ImmuneMirror for evaluation of the genomic and transcriptomic features of resistance to immunotherapy for gastrointestinal tract cancer: abridged secondary publication

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KEY MESSAGES

1. We developed an integrative ImmuneMirror pipeline to evaluate tumour mutation burden, microsatellite instability status, human leukocyte antigen type, predicted neoantigen load, top-ranked neoantigens with T cell immunogenicity, and expression of innate anti-PD-1 resistance signatures.
2. We established a web server incorporating a machine learning model for neoantigen prediction and prioritisation.
3. In gastrointestinal tract cancers (including colorectal cancer, oesophageal squamous cell carcinoma, and hepatocellular carcinoma), an elevated neoantigen load was associated with good clinical outcomes in patients with oesophageal squamous cell carcinoma and poor clinical outcomes in patients with hepatocellular carcinoma.
4. A subset of microsatellite instability–high colorectal cancers with lower neoantigen load was shown to exhibit an advanced T stage.
5. The neopeptide YMCNSSCMGV derived from the TP53 hotpot mutation G245V restricted by HLA-A02 was identified in a patient with oesophageal squamous cell carcinoma. Experimental validation revealed high binding affinity between HLA-A02 and TP53G245V (YMCNSSCMGV).

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Introduction

Gastrointestinal tract (GIT) cancers have become the leading cause of cancer mortality in Hong Kong, responsible for 29.6% of cancer-relevant deaths. In particular, colorectal cancer (CRC), oesophageal squamous cell carcinoma (OSCC), and hepatocellular carcinoma (HCC) are among the top 10 major causes of cancer-related death. Clinical outcomes for patients with advanced-stage cancer metastasis remain poor.

The Food and Drug Administration has approved the clinical use of programmed death-1 (PD-1)/programmed death-ligand 1 (PDL-1) inhibitors (pembrolizumab and nivolumab) for metastatic CRC and gastric cancer. Nonetheless, response rates to these immunotherapies remain low in unselected patients; most patients only achieve a partial response. Thus, it is important to understand the mechanisms of resistance and identify biomarkers that predict treatment outcomes, thereby facilitating patient stratification.[1]

Multiple mechanisms are involved in resistance to cancer immunotherapy.[2] Although key genomic and transcriptomic features (mismatch repair deficiency; mutations of specific genes such as *BRCA2*, *B2M*, *JAK1/JAK2*, *PTEN*, *AKT1*, and *EGFR*; and enrichment of innate anti-PD-1 resistance signatures) have been associated with treatment efficacy,[2,3] the use of multiple standalone and web-based tools to extract these features complicated next-generation sequencing data analysis. Moreover, a lack of user-friendly integrative tools hinders the translation of these valuable findings into relevant clinical trials. Therefore, we established an all-in-one computational framework (ie, ImmuneMirror) that incorporates benchmark tools to characterise key genomic features and gene expression signatures associated with responses to PD-1/PDL-1 inhibitors.

Methods

In total, 805 normal-tumour paired GIT cancer samples were analysed. Whole-exome sequencing data and RNASeq data were obtained from various archives.

The ImmuneMirror web server was implemented using the Shiny web framework.

Various R packages were used to design the interactive web interface. The R package *emayili* was used to send automatic emails from the server to users.

To build a prediction model for neoantigen identification, we first gathered neopeptides (from 19 studies) with experimentally confirmed T cell responses as the training data. The class distribution of the dataset was extremely unbalanced due to the low proportion of T cell-positive neoantigens. Consequently, conventional classification algorithms were expected to bias towards the minority class, resulting in poor prediction performance. Therefore, we used random forest learning algorithms, which were evaluated using the area under the receiver operating characteristic curve (AUC). We evaluated synthetic minority over-sampling and balanced sampling techniques for the random forest method; the estimated AUCs for the test set were 0.8294 and 0.8679, respectively. The balanced random forest method outperformed the other method, indicating that it was the optimal model for neoantigen prediction; thus, it was implemented in our computational platform.

The accuracy of identifying human leukocyte antigen (HLA) types at 4-digit resolution was assessed using the benchmark dataset from the 1000 Genomes Project, which included 271 Asian samples with known HLA typing results. The accuracies of HLA type identification were 98.3% for class I alleles and 85.3% for class II alleles. We evaluated the accuracy of microsatellite instability subtype prediction in ImmuneMirror, compared with The Cancer Genome Atlas database for CRC patients. The sensitivity, specificity, and accuracy were 97.37%, 99.56%, and 99.25%, respectively.

