Figure 1: Manufacturing scientist working in a glove box to weigh HPAPIs used in ADC production

Playing it Safe

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Although antibody-drug conjugates show promise in the development of effective cancer therapies, there are still a variety of challenges involved in enabling the safe manufacture of these hazardous drugs

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Antibody-drug conjugates (ADCs) – anti-cancer drugs which combine the specificity of a monoclonal antibody with the cell-killing efficacy of a highly potent active pharmaceutical ingredient (HPAPI) – continue to gain popularity in the biopharmaceutical industry. As initial trials with these targeted treatments show success, more companies are looking to produce these drugs. However, there are many difficulties that come with manufacturing ADCs – most notably, handling their cytotoxic aspect. This article provides a deeper look at the ADC market and discusses why it is so challenging to implement the technology, as well as why working with a contract manufacturer can help.

Existing Market

Numerous biopharma companies, ranging from Big Pharma to small biotech, have been working to create ADCs for particular cancer treatments. However, there is also significant research exploring the potential of ADCs for other therapeutic areas, such as autoimmune diseases, or the use of conjugates to create novel diagnostics.

The first such ADC drug on the market – Mylotarg® from Wyeth (now Pfizer) – was approved for the treatment of acute myeloid leukaemia back in 2000. While it was withdrawn a decade later in light of concerns over its safety and lack of additional therapeutic benefit over more conventional chemotherapy options, it proved the principle that such drugs represent an opportunity in cancer therapy.

Since the withdrawal of Mylotarg, two further ADCs have reached the market. Adcetris® (brentuximab vedotin) from Seattle Genetics was approved by the FDA in 2011 for the treatment of Hodgkin's lymphoma and systemic anaplastic

large cell lymphoma. The antibody portion, brentuximab, targets the cell membrane protein CD30, delivering the cytotoxic monomethyl auristatin E to the cancer cells. Meanwhile, Genentech's Kadcyla® (ado-trastuzumab emtansine) was given FDA approval in early 2013. This product combines a cytotoxic drug with trastuzumab, the HER2-targeting monoclonal antibody that is also used to treat HER2+ breast cancers under the brand name Herceptin.

In an ADC, the two primary components are joined via a linker that is typically cleavable and will release the small-molecule drug once the antibody has found its target. Yet, each of the marketed ADCs uses different conjugation technologies. Adcetris employs Seattle Genetics' proprietary linker chemistry based on cysteine; while Kadcyla uses the lysine-based method developed by Immunogen. In both cases, the relevant amino acid in the antibody chain is attached to a short linker group, which in turn is attached to the cytotoxic molecule.

Manufacturing Complexity

ADCs are far from straightforward to manufacture. They require specialised equipment and careful handling techniques, representing a huge capital investment. With manufacturing capability having to be in place long before a drug is marketed and bringing in revenue, even if a biopharma company has its own biologics production line, it might be better advised to use a contract manufacturing organisation (CMO) to make its developmental ADCs because of the high cost and added complexity of upgrading facilities. Small biotech companies are rarely in a position to invest in significant in-house manufacturing facilities, particularly those of an extremely demanding and expensive nature. Instead, CMOs can offset that hefty investment requirement against the ability to work for multiple customers, all of whom need these specialised manufacturing capabilities.

The biggest challenge comes with the need to balance the conflicting handling requirements for the antibody and HPAPI components. Standard handling techniques in terms of maintaining sterility are required for both the antibody and final conjugated drug product. This is typically achieved by operating under positive pressure to prevent particles and microbes from entering the manufacturing suite. However, this is not appropriate when working with highly potent compounds, because they must be contained within the suite to prevent exposure of operators to these hazardous materials or wider environmental contamination. This implies that the operations should be carried out under negative pressure.

To overcome this dichotomy, the most hazardous step – the weighing and dissolution of the HPAPI – can be carried out in an isolator located in a separate room, which is under negative pressure. Once the HPAPI is in solution, containment is much more straightforward, as the risk of airborne contamination has been reduced. The conjugation, purification and filling of the ADC can then be safely carried out in other rooms under positive pressure to protect the product's sterility. A single-pass, uni-directional airflow ensures the air within the room meets the required classifications.

Handling HPAPIs is certainly problematic. Approximately each kilogram of ADC will require about 25g of cytotoxic active pharmaceutical ingredient (API) to manufacture. For a molecule with picomolar activity, this is a substantial quantity and represents a significant hazard. The HPAPI will be weighed in an isolator under negative pressure, which is both vented and scrubbed, to prevent the operator from coming into direct contact with the harmful material. The risks are lower – but not completely removed – once the HPAPI is in solution. Therefore, the conjugation chemistry must be carried out in a closed system to contain the hazardous material, with appropriate engineering controls.

