A Fast & Low Cost Process for Neo-Epitope Based Immune Monitoring

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Cancer neo-antigens are considered optimal targets for truly individualized cancer immunotherapy. However, development and application of patient-specific cancer vaccines pose a challenge for fast, flexible and cost-effective immune monitoring of T-cell responses. Most immune monitoring protocols use short synthetic peptides originating from each vaccine target antigen. In contrast to the detection of antigen-specific T-cells specific for shared tumor associated antigens, for which the same peptide batch can be used for all patients monitoring of individual neo-antigen-specific T-cell responses requires the availability of different set of peptides for every single patient. Standard commercial peptide synthesis cannot accommodate this need in the capacity and speed required for large trials. Here, we present ex-vivo ELISPOT data monitoring neo-antigen-specific immune responses using peptides produced by JPT's high-throughput, low-cost FastTrack™ peptide synthesis method.

Introduction

Advances in genomic technologies have paved the way for the development of personalized cancer vaccines targeting neo-antigens. Patient-specific cancer vaccines (PCV) targeting several neo-antigens at the same time are being evaluated in clinical studies. Such a personalized, multi-target approach poses a challenge with respect to monitoring vaccine-induced T-cell responses in a fast, flexible and cost-effective fashion.

Most immune monitoring protocols use 9-15 amino acid (aa) long synthetic peptides derived from the vaccine target antigen. In contrast to the detection of antigen-specific T-cells from shared tumor associated antigens, for which the same peptide batch can be used for several patients, the monitoring of individual neo-antigen-specific T-cell responses requires large numbers of peptides in small amounts, as they can only be used for one single patient only. For example, patients participating within the IVAC® MUTANOME trial [1, 2], were vaccinated against 10 individual neo-antigens formulated as mRNA, each translating into a 27 amino acid long peptide. For the monitoring of each study participant 10 sets of 4 overlapping 15 aa peptides were required, each set of 4 peptides covering one neo-epitope (Figure 1).

Standard commercial peptide synthesis not only lacks the capacity and speed required for large trials of this kind, but is also too costly.

Here, we present ex-vivo ELISPOT data on the monitoring of neo-antigen-specific immune responses using peptides produced by our high-throughput, low-cost FastTrack™ peptide synthesis method. Results are compared with those obtained with peptides generated by standard methods with several different specifications.

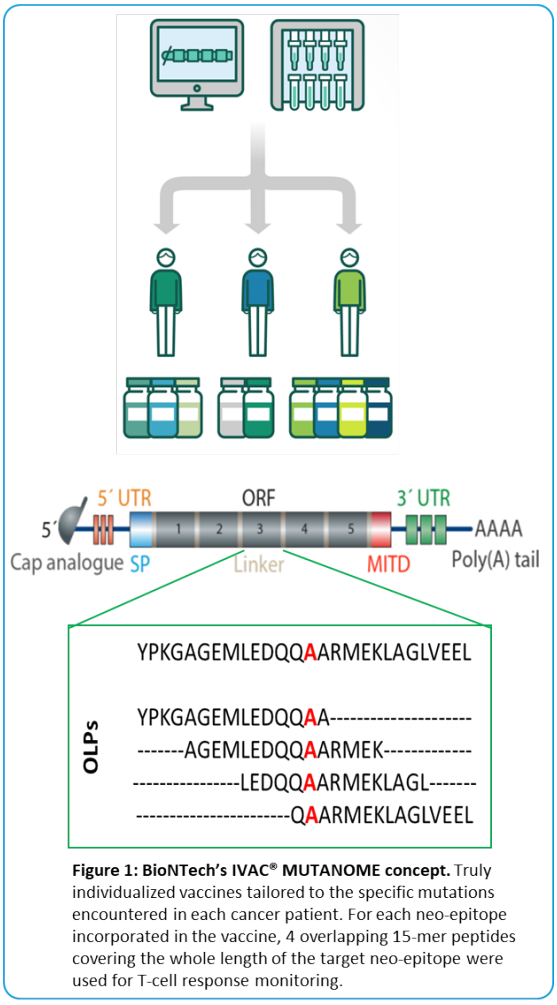

Figure 1: BioNTech's IVAC® MUTANOME concept. Truly individualized vaccines tailored to the specific mutations encountered in each cancer patient. For each neo-epitope incorporated in the vaccine, 4 overlapping 15-mer peptides covering the whole length of the target neo-epitope were used for T-cell response monitoring.

Materials & Methods

Standard Peptide Synthesis: Synthesis was performed on a Syro II peptide synthesizer (MultiSyntech) using Fmoc-based solid phase technology using a TentaGel PHB resin with PyBOP (5 eq.) activation and a double coupling protocol (2 x 30 min). Peptide resin cleavage with TFA/water/EDT (94/3/3) was followed by peptide precipitation in diethyl ether and purification (where applicable) by RP-HPLC. Purity was determined by HPLC-MS analysis.