Results

We developed the ImmuneMirror pipeline to evaluate tumour mutation burden, microsatellite instability status, HLA type, predicted neoantigen load, top-ranked neoantigens with T cell immunogenicity, and the expression of selected gene signatures (Fig 1). The pipeline can support Linux, Mac, and Windows operating systems. We implemented the machine learning model to prioritise neoantigens for HLA class I. Final outputs of the pipeline included germline and somatic estimated tumour mutation burdens, microsatellite instability status, HLA type, predicted neoantigen load, top-ranked putative neoantigens with T cell immunogenicity, and the expression of selected gene signatures. The ImmuneMirror pipeline can be obtained from the GitHub repository (https://github.com/weidai2/ImmuneMirror/).

We also developed the ImmuneMirror web server to identify potential neoantigens restricted by major histocompatibility complex (MHC) classes I and II molecules. Users can upload a VCF file, specify a set of alleles for both MHC classes I and II, and select peptide lengths via the interface. A URL link to download the results is automatically emailed to the user. The web server is freely available at http://immunemirror.hku.hk/App/.

We tested the pipeline on a Linux operating system (Ubuntu 20.04). One pair of samples with 13 threads required 24 hours of processing time. Moreover, we used the pipeline to process multiple pairs of samples with different types of cancers. Users can use the pipeline to process a list of samples; the actual processing time is dependent on the computation speed and resources of the user's device. For successful pipeline implementation, we recommend using a device with at least 64 GB of

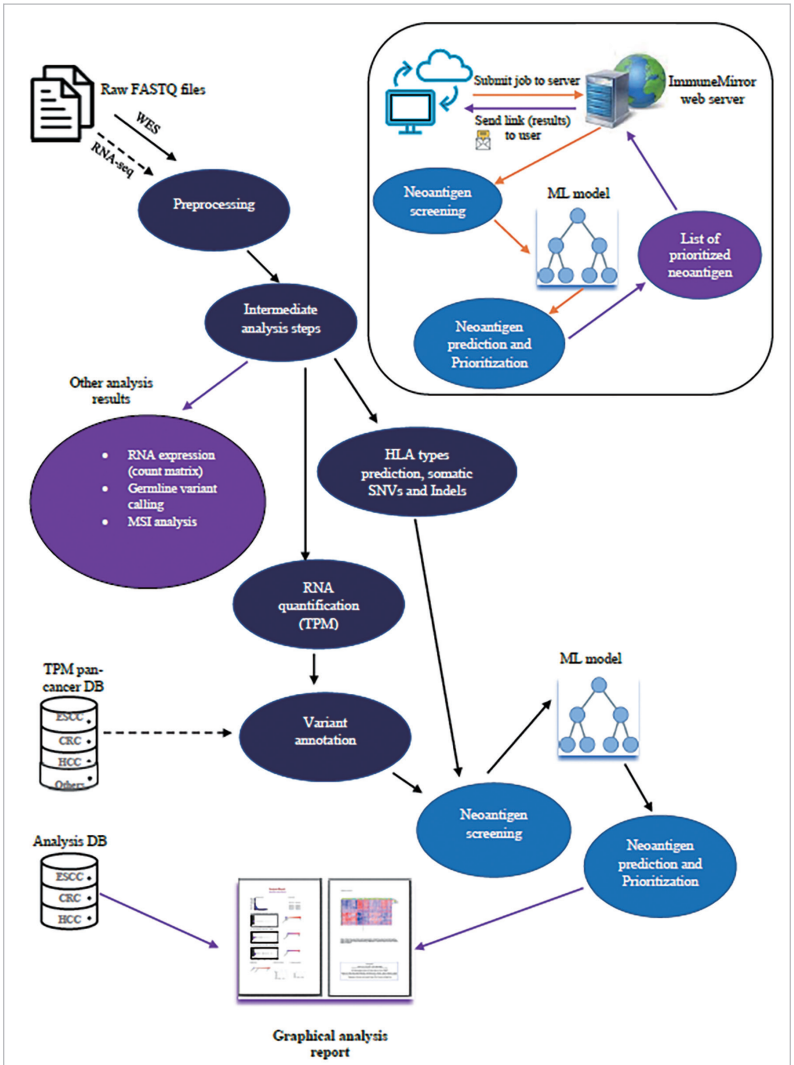

FIG 1. ImmuneMirror workflow: raw FASTQ files are processed for human leukocyte antigen (HLA) subtype prediction, single-nucleotide variant and Indel detection, variant annotation, and neoantigen prediction and prioritisation. A graphical analysis report is generated for each patient. The web server accepts VCF files as input, and a web link to the analysis results (list of prioritised neoantigens) is emailed to the user.

