Exploring novel T-cell antigens through AI-driven genomic analysis in breast cancer

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This research was conducted as part of Japan's national whole-genome sequencing initiative, the "Action Plan for Whole Genome Analysis 2022", supported by AMED.

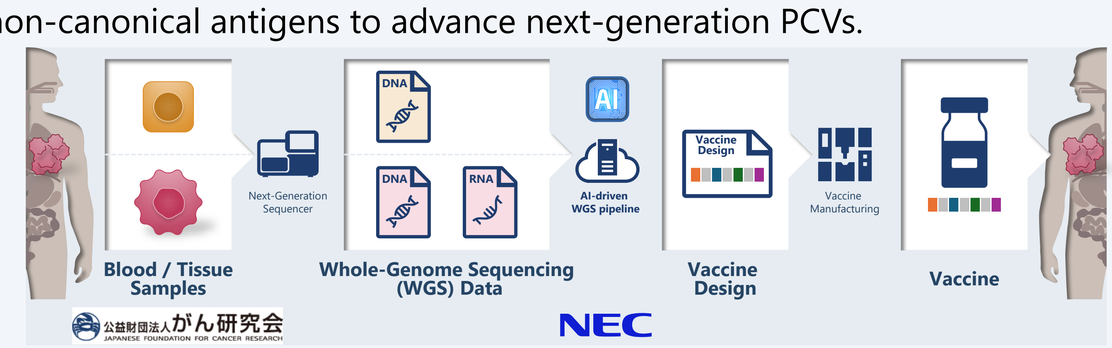
1. Introduction

- Personalized cancer vaccines (PCVs) are promising novel immunotherapy.
- In the phase II KEYNOTE-942 trial[1], the addition of a PCV to anti-PD-1 therapy improved recurrence-free survival in melanoma, and several phase III trials are now ongoing[2-4].
- Vaccine development has mainly focused on canonical antigens derived from SNVs and indels identified by whole-exome sequencing (WES).
- Recently, non-canonical antigens, including those arising from splicing variants and exon-transposon junctions, have been reported as potential targets[5-6].

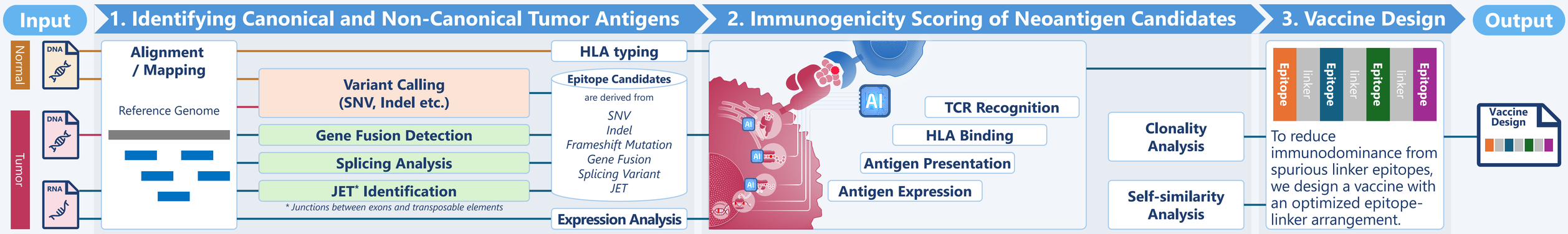
1) *Lancet.* 2024;403:632-644. 2) *JCO* 42, TPS9616 (2024). 3) *JCO* 42, TPS8116 (2024). 4) *Cancer Res* 2025; 85: CT251. 5) *Nature* 639, 463-473 (2025). 6) *Nat Rev Cancer* 24, 123-140 (2024)

2. Objectives

- To develop and apply a Whole-Genome Sequencing (WGS)- and RNA-seq-based AI-driven pipeline for the comprehensive prediction for canonical and non-canonical antigens to advance next-generation PCVs.



3. Methods



Data from 43 patients with breast cancer who participated in the national WGS project at our institution.

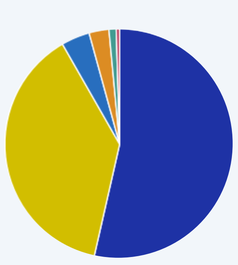
- **Canonical antigen:** generated by SNV, In-frame insertion/deletion, or frameshift mutations that result in abnormal protein expression with potential immunogenicity, and are usually detected by WES.
- **Non-canonical antigen:** Derived from gene fusion, splicing variant, or exon-transposon junctions (JETs), not necessarily mutation-associated, but resulting in aberrant protein expression.

4. Results

Patient Characteristics (n=43)

Median age; years (range)	63（24-80）
Sex, female; n (%)	43 (100)
Settings; n (%)	
Stage I-III	32 (74.4)
Metastatic/Recurrent	11 (25.6)
Subtype; n (%)	
Luminal	23 (53.5)
TNBC	14 (32.6)
HER2	1 (2.3)
Unknown	5 (11.6)

