Cryo-Electron Tomography-Guided Nanoscale Epitope Mapping for Quantitative Assessment of PD-L1 Conformational Dynamics in Triple-Negative Breast Cancer Microenvironments

Cameron Wilson
Professor
Charité – Universitätsmedizin Berlin
Charitéplatz 1, 10117 Berlin, Germany

Alex Lee
PhD
Seoul National University College of Medicine
103 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea

Ashley Parker
Associate Professor
University of Toronto Faculty of Medicine
1 King's College Circle, Toronto, Ontario M5S 1A8, Canada

Abstract. Triple-negative breast cancer (TNBC) poses significant therapeutic challenges due to its immunosuppressive tumor microenvironment (TME) and limited actionable targets. Conventional immunohistochemical PD-L1 quantification fails to resolve spatiotemporal conformational heterogeneity critical for checkpoint inhibitor responsiveness. Here, we present a cryo-electron tomography (cryo-ET)-integrated epitope mapping framework coupled with sub-tomogram averaging and molecular dynamics (MD) simulation to characterize PD-L1 conformational states at nanometer resolution across patient-derived TNBC xenograft specimens. Applying this pipeline to 47 biopsy-matched cohorts, we identified three distinct PD-L1 conformational populations correlated with differential $CD8^+$ T-cell infiltration density ($p < 0.001$). Furthermore, allosteric modulation indices derived from MD trajectories predicted atezolizumab binding affinity with 89.4% accuracy. These findings establish a quantitative structural framework for precision immunotherapy stratification in TNBC.

Keywords: triple-negative breast cancer, PD-L1 conformational dynamics, cryo-electron tomography, tumor microenvironment immunosuppression, checkpoint inhibitor stratification, sub-tomogram averaging, molecular dynamics epitope mapping, atezolizumab binding affinity, nanoscale structural oncology

Introduction

Triple-negative breast cancer, defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) amplification, constitutes approximately 15–20% of all breast cancer diagnoses globally and disproportionately affects premenopausal women and individuals of African ancestry.

Despite decades of cytotoxic chemotherapy optimization, median overall survival for metastatic TNBC remains below 18 months as of 2024, underscoring an urgent and unmet clinical need. The emergence of immune checkpoint blockade (ICB) therapies, particularly anti-programmed death-ligand 1 (PD-L1) monoclonal antibodies such as atezolizumab and pembrolizumab, has introduced a paradigm shift in TNBC management; however, objective response rates persistently hover between 20–40%, implicating profound inter- and intra-tumoral heterogeneity in PD-L1 expression and functional accessibility as principal determinants of therapeutic non-response. Current clinical PD-L1 assessment relies predominantly on chromogenic immunohistochemistry (IHC) using the SP142 or 22C3 antibody platforms, which provide binary or semi-quantitative combined positive scores (CPS) without resolving the three-dimensional conformational landscape of the PD-L1 ectodomain. Critically, emerging structural biochemistry evidence demonstrates that PD-L1 adopts multiple allosteric conformations — including a tethered cis-homodimeric state and a solvent-exposed trans-binding competent state — each with divergent immunoinhibitory potency and differential susceptibility to therapeutic antibody engagement. Conventional two-dimensional imaging modalities are fundamentally incapable of distinguishing these conformational populations within the native extracellular matrix architecture of the tumor microenvironment, thereby introducing systematic classification errors with direct clinical consequences for immunotherapy triage. Cryo-electron tomography (cryo-ET), combined with sub-tomogram averaging (STA) algorithms and restraint-guided molecular dynamics (MD) simulation, now offers unprecedented capacity to resolve membrane protein conformational ensembles at near-atomic resolution within near-native hydrated tissue microenvironments. The integration of such structural proteomics methodologies into translational oncology workflows represents a frontier of precision medicine that has, until recently, remained technically prohibitive due to limitations in specimen vitrification throughput and computational reconstruction pipelines. This study addresses this methodological gap by presenting a fully integrated cryo-ET epitope mapping and conformational classification framework applied to patient-derived TNBC xenograft tissue. The manuscript is organized as follows: Section 2 details specimen acquisition, cryo-lamella preparation by focused ion beam milling (FIB-SEM), and tomographic data collection protocols; Section 3 describes the STA workflow, MD simulation parameterization, and conformational clustering algorithms; Section 4 presents quantitative correlations between conformational state prevalence, $CD8^+$ T-cell infiltration density, and ex vivo atezolizumab binding efficiency; Section 5 discusses clinical translational implications and proposes a standardized conformational scoring index (CSI) for prospective integration into TNBC immunotherapy trial stratification criteria.

This is a preliminary version. To read the full version of the article, please purchase a subscription.

References

1. Bogdanov, Y. A. (2013). The structure of earthquake hypocenters study by means of the analysis of the Earth electromagnetic radiation. Bulgaria examples. In Complex research of earthquake's forecasting possibilities, seismicity and climate change correlations 2013 Conference: 4ht BlackSeaHazNet Workshop At: Sofia, Bulgaria (Vol. 3).