

Case Study

NeoPre™; GenScript's Proprietary Bioinformatics Tool for Difficult Synthesis of Neoantigen Peptides for Personalized Medicine

Stop Wasting Precious Time on Untrustworthy Neoantigen Peptide Production



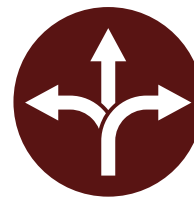
Order GenScript's **Reliable** Neoantigen Peptide Synthesis Service Today!



Trustworthy Partner in Neoantigen Synthesis



High Success Rate for Synthesis of Difficult Peptides



Service Flexibility for Personalized Therapy

I. Abstract

Neoantigen based personalized immuno-oncology drugs are a relatively new therapeutic avenue that have shown strong anti-tumor efficacy in the clinic. Neoantigen peptides have been widely reported to be difficult to synthesize due to their hydrophobicity, length, and charge. Leveraging extensive peptide synthesis experience, GenScript has developed NeoPre™, a predictive algorithm which is able to determine peptide synthesis difficulty based on sequence alone. NeoPre can then recommend the most efficient approach to successfully synthesizing peptides using one of GenScript's many synthesis platforms. This case study will highlight how NeoPre identifies synthesis difficulty and review successful cases of difficult neoantigen peptide synthesis.

II. Introduction

Even with standard methods of care, 55% of the 2 million people diagnosed with cancer this year will unfortunately pass. With this devastating statistic in mind, researchers and clinicians worked together to stray from one size fits all treatments and moved to precision medicine. Personalized immuno-oncology utilizes next generation sequencing technologies (NGS) to determine the genome of a patient's tumor in order to identify unique biomarkers known as neoantigens which can be used to initiate an anti-tumor immune response.

The two most common forms of neoantigen based therapies are personalized cancer vaccines (PCVs) and T-Cell therapy (ATCT). PCV's introduce a range of about 20-50 different neoantigen peptides, DNA, RNA or loaded dendritic cells directly into a patient's lymphatic system in order to induce T-cell mediated tumor killing. During ATCT patient derived naïve T cells are isolated in order to identify those which when bound to a neoantigen, can initiate a strong immune response when reinfused back into the patient. The first stage of either of these therapeutics is to identify neoantigen candidates through comparative NGS between cancerous and wild type tissue to identify the mutanome. After identifying neoantigen candidates through bioinformatics, most researchers will move on to pre-clinical in vitro functional screening of 1-1000's of predicted peptides. Once the top neoantigens are identified for either PCV or ATCT, researchers will move on to drug development and clinical testing. After treatment with either a PCV or ATCT, patient PBMCs will be isolated and incubated with neoantigen peptides in order to analyze the efficacy of the treatment.

Regardless of the therapeutic avenue, one commonality linking all forms of neoantigen treatments is the requirement for reliable neoantigen peptides as they are the only reagent required for therapeutic discovery, development, and efficacy screening. However, neoantigen peptides are commonly quite difficult to synthesize as they can be highly hydrophobic, vary significantly in length and charge, and have a strong tendency to aggregate (Table 1). Therefore, neoantigen peptide synthesis can be a significant bottleneck in the timeline of clinicians generating PCV's and ATCTs. In order to help the neoantigen community circumvent this bottleneck, GenScript has launched their neoantigen peptide synthesis platform featuring their proprietary NeoPre algorithm which identifies synthetic difficulty and recommends the best synthetic technology based on each individual peptide's intrinsic characteristics.

Characteristic	Description	Difficulty
Length	The number of amino acids within a peptide	Neoantigen peptides range from 8-50 amino acids. Peptides over 20 AA are difficult to synthesize
Charge	The isoelectric point (pI) of a peptide chain	Many synthetic steps only work with specific charges, the further from neutral a peptide is, the synthesis will be more difficult
hydrophobicity	Amino acids which naturally repel water due to their charge	The more hydrophobic a peptide, the more difficult purity will be due to its insolubility in water
Yield	The amount of one peptide in mass (mg)	It is very difficult to find a provider who can generate a range of yields for difficult peptides
Purity	The percentage of an individual peptide in a solution of peptides	The charge and hydrophobicity (solubility & aggregation) make standard purification difficult

Table1. The Intrinsic Characteristics of Neoantigen Peptides Which Make Them Difficult to Synthesize.

Neoantigen peptides are usually expressed on the cell surface, and arise due to single amino acid deletions, insertions, or substitutions. Because of this, they vary in length, charge, and hydrophobicity, leading to difficulty in synthesis and purification.

III. Results

A. NeoPre™ analyzes individual neoantigen peptide sequences to determine synthetic difficulty and methodology

GenScript peptide experts place every neoantigen peptide sequence into GenScript's proprietary NeoPre™ bioinformatics algorithm at the start of each project. The algorithm will analyze the individual peptide sequences and convert its intrinsic characteristics to numbers which correspond to how difficult the peptides synthesis will be due to that specific characteristic. The 4 characteristics that NeoPre™ analyzes are tendency of the peptide to aggregate (Na4vSS), isoelectric point (PI), hydrophobicity, and sequence length. Once the algorithm has assessed each peptides difficulty it will determine the predicted difficulty of synthesizing each peptide as well as recommend the best means of successfully synthesizing each individual peptide. Once the GenScript peptide expert reviews the results, they will then adjust the synthetic methodology as needed based off of GenScript's 15 years of peptide synthesis. An example of the read of from NeoPre™ is shown in table 2.