Figure 2: Specialised containment equipment is used for handling HPAPIs

Regulatory Implications

Regulators are also taking a greater interest in quality and control of ADC manufacture. This is because there is a growing realisation that there is more to an ADC than an antibody component and a HPAPI. The linker between them must also be taken into account because of its impact on the way the drug is delivered within the cell and its mechanism of action. The ADC as a whole – and every one of its component parts – must be considered when designing a

Figure 3: Manufacturing scientist preparing for conjugation step used in ADC production

manufacturing process and the facility in which to make it. A quality failure in one component part means a quality failure for the whole product.

The FDA and other regulators want reassurance that any company producing ADCs has a complete understanding of the entire manufacturing process, and can supply all the information required to be certain that all systems will be adequately controlled. The ICH guidelines now being implemented are leading to a real push for greater insight into the process from the earliest stages of preclinical development. Therefore, a company would be well advised to work closely with a CMO from the preclinical stage.

Chemical Conjugation

To carry out a conjugation reaction, two chemical processes are usually required. First, the antibody is treated to create sites ripe for conjugation. If the product uses Seattle Genetics technology, cysteine residues will be modified via a partial reduction of their disulfide bonds, releasing thiols. These can be reacted with a suitable group on a linker attached to the cytotoxic payload to create an ADC. The linker in this case is a maleimide-activated peptide.

For Immunogen chemistry, the primary amine of lysine residues within the antibody is coupled to a bi-functional stable amide linker, usually via an N-hydroxysuccinimide-activated ester. As the linker contains a second reactive site, it can then be reacted with a small excess of HPAPI with a suitable functional group to form the ADC.

In either case, this process controls the number of toxin molecules that can be conjugated to each antibody – one for each modified amino acid. The total number for each antibody is usually about four.

Typically, an analytical-scale design-of-experiment optimisation will be carried out for the conjugation process at the outset to ensure the density of modified amino acids is similar across the entire antibody population. The FDA wants tight control of the number of HPAPI molecules conjugated to each antibody because variability affects the way the ADC will behave *in vivo*.

The second reaction, to introduce the HPAPI (or HPAPI-plus-linker combination for Seattle Genetics technology), also requires careful process control. It is important to drive the reaction to completion so that those carefully introduced, modified amino acids are all conjugated. Residual HPAPI will be removed from the conjugate by a purification step. Free toxin must not be left behind in the drug product, as this would pose a real risk to patient safety.

Figure 4: Process development scientist performing a conjugation as part of a technology transfer for an ADC

Decontamination

If a manufacturing line used to conjugate ADCs was dedicated solely to one product, then decontamination between runs would be much less of an issue. A CMO is well-acquainted with the complexities of ensuring that all traces of the previous run are removed from its multi-purpose equipment before the process of making the next product begins.

For HPAPIs, the need is particularly acute because of the extremely small amounts of the molecule required to elicit a biological effect. Comprehensive cleaning protocols must be in place for multiple-use equipment and, ultimately, any material that might be contaminated with the HPAPI should be incinerated.

Protecting the operators as they carry out the decontamination is particularly important. If no HPAPIs are present, then overalls, gloves and safety goggles will suffice as protection. But when decontaminating equipment and working areas where HPAPIs have been handled, a 'moon suit' with self-contained breathing apparatus is more appropriate.

Standard operating procedures must be in place to ensure all traces of previous product are removed via the cleaning protocol. These must also be fully validated via challenge tests to prove their efficacy. Cross-contamination must always be avoided between runs – and for HPAPIs, the potential problems arising from failure in this area are particularly acute.

Future Developments

While the technologies developed by Seattle Genetics and Immunogen are now both well-established and well-understood, future ADC developments will include novel or repurposed areas of technology, which may require additional investments from CMOs working in the area and could benefit from their technical expertise.

In particular, there is a trend toward using even more potent HPAPI payloads in ADCs, and containment and decontamination capabilities will need to be enhanced to address these. There is also interest in using non-monoclonal antibody targeting portions. Whether these are fragment antigen-binding, bi-specific antibodies, or something completely new, manufacturing protocols will need to be designed to address the changed requirements. As drug design moves forward, so process capabilities will need to be advanced to meet the manufacturing needs for the ADC products of the future.

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