FastTrack Peptide Synthesis: Synthesis was performed on fully automated SPOT-synthesizers. A cellulose membrane was loaded with the individual C-terminal amino acid for each peptide. After Fmoc de-protection and washing, the next activated subsequent amino acid was spotted to the membrane in a computer controlled fashion. De-protection, washing and coupling cycles were repeated until full length peptides were assembled. Following side-chain de-protection peptide spots were transferred into microtiter-plate wells, peptides released from the membrane, analyzed and quantified by HPLC-MS. Finally, peptides were aliquoted and dried.

Ex-vivo ELISPOT: Cryopreserved PBMCs were used in an overnight (16-20 h) ELISpot assay after a resting period of 2-5 h at 37°C following thawing. $3x10^5$ cells/well were tested in triplicates. Cells were either left untreated or stimulated with peptides of different quality. Plates were scanned using CTL's ImmunoSpot® Series S five Versa ELISpot Analyzer (S5Versa-02-9038) and analyzed by ImmunoCapture V6.3 software.

Results

25 peptides originating from 7 mutations in 4 IVAC Mutanome patients have been tested in the ex-vivo ELISPOT setting. 4 different peptide specifications were compared for each peptide sequence:

- Crude (unpurified), standard peptide synthesis
- Purity >70%, standard peptide synthesis
- Purity >90%, standard peptide synthesis
- FastTrack, unpurified, SPOT peptide synthesis

As shown in Figure 3, peptides synthesized using the FastTrack approach as well as those synthesized using the standard approach performed equally well in the ex-vivo ELISPOT by stimulating T-cell responses in case of immunogenic peptides (e.g. 004-M05A2-ID02 and 004-M05A2-ID02 shown in the ELISPOT picture below) without inducing any non-specific response in non-immunogenic peptides (e.g. 004-M05A2-ID01 and 004-M05A2-ID04).

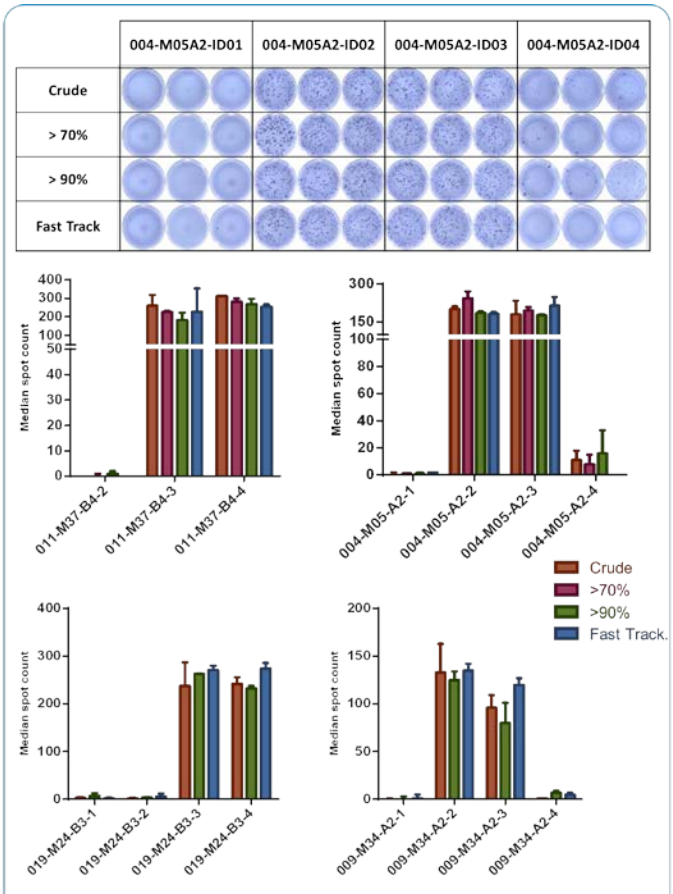

Figure 3: **Ex-vivo ELISPOT data.** Exemplary ELISPOT data, as well as a summary of data obtained in 4 patients is shown. Bars represent spot count median values for each triplicate.

Discussion & Conclusions

The study shows that the FastTrack peptide synthesis approach provides peptides of sufficient quality for immune monitoring. It is fast, inexpensive, and gives flexible access to thousands of peptides. This novel peptide synthesis method may provide batches of up to 1.200 peptides in less than 3 weeks at a fraction of the cost of standard peptides which makes it the ideal choice for individualized neo-epitope based immune monitoring.

References

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The Company

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