as well as unacceptable toxicity. A new study presented at the American Society of Hematology (2011) suggest Mylotarg along with chemotherapy, helped prolong the lives of patients just diagnosed with acute myeloid leukemia compared to chemotherapy alone which may lead to a second chance for the drug.

In August of last year, the FDA announced it granted accelerated approval to brentuximab vedotin (Adcetris®), the first of a new generation of Antibody Drug Conjugates (ADCs), which was approved for two indications: Hodgkin’s Lymphoma and Anaplastic Large Cell Lymphoma. Adcetris® is the first new therapy approved for use against Hodgkin’s Lymphoma since 1977 and if the first therapy ever to receive approval for treatment of Anaplastic Large Cell Lymphoma (2). While ADCs represent a unique treatment strategy and offer hope to patients faced with limited treatment options, manufacturing these unique highly potent biopharmaceuticals creates a series of unique engineering, chemistry, logistics and safety challenges. The unique characteristics of this potent new class of oncology drugs requires unique infrastructure to support clinical and licenses product manufacturing.
HYBRID TECHNOLOGY

Combining the specificity of antibody targeting agent with the potency of a small molecule cytotoxin requires a chemical linker. The linker must be stable after introduction into systemic circulation, but after internalization into the target cell, it must efficiently release the cytotoxic drug in its active form (Figure 1). Additionally, the inherent potency of the released drug must be sufficient to kill the tumor cells at a low concentration. Usually this requires very potent drugs with subnanomolar IC₅₀ - the cytotoxins used in ADCs are 100-1000 fold more potent than traditional anticancer agents.

CYTOTOXIC DRUGS USED IN ADCS

Currently several highly potent drugs are being conjugated to humanized mAb targeting agents via cleavable and non-cleavable linkers. These drugs are anti-microtubule agents, alkylating agents and DNA minor groove binding agents which are biologically active at the ng/Kg level placing them in the most potent class of advanced cancer drugs. The cytotoxicity of the maytansine and auristatin drug analogues are related to their ability to inhibit cell division by binding tubulin, which arrest the target cell in the G2/M stage of the cell cycle resulting in apoptosis. The duocarmycin derivatives such as CC-1065 are DNA alkylating agents. Calicheamicin and Pyrolobenodiazepine (PBD) are DNA minor groove binding agents. Pyrolobenodiazepine (PBD) has displayed an in vitro cytotoxic potency against human tumor cell lines at 20pmol/L in cell culture medium (6, 7).

SAFETY CHALLENGE

Potent drugs used to create ADCs present an interesting safety challenge for those engaged in development and clinical product manufacturing of the active pharmaceutical ingredient (API). Lonza has established a 6 band classification system for highly active and potent compounds and ADCs. Unconjugated cytotoxins used in ADC manufacturing are classified as band 5 which refers to both a primary containment strategy that prevents inhalation and skin contact of employees working with these potent cytotoxins and a secondary containment strategy that prevents personnel outside the manufacturing suite area from exposure to the cytotoxins.

The primary containment strategy for the class 5 safety category listed in Figure 3 would consist of handling unconjugated cytotoxin in a closed front isolator cabinet under negative pressure in a room designed for negative pressure relative to surrounding clean rooms. Hermetically sealed process vessels under nitrogen overpressure are then used to execute the antibody modification and conjugation reaction while protecting the valuable raw materials from microbial contamination. The integrity of the process equipment is confirmed prior to process execution to prevent exposure of manufacturing personnel. In the event of a failure of the primary containment envelope, a secondary containment strategy would reduce exposure of manufacturing personnel to cytotoxin by exchanging the air >30 times per hour in the manufacturing suite. Personnel working in the manufacturing area are gowned in one-way clothing which is removed before exiting the protective air locks. High Efficiency Particle Air (HEPA) filters serving the manufacturing area are designed as safe change filters to protect personnel during facility preventive maintenance. As an environmental safety precaution at solid and liquid waste is incinerated on site.

One of the most important aspects of safety however is a comprehensive occupational hygiene monitoring program that uses analytical methods to detect 10⁻⁹ gram levels of cytotoxin in the production suite air and surfaces exposed to cytotoxins.