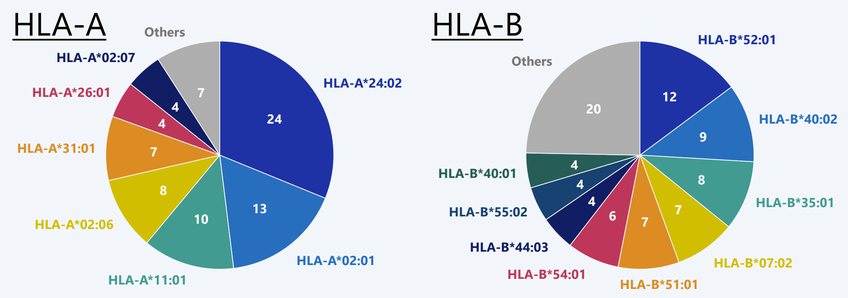
Distribution of the Detected Variant Types



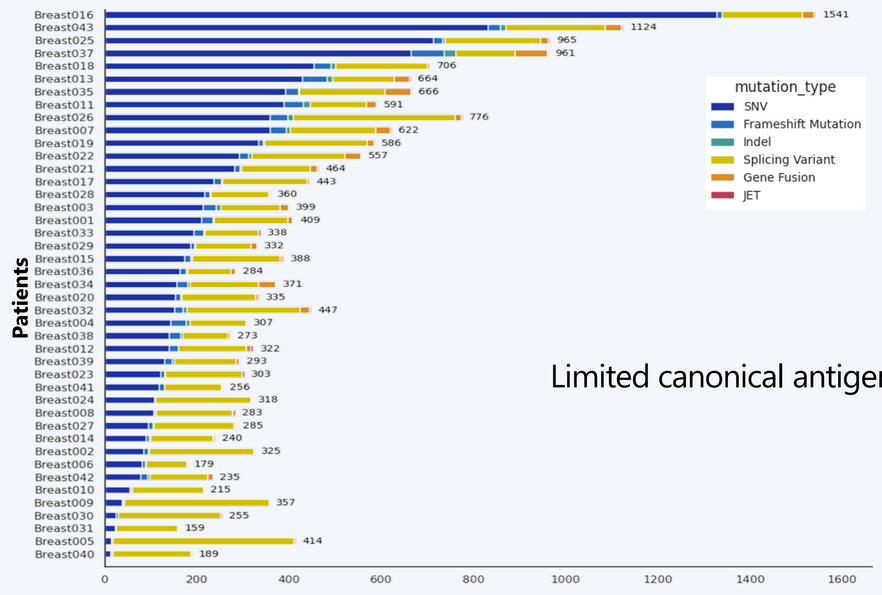
Variant Type		
SNV:	53.6%	(10468)
Splicing Variant:	38.1%	(7446)
Frameshift Mutation:	4.0%	(784)
Gene Fusion:	2.8%	(547)
Indel:	1.0%	(199)
JET:	0.5%	(93)

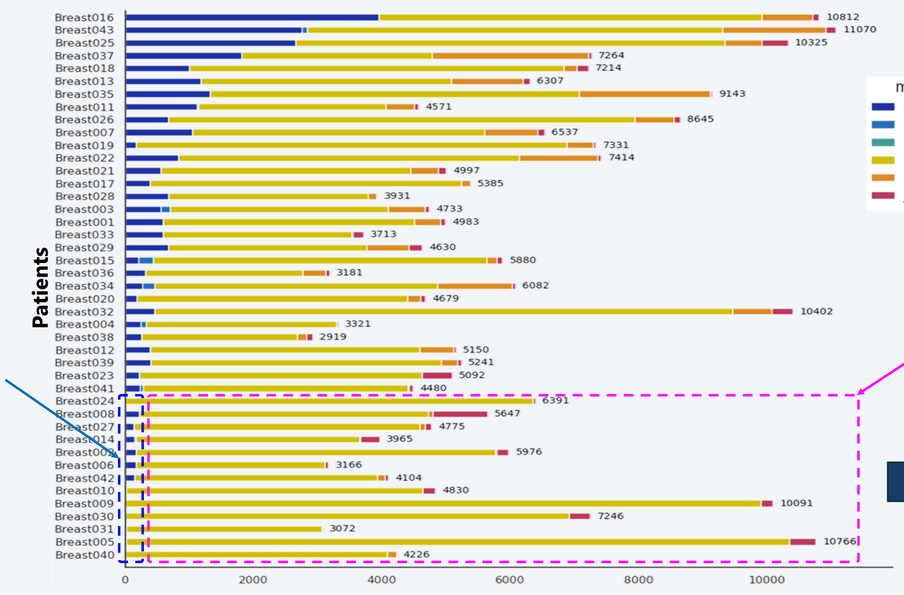
A total of 19,537 variants were identified, with SNVs being the most prevalent. Splicing Variants, Gene fusions, and JETs were also observed.

HLA allele frequency



Patient-Level Analysis of Variant Detection and Epitope Derivation





Even patients without canonical antigens may have the opportunity to receive personalized cancer vaccines through our pipeline that can identify both canonical and non-canonical antigens.

5. Conclusion and Further Studies

- We developed a WGS- and RNA-seq-based AI-driven prediction pipeline.
- Our pipeline identified numerous non-canonical antigens beyond WES.
- These findings may contribute to the development of next-generation PCVs targeting both non-canonical and canonical antigens.

Future work:

- Validate the immunogenicity of non-canonical antigens in vivo/ex vivo.
- Conduct clinical trials of next-generation PCVs incorporating canonical and non-canonical antigens predicted by our pipeline.

Acknowledgements

We would like to express our sincere gratitude to all participants and collaborators involved in this study. Special thanks to our colleagues and mentors for their valuable support and guidance throughout the research process. This research was supported by AMED under Grant Number JP24ck0106869.

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