'Needle-to-needle': Manufacturing neoantigen peptides

Microwave synthesiser

Trishul Shah of PolyPeptide Laboratories looks at the prospects for individualised peptide therapeutics*

TRADITIONAL TREATMENTS FOR cancer have mainly used surgery, chemotherapy and/or radiation to remove and destroy cancer cells. This means they rely on processes and systems external and foreign to the human biome. Chemotherapy and radiation treatments destroy normal cells as well as cancer cells and they have unpleasant side effects. In the late 1990s and early 2000s, some progress was made through better monitoring and more focused chemotherapy agents. The advent of significantly improved genetic analysis has opened the door to better options. With more understanding of genetic mutations, more individualised treatment can be offered.

Neoantigen peptides

One key area of focus is the use of patient-specific neoantigens to generate or enhance an immune response from the patient's own disease-fighting systems. Peptides called antigens adorn the surface of all cells. Cancer cells are prone to developing mutations that may result in a change in the amino acid sequence to the surface peptides. These 'neoantigens' stimulate the patient's own immune system to produce T-cells that attack cancer cells.

Individualised neoantigen peptide cocktails, synthetically manufactured, are now being explored to treat cancer. During this 'needle-to-needle' process, the tumour cells undergo a biopsy, the cells are subsequently sequenced and, with the use of bioinformatics, patient-specific neoantigen peptides are predicted. The unique cocktail is then manufactured and administered to treat the specific cancer.

During the sequencing of the tumour cell, over 100 different neoantigen peptide sequences are identified but, with the use of proprietary bioinformatics algorithms, the number can be reduced to ten to 80, helping to speed their manufacture. Depending on the type of cancer and the algorithm used, the peptides range in length from ten to 40 amino acids.

Typically, patients would require these peptides to be administered within two months of the biopsy. Thus, up to 80 peptides containing up to 40 amino acids need to be manufactured and formulated in six to eight weeks, whereas in traditional peptide therapeutic manufacturing the typical timeline is five months for the drug substance and two more months to formulate the drug product. There is also very little leeway in the timelines. Manufacturing neoantigen peptides requires a completely different and pragmatic approach, where high throughput, low cost and GMP production are significant.

Co-ordination for success

A high level of coordination is required between the various departments of the manufacturing site. Strong project management is critical to success. To avoid any conflict or disruption to the normal manufacturing environment, it is essential to set up resources and personnel dedicated to and trained for neoantigen peptide manufacturing. The use of GMP-released raw materials is imperative to ensure control and quality of the manufacturing process. Such materials are readily available at organisations that focus on GMP manufacturing, like the PolyPeptide Group.

Quality assurance (QA) plays a key role in the oversight of the manufacturing process, so a close partnership between QA and manufacturing is paramount for high throughput and quality. Standard batch records used in traditional peptide manufacturing do not allow the flexibility and speed needed for neoantigen peptide manufacturing, therefore a simple GMP batch record format needs to be developed and used. In the long term, to avoid transcription errors, the sponsor and the manufacturer can integrate a laboratory information management system (LIMS) to transfer the peptide sequences and the critical quality attributes of the peptides.

Alternative routes

In traditional peptide therapeutics, quantities range from tens of grams to 100kg, depending on the indication. Neoantigen peptides are needed in mg quantities, so automation, which is critical for high throughput and low cost, is more easily implemented. To ensure high throughput, different types of automated synthesisers can be implemented depending on the need – microwave or parallel. The former uses microwave heating to increase the rate of peptide-resin production but each is synthesised individually. The latter can produce up to 24 peptide resins in one go.

With microwave synthesisers, each peptide-resin is processed further while new ones are being synthesised, providing an efficient flow. Those produced from parallel synthesisers are only processed once all of those in a group have been synthesised and the time taken to produce them is significantly longer. This does not offer an efficient flow because not all the peptide-resins in that group can be further processed at once. Any failure in the parallel synthesiser would impact them all, while any failure in the microwave synthesiser would only impact the one under synthesis.

Parallel synthesisers allow in-line cleavage capabilities, whereas separate operations to cleave the peptide-resin are required in microwave synthesisers. Both types have their advantages and disadvantages, and both may work better for different types of sequences. Therefore, both are viable options for neoantigen peptides. It is nevertheless more important for contract manufacturers and peptide synthesiser manufacturers to collaborate closely and thus quickly alleviate any challenges that arise.

Following the cleavage of the peptide-resins, the crude peptides are purified using the semi-preparatory automated purification systems that contract manufacturers use for design of experiment studies. A platform approach is followed, where the purification resin is dedicated to each patient rather than each peptide. Cleaning verification is performed between each patient sub-set. Each purified peptide is isolated on a manifold lyophiliser, with each lyophiliser dedicated to a patient sub-set. This approach permits high throughput and lower costs.