OVERVIEW OF ADC MANUFACTURING PROCESS

Figure 4 displays a generic process overview of various process steps involved in ADC manufacturing using a non-cleavable Succinimidyl-4-(N-maleimidomethyl) cyclohexan-1-1-carboxylate (SMCC) linker. The Succinimidyl-4-(N-maleimidomethyl) cyclohexan-1-1-carboxylate (SMCC) linker is an amine-to-sulfhydryl crosslinker that contains NHS-ester and maleimide reactive groups at opposite ends of a cyclohexane-stabilized spacer arm. The NHS esters react with primary amines at pH 7.9 to form stable amide bonds. Maleimide reacts with sulfhydryl groups at pH 6.5-7.5 to form stable thioether bonds. The maleimide group of SMCC is stable up to pH 7.5 because of the cyclohexane bridge in the spacer arm (3, 4).

ADC production process using the SMCC linker is characterized by steps controlling the antibody modification (preparing the antibody for the conjugation reaction) and conjugation reaction (introduction of drug moiety) to achieve the desired level of drug loading. The molar ratio of drug to antibody can be adjusted by changing the reaction stoichiometry to deliver the desired level of potency to the target tissue (5). Additional steps such as removal of process related contamination, concentration of the active pharmaceutical ingredient (API) and stabilization of the resulting bulk drug substance (BDS) are also critical steps in the manufacturing process.
Personnel safety and product change-over were important considerations during the design phase of the manufacturing facility.

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The project was reviewed by a cross functional team within Lonza that represented Safety, Process Development and Manufacturing groups. Occupational safety concepts were given a secondary review by an external consulting company to catch any potential safety problems before construction began. Execution of this unique facility design concept was possible because of the high level of synergy between Lonza’s biopharmaceutical and small molecule business units and utilization of existing site infrastructure. Since the facility was designed for multiple-product manufacturing, analytical techniques to detect residual cytotoxin in the 10⁴ gram range were developed so a maximum acceptable carryover (MAC) calculation could be performed at the conclusion of each change-over. The MAC calculation takes into consideration the amount of cytotoxin recovered from various product contact locations in the process equipment and scales the maximum potential carryover based on the product contract surface area used by the new process. Scale-up capabilities must support the product throughout the product life cycle so the facility will need small scale R&D capacity to perform effective technology transfer, scale-up and transition to manufacturing scale. Reactor volumes in the Lonza facility range from 2 ml to 4L in the R&D labs. cGMP facilities can accommodate 10L – 600L depending on BDS demand (Figure 5).

MANUFACTURING ADC(s)

Clinical ADC manufacturing must be executed in an aseptic biological manufacturing environment which operates under cGMP since the antibody modification and cytotoxin conjugation reaction are usually executed under process conditions which will support growth of environmental microbial contaminates. Batch failures resulting from human error or equipment failure are typically low for biological manufacturing. The potential for larger financial losses in ADC manufacturing dictate the need for investment in higher levels of utility redundancy, automation and employee training to further reduce the potential for batch failure. Figure 6 exhibits the suite layout required for effective large scale ADC manufacturing. The figure displays four separate suites required to support the typical ADC manufacturing process as it moves through Lonza’s large scale manufacturing facility.

In the antibody modification suite the naked antibody raw material which is typically frozen at -20°C is thawed and the storage buffer is exchanged via tangential flow filtration. Buffer exchange is often required to alter the pH, salt concentration and remove excipients used to improve storage stability in the bulk antibody solution which may hinder or alter the performance of the conjugation reaction. The red circle indicates the location of the primary containment envelope which isolates process steps with the greatest theoretical risk to manufacturing personnel. These steps include the isolator used for weighing and preparation of the solution containing the cytotoxin and the conjugation suite, in which the cytotoxin is combined stoichiometrically with the antibody linker complex. Bulk ultrafiltration/diffiltration (UF/DF) or chromatography is then employed to remove process contaminants such as free cytotoxin and organic solvent. In the isolator suite cytotoxins can be weighed and solubilized in organic solvent for introduction into the antibody conjugation suite via a hard piped transfer line into the conjugation vessel. The isolator permits safe handling of the cytotoxins in a negative pressure environment and a means of addressing the electrostatic properties of cytotoxins through humidity control. Since the interior of the hood comes in direct contact with potent cytotoxins the hood is designed with a clean in place (CIP) system that permits inactivation of the cytotoxin and/ or multiple detergent and rinse cycles to remove cytotoxin contamination.