Abbreviations: CRC = colorectal cancer, HCC = hepatocellular carcinoma, and ESCC = oesophageal squamous cell carcinoma

TABLE. Comparison of bioinformatics tools currently available for neoantigen prediction

Index	Software	Input	Raw data type	Source	Method for prioritisation	Docker image	Web server/app	Class I prediction	Class II prediction	Multiple prediction methods
1	ImmuneMirror (current study)	FASTQ, VCF	Whole-exome sequencing, RNASeq	Mutation	✓	✓	✓	✓	✓	✓
2	NeoPredPipe	VCF	-	Mutation				✓	✓	
3	MuPeXI	VCF	-	Mutation			✓	✓		
4	TSNAD	FASTQ	RNASeq	Gene fusion		✓		✓		
5	pVAC-Seq	VCF	-	Mutation	✓	✓		✓	✓	✓
6	CloudNeo	VCF, BAM	RNASeq	Mutation			✓	✓		
7	TIminer	VCF, FASTQ	RNASeq	Mutation	✓			✓		
8	INTEGRATE-Neo	FASTQ	Whole-genome sequencing, RNASeq	Gene fusion				✓		
9	Neopepsee	FASTQ, VCF	RNASeq	Mutation	✓			✓		
10	Vaxrank	VCF, BAM	RNASeq	Mutation				✓		
11	OpenVax	FASTQ	Whole-exome sequencing, RNASeq	Mutation	✓	✓		✓	✓	✓
12	TruNeo	FASTQ	Whole-exome sequencing, RNASeq	Mutation, gene fusion	✓			✓		✓
13	ScanNeo	BAM	RNASeq	Indels				✓		✓
14	NeoFuse	FASTQ	RNASeq	Gene fusion				✓		

RAM and sufficient storage for the pipeline (80 GB), its supporting files (483 GB), and analysis results directory (45 GB for one pair of samples). Input/output file formats and detailed instructions are provided on the website.

We compared the bioinformatics tools currently available for neoantigen prediction (Table). Only ImmuneMirror has all six features (prioritisation method, docker image, web server, MHC class I prediction, MHC class II prediction, and multiple prediction algorithms). ImmuneMirror accepts inputs of raw fastq files from both RNASeq (tumour) and whole-exome sequencing (matched normal-tumour pairs) data. Similar to pVAC-Seq, ImmuneMirror can be used for neoantigen prediction restricted by MHC classes I and II, whereas pVAC-Seq accepts VCF files only. ImmuneMirror accepts VCF files that contain somatic mutations detected by MuTect2 for identification of potential neoantigens from both MHC classes I and II molecules.

We collected a total of 805 samples of GIT cancers. After quality checking, we analysed 691 samples with valid data, including 316 CRCs, 290 OSCCs, and 85 HCCs. On average, ImmuneMirror identified 17 (range, 0-316), 5 (range, 0-76), and 6 (range, 0-64) neoantigens per patient with CRC, ESCC, and HCC, respectively. Importantly, the neoantigen load was associated with favourable clinical outcomes and longer overall survival in patients with OSCC, whereas it was associated with poor clinical outcomes and shorter overall survival in patients with HCC ($P<0.05$). In patients with CRC, although the neoantigen load was not associated with overall survival, we identified a subgroup of patients with mismatch repair deficiency who had much lower neoantigen loads for both MHC classes I and II (Fig 2); these patients exhibited an advanced T stage (T4 vs others, 30.8% vs 0%, $P=0.011$).

We compared neoepitopes in 10 cancer-related genes with hotspot mutations and identified 12 putative neoepitopes derived from TP53, STAT3, and RAB35 that demonstrated high affinity with HLA-A*02:01, HLA-A*11:01, HLA-A*33:03, HLA-A*33:01, HLA-A*03:01, and HLA-A*02:06 HLA alleles. The neoepitope $TP53^{G245V}$ (YMCNSSCMGV), which was restricted by HLA-A*02, was identified in a patient with OSCC (Fig 2). This mutation affected the binding of p53 to DNA and interfered with the protein's transcriptional activity. RNASeq data indicated that this mutant was widely expressed in tumour tissue.