A similar approach should be employed to test the peptides' critical quality attributes. A UPLC-MS method should be developed and qualified to test them all for purity and to confirm identity. Additional testing parameters, such as residual solvents, counter-ion, peptide content, moisture, bioburden and endotoxin, can be tested on a platform basis on a statistical number of batches. The sponsor and the contract manufacturer should agree on a strategy

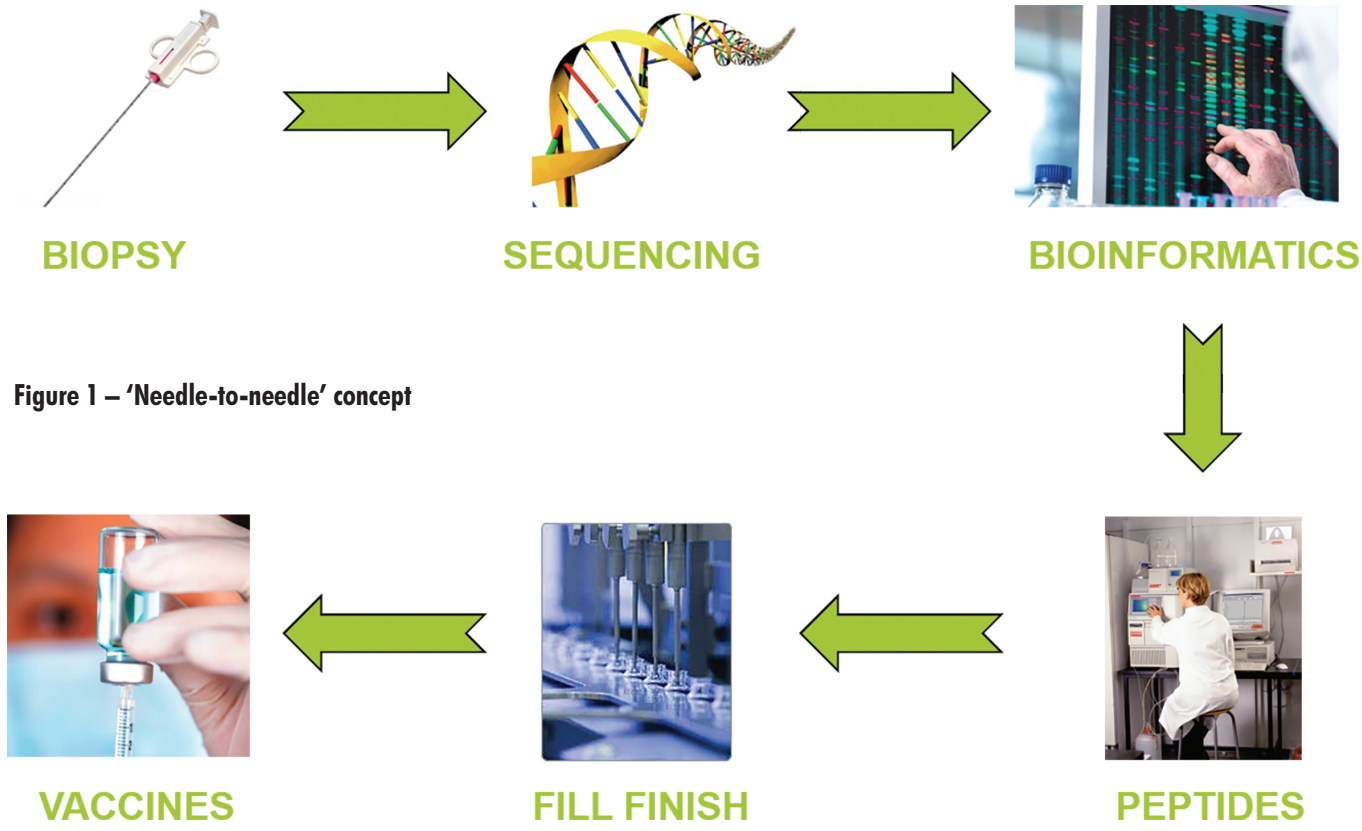

Figure 1 – 'Needle-to-needle' concept

Parallel synthesiser

for these additional tests and understand their impact on throughput and costs.

Additional challenges

As stated above, the number of peptides required per patient can vary from ten to 80. The probability of successful manufacture decreases with increased number, greater length and greater complexity. To ensure the success of the programme, it is important for the manufacturer and the sponsor to align on an acceptable attrition rate, so as to ensure that not too much time is spent on challenging peptide sequences.

To further complicate the matter, these challenging sequences tend to be immunogenic and preferred for stimulating T-cell production. Quite a lot of work is being performed to improve the predictive nature of the bioinformatics algorithms in order to reduce the number of sequences, thus improving the number of shots on target.

Peptide manufacturers can take a similar approach to predict the ease of manufacture and thereby further improve the success rate. For instance, PolyPeptide has proprietary internal algorithms based on data collected over the years that help to predict challenges in manufacturing specific peptide sequences. This allows the organisation to make educated decisions on manufacturing strategies for each individual peptide, increasing the likelihood of success.

The long-term success of individualised peptide therapeutics raises questions about the validation strategy for their manufacture. Late-stage traditional peptide therapeutics manufacturing undergoes 'product' validation, where a full quality by design approach is applied to each individual peptide. This approach would be very time-consuming and costly if applied in individualised peptide therapeutics.

Additionally, using the resources required to validate each individual peptide would not be practical. Rather a platform approach where the overall process is validated would be more appropriate. Once process validation is completed, continuous process verification can be performed to ensure the process is operating within control.

Outlook

Currently, neoantigen peptide treatments are offered in conjunction with standard, first-in-line, traditional treatments for late-stage cancers. If these new individual treatments prove successful, they could be used as a primary standard of care option for a wide variety of cancers. These therapeutics provide a highly fascinating approach to cancer treatment requiring a pragmatic process for 'needle-to-needle'. The success of this process depends on close partnerships between stakeholders, including investigators, sponsors, manufacturers and regulatory authorities, to establish an effective and safe pathway.

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