In the conjugation suite the coupling between antibody and the cytotoxin is performed in a sealed temperature and pH controlled stainless steel tank with nitrogen over-lay. Special equipment modification in this area such as double mechanical seals, overflow trays and positive displacement pumps with both primary mechanical seals and secondary water seals strengthen the primary containment envelope to protect employees working in the suite. After removal of the process contaminants the buffer containing the ADC is transferred to the formulation suite for transition into the BDS formulation buffer. All liquid and solid waste from the process are then inactivated and incinerated on site depending on process requirements. Following API concentration adjustment and excipient addition in the formulation suite the BDS is then stored frozen at -20 to -60°C using a controlled rate shelf freezer or aseptically dispensed into multiple PETG bottles for bulk storage.
ANALYTICAL SUPPORT FOR ADC MANUFACTURING

Analytics are especially challenging for ADCs because they require expertise in both small and large molecule analytical techniques to support in-process, bulk drug substance release, reference standard preparation and drug product stability testing. Reference standard will need to be created, and these methods will include carbohydrate analysis and various methods to assess the level of deamination, oxidation and adducts which may have formed during the production process (Figure 7). Analytical methods selected for testing BDS are designed to assess the targeting ability of the antibody but must also demonstrate the potency of the cytotoxin. Techniques that determine the amount of process and product related contaminants are critical methods for establishing a predictable manufacturing process. Recent audits of project hours have revealed approximately 58 percent of the total project hours for Technology Transfer are related to developing analytical methods, validation and supporting R&D activities. The same project hour distribution is found with GMP manufacturing in which 55 percent of the total hour are related to QC activities associated with in-process testing and release testing of BDS. Important for multi-product facilities are development of analytical methods which permit detection of cytotoxin in the 10^-9 to 10^-12 gram range for product change-over. Additional complications such as quality control staff testing raw materials and process samples with free cytotoxin require a lab environment designed to prevent potential exposure to highly potent cytotoxins. Laboratories used for testing will require isolators for sample manipulation and analytical equipment with enclosed auto injectors to prevent exposure to aerosols containing free cytotoxin.

RAW MATERIAL SUPPLY CHAIN

ADC(s) have the most extensive supply chain of any biological manufacturing requiring sourcing of an antibody, linker and potent small molecule cytotoxin. The additive risk associated with supply chain synchronization of multiple vendors results in a large increase in the number of project management hours required to assure successful project execution. Most bio/pharmaceutical companies currently developing ADC(s) prefer to use an intact monoclonal antibody targeting agent. The cell bank used to produce the antibody will require extensive virus testing to prove the cell bank is free from adventitious agents. Also the associated downstream purification process for the antibody will require evidence the manufacturing process can inactivate, clear, and remove virus contamination if present. The potent cytotoxins currently used in ADC manufacturing could be semi-synthetic (fermentation derived intermediate) or fully synthetic (chemical synthesis). If a semi-synthetic cytotoxin is required there are a limited number of CMOs with fermentation, recovery and purification suites designed to manufacturer cytotoxic intermediates in the Lonza class 5 category often resulting in availability of the cytotoxin controlling the overall project time line.

The fractional cost of raw materials required for phase 1 ADC manufacturing are contained in (Figure 8) with manufacturing assumptions. Since access to some of the most innovative technology for the new generation of ADC(s) will likely require a licensing fee Figure 7 displays how the raw material cost distribution shifts after applying a 10 million CHF licensing fee.

CONCLUSIONS

Despite reduced research budgets the Pharmaceutical industry appears poised to invest precious resources into ADC(s) since they appear to offer. A dramatic increase in efficacy over naked antibodies while sparing patients from the systemic toxicity that often limits current therapeutic regiments using small molecule cytotoxins. Increased efficacy and decreased systemic toxicity are metrics that will certainly improve the quality of life for patients receiving these novel therapies. Manufacturing ADC(s) and maintaining the clinical supply will require unique infrastructure to provide a safe working environment for manufacturing personnel and a robust raw material supply chain to deliver these unique and lifesaving therapies to patients.

REFERENCES AND NOTES