Conclusion

ImmuneMirror is reliable and effective for identification of genomic and transcriptomic features

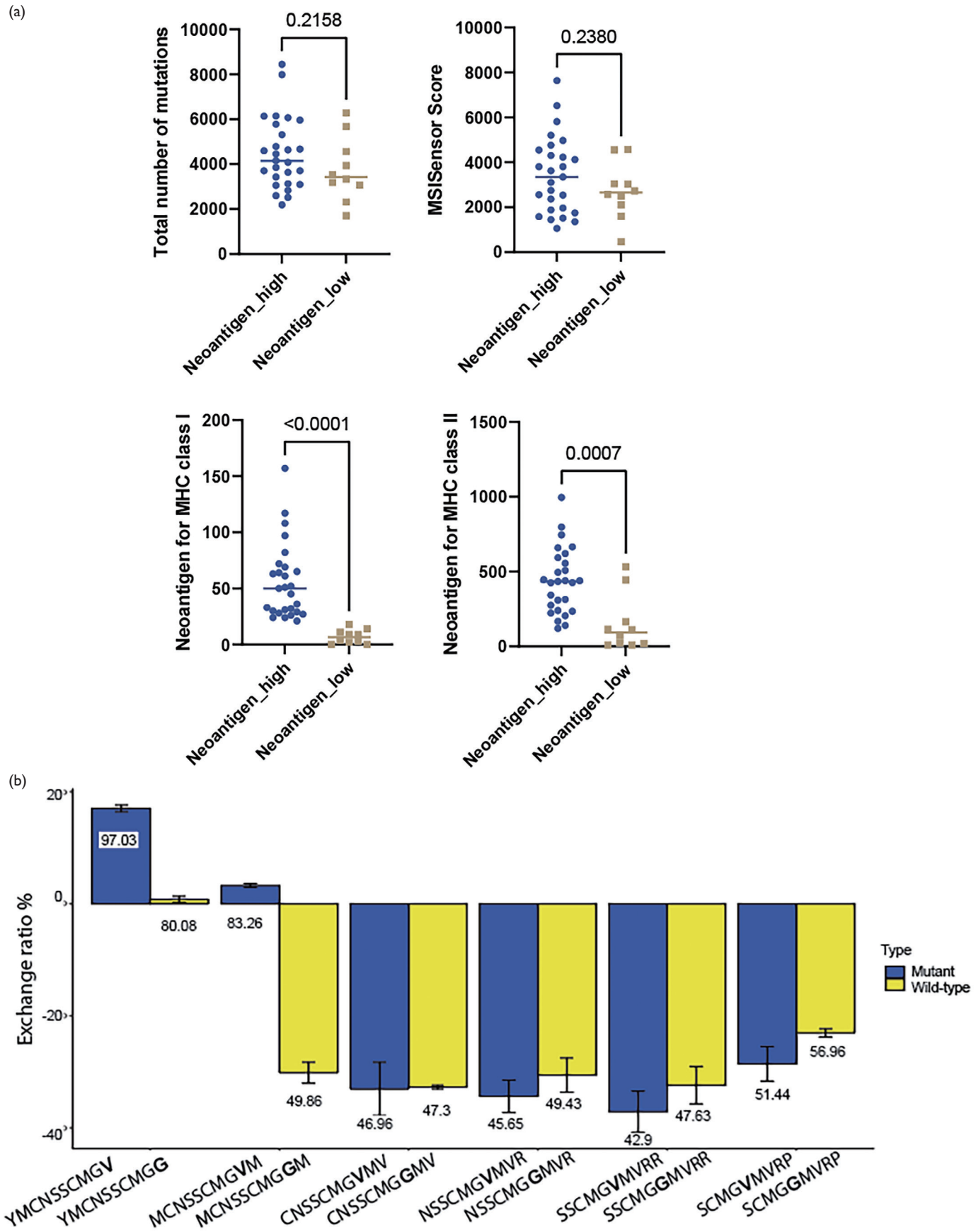

FIG 2. (a) A subgroup of patients with colorectal cancer who have mismatch repair deficiency and low neoantigen loads for major histocompatibility complex (MHC) classes I and II. (b) Validation of binding affinity between human leukocyte antigen–A02 and neoepitopes derived from TP53 mutations. Exchange ratios of $TP53^{G245V}$ mutants to wild-type peptide are shown (>80% is the cut-off for positive values).

associated with responses to immunotherapy. We developed a machine learning model to predict putative neoantigens and identify neoantigens derived from hotspot mutations that can serve as actionable targets in cancers.

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Disclosure

The results of this research have been previously published in:

1. Chuwdhury GS, Guo Y, Chiang CL, et al. ImmuneMirror: a machine learning-based integrative pipeline and web server for neoantigen prediction. Brief Bioinform 2024;25:bbae024.

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