GenScript offers a wide range of neoantigen peptide synthesis technologies including manual; (mankind handling) a combination of solid and liquid phase technologies, semi-automatic; a combination of manual and automatic, and automatic; using machine driven microwave technology. Each synthetic technology is preferred for a specific type of difficult synthesis (table 3). NeoPre™ is able to recommend the best synthetic technology for each individual peptide based off of the numerical values given to each intrinsic characteristic.

length	Na4vSS	PI	hydrophobic	Difficulty	Synthesis Method
24	4.18	11.82977	-0.032	Middle	Semi-automatic synthesis, monitored under all process to ensure coupling efficiency
27	27.2128	6.069031	0.48	Hard	Mankind handling with improved coupling reagent, monitored under all process, involving Mid-control process to detect MS results
16	16.3136	8.430237	0.256	Middle	Semi-automatic synthesis, monitored under all process to ensure coupling efficiency, preparation for a dimer peptide fragment in advance to improve difficult coupling; involving Mid-control process to detect MS results
26	31.6304	10.06195	0.492	Hard	Mankind handling with improved coupling reagent, monitored under all process, involving Mid-control process to detect MS results
18	9.2056	6.616516	-0.12	Middle	Semi-automatic synthesis, monitored under all process to ensure coupling efficiency
11	-11.3712	11.16608	-0.288	Simple	Automatic synthesis, reduced purification time due to unstable terminal amino acid
15	-12.128	9.994324	-0.336	Simple	Automatic synthesis

Table2. Example Read Out of NeoPre™.

NeoPre™ will analyze individual peptide sequence and assign numerical difficulty values for aggregation (Na4vSS), isoelectric point (PI), and hydrophobicity before determining synthetic difficulty and methodology. Amino acids designated in red were shown to have a strong tendency to aggregate. For aggregation, less than 0 is simple, 0-20 is middle or hard, and over 20 is hard, while larger hydrophobicity numbers indicate more difficult sequences

Technology	Use
Liquid-Phase	synthesis of modified peptides
Solid-Phase	preferred for high purity peptides
Microwave	automated for high yield & fast TAT
Ligation	synthesizing peptides up to 200 AA
Recombinant	production with > 3 disulfide bonds

Table3 . Synthetic Technologies for Neoantigen Peptides.

NeoPre™ will predict the best mode of synthesis for each individual peptide based on its intrinsic characteristics. GenScript peptide experts will also review each read out of NeoPre and determine what modifications in synthetic method need to be done in order to successfully synthesize each peptide

B. NeoPre™ has an 86% accurate predictability rate of synthetic difficulty leading to a 95% success rate for difficult neoantigen peptide synthesis

NeoPre™ is able to analyze the numerical values assigned to individual characteristics of each peptide in order to predict the synthetic difficulty. Overall, GenScript has used NeoPre™ for 171 peptides within this case study, of those 71 were shown to be difficult to synthesize. Of the 71 difficult peptides, 61 (86%) were actually difficult to synthesize while 10 (14%) were actually easier to synthesize than predicted. Digging deeper, 100 peptides were shown to be easy to synthesize, with 78 (78%) of them accurately predicted and 22 of them actually difficult to synthesize. With this tool, those 83 total difficult peptides were able to be synthesized despite their hydrophobicity, length and aggregation (table 4).

Predict/Actual	Easy	Difficult	Total	Accuracy Rate
Easy	78	22	100	78%
Difficult	10	61	71	86%
Total	88	83	171	82%

Table 4. Prediction Accuracy of Synthetic Difficulty Using NeoPre™.

Overall NeoPre™ has an 82% accuracy rate, with a higher 86% accuracy rate for difficult neoantigen peptides. Predictions are based off of intrinsic characteristics such as length, charge, hydrophobicity, and aggregation.

Since using NeoPre™, GenScript has been able to synthesize 2,158 out of 2,267 peptides ordered, giving an overall success rate of 95.15%, 37.5% of which were delivered using GenScript's Rush peptide Service within 67 days. Neoantigen peptides tend to either be focused on epitopes presented on MHCII/III which range from 9-15 amino acids. For this length, GenScript has synthesized 1,835 out of the 1,869 peptides ordered, giving an overall success rate of 98.18%, with the majority of these peptides being deemed as difficult. Synthesizing larger peptides over 20 amino acids always tend to be more difficult and require more attention in synthetic methodology and take slightly longer. Because of this, GenScript has synthesized 85.31% of ordered large peptides (Table 5).

	Total	Delivered	Successful rate	Avg TAT (Days)
short (<19 AA)	1869	1835	98.18%	10
Difficult	957	937	97.91%	12
Easy	912	898	98.46%	8
long (>20 AA)	429	366	85.31%	19
Difficult	158	123	77.8%	23
Easy	271	243	89.67%	15
Total	2267	2158	95.15%	
Difficult	1098	1030	93.81%	36% ≤ 6 days
Easy	1169	1128	96.49%	39% ≤ 6 days

Table 5. Synthetic Ability of Neoantigen Peptides Using NeoPre™.

GenScript has delivered a total of 2,158 neoantigen peptides with a 95.15% success rate. Of these peptides, 1,030 were deemed as difficult to produce, and still 36% of them were able to be delivered within 6 days.

V. Conclusion

With GenScript's 15 years of peptide production experience, NeoPre™ was designed to analyze neoantigen peptide sequences the same way in which GenScript's neoantigen peptide experts would. The main difference, is that through the bioinformatics algorithm, every individual peptide is tracked and used to help suggest the synthetic technology used for future peptides based on experience. Also, thanks to NeoPre™, neoantigen peptides can be produced quickly, as time is of the essence for these crucial personalized medicine reagents. GenScript's neoantigen peptide synthesis service offers the technical means & experience to synthesize large numbers of challenging neoantigen peptides and provide tailored optimization tools for your downstream applications. Allowing them to generate Reliable neoantigen peptides!



For More Information Check Out Our Website at
www.GenScript.com/neoantigen-peptide-service.html