Sequence analysis
nextNEOpi: a comprehensive pipeline for computational neoantigen prediction

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Abstract
Summary: Somatic mutations and gene fusions can produce immunogenic neoantigens mediating anticancer immune responses. However, their computational prediction from sequencing data requires complex computational workflows to identify tumor-specific aberrations, derive the resulting peptides, infer patients’ Human Leukocyte Antigen types and predict neoepitopes binding to them, together with a set of features underlying their immunogenicity. Here, we present nextNEOpi (nextflow NEOantigen prediction pipeline) a comprehensive and fully automated bioinformatic pipeline to predict tumor neoantigens from raw DNA and RNA sequencing data. In addition, nextNEOpi quantifies neoepitope- and patient-specific features associated with tumor immunogenicity and response to immunotherapy.

Availability and implementation: nextNEOpi source code and documentation are available at https://github.com/icbi-lab/nextNEOpi

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction
T-cell mediated recognition of tumor neoantigens is pivotal for the success of anticancer immunotherapies (Schumacher et al., 2019). Thus, in silico prediction of patient-specific neoepitopes from whole-exome (WES), whole-genome (WGS), and RNA sequencing (RNA-seq) data is a fundamental task in immuno-oncology. To this end, complex computational pipelines must be assembled to predict tumor-specific, mutated peptides and their likelihood of binding the patients’ Human Leukocyte Antigen (HLA) molecules and being recognized by T cells (Finotello et al., 2019; Wells et al., 2020). In addition to neoantigens derived from single-nucleotide variants (SNVs) and insertions or deletions (indels), gene fusions can be a source of noncanonical neoantigens (Yang et al., 2019).

In recent years, several pipelines for the prediction of neoantigens have been developed (see recent review, Finotello et al., 2019), but most of them require cumbersome software installation and extensive data preprocessing with third-party tools to predict somatic mutations and HLA types. Moreover, to the best of our knowledge, most of the available pipelines are not able to predict class-II and noncanonical neoantigens, or to extract features associated to anticancer immune responses like mutation clonality and immune-cell receptor repertoires (Supplementary Table S1). Here, we present nextNEOpi (nextflow NEOantigen prediction pipeline), a fully automated and comprehensive computational workflow that overcomes these shortcomings. nextNEOpi predicts class-I and -II neoantigens originating from SNVs, indels and gene fusions through the analysis of raw sequencing data and derives a set of features associated with tumor immunogenicity and response to immunotherapy.

2 The nextNEOpi pipeline
nextNEOpi takes as input raw WES or WGS data from matched tumor-normal samples and, optionally, bulk-tumor RNA-seq data (Fig. 1 and Supplementary Fig. S1). After data preprocessing, nextNEOpi derives germline and phased somatic mutations, copy number variants, tumor purity and ploidy, and selects high-confidence variants through majority voting (Supplementary Methods).

nextNEOpi infers class-I and -II HLA types from WES/WGS (DNA-seq) and RNA-seq data using OptiType (Szolek et al., 2014) and HLA-HD (Kawaguchi et al., 2017), respectively, and can employ an RNA-seq-informed strategy to correct DNA-seq calls for
missing HLA genes or alleles (Supplementary Methods). HLA typing benchmarking using data from the 1000 Genomes Project confirmed the high performance of OptiType and HLA-HD, especially on RNA-seq data (Supplementary Figs S2–S4). DNA-seq calls showed a lower accuracy, a systematic underestimation of zygosity and a lower number of missing calls likely due to the low sequencing depth of WGS data (~4–29 million reads per sample), which were improved using the RNA-seq-informed approach.

nextNEOpi exploits tumor purity information to derive the cancer cell fraction (CCF) and clonality of mutations and resulting neoantigens. Tumor mutational burden (TMB) is computed as the number of somatic mutations over the entire read-covered genome, with the former having higher CCF 5% confidence interval ($P = 0.017$) and probability of being clonal ($P = 0.024$). Clonal neoantigens were enriched in patients responding to immune checkpoint blockers, whereas subclonal mutations were associated with a single patient (Pat_8) with progressive disease (PD).

The investigation of TMB and diversity of immune receptor repertoires can provide further insights into the antigenicity of the tumors and immune-cell infiltration and expansion in the single patients (Supplementary Fig. S6).

4 Conclusions

textnextNEOpi is a comprehensive and fully automated pipeline that predicts tumor neoepitopes from raw sequencing data. It is implemented in Nextflow to ensure easy installation and usage, as well as high portability, scalability (see example computational times in Supplementary Table S7), and reproducibility. nextNEOpi quantifies neoepitope- and patient-specific features associated with tumor immunogenicity and response to immunotherapy, and uses multi-method consensus approaches to guarantee robust results in case of suboptimal data. In the near future, we plan to extend nextNEOpi to other classes of noncanonical neoantigens and to reduce ESL 2 Nextflow syntax to facilitate the integration of additional features relevant for neoantigen prioritization.

Data availability

References and accession numbers to the published data analyzed in this work are reported in the Supplementary Material.

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3 Analysis of TESLA data with nextNEOpi

To benchmark nextNEOpi, we considered WES and RNA-seq data from two cohorts of melanoma and non-small cell lung cancer patients ($n = 8$) generated by the Tumor Neoantigen Selection Alliance (TESLA) initiative (Wells et al., 2020). nextNEOpi predicted 30 912–364 532 putative HLA-binding peptides (pMHC) per patient, spanning 5 152–90 819 unique peptides (Supplementary Table S5). The identified pMHC represented 76.40–92.59% of the total immunogenic pMHC. In total, 36 over 38 immunogenic pMHC were identified. Prioritization of candidate neoepitopes based on relaxed filtering (Supplementary Methods) resulted in the identification of 32 over 38 immunogenic pMHC (Supplementary Table S6).

We considered all pMHC experimentally assessed by TESLA to investigate the features associated with immunogenicity. All scores related to HLA-binding affinity of the mutated peptides were strongly associated with immunogenicity (Supplementary Fig. S5): immunogenic peptides showed a lower IC50 ($P = 5.8e–11$) and percentile rank ($P = 1.6e–10$), whereas the expression of the mutated gene was higher for immunogenic peptides ($P = 9.9e–5$). Clonality features showed different distributions for immunogenic and non-immunogenic peptides, with the former having higher CCF 5% confidence interval ($P = 0.017$) and probability of being clonal ($P = 0.024$). Clonal neoantigens were enriched in patients responding to immune checkpoint blockers, whereas subclonal mutations were associated with a single patient (Pat_8) with progressive disease (PD).

The investigation of TMB and diversity of immune receptor repertoires can provide further insights into the antigenicity of the tumors and immune-cell infiltration and expansion in the single patients (Supplementary Fig. S6).

Fig. 1. Schematization of nextNEOpi pipeline with main input data (white boxes), data flow (lines), intermediate (grey boxes) and final (grey boxes with borders) outputs.