## Unveiling surface Tumor-Associated Antigens across Diverse Immune Scenarios for Osteosarcoma

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INTRODUCTION: Tumor development and drug resistance are intricately influenced by the tumor immune microenvironment and tumor-associated antigens (TAAs). However, the identification of universally applicable TAAs across diverse immune scenarios, named uiTAAs, is a challenge, attributed to immunerelated biases in the cancer transcriptome.

METHODS: To address this challenge, we introduce a Cell-Surface Antigen Targeting (CSAT) system that integrates machine learning techniques to identify TAAs. Applied to osteosarcoma (OS), a tumor thriving within an immune-rich bone marrow microenvironment, we employed the CSAT system on 73 human primary OS transcriptomes. Clinical information and RNA-seq counts of 73 primary osteosarcoma patients were obtained from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database.

RESULTS SECTION: This approach yielded 153 potential surface uiTAAs universally relevant across varying immune infiltration levels. Bioinformatics analysis highlighted their association with immune systems and G-protein-coupled receptors. Literature scrutiny revealed that these CSAT findings were predominantly assessed in cancer studies, with a subset evaluated in OS contexts. From these, 26 surface uiTAAs (CD34, CXCR4, KDR, GGT1, PTPRC, CD68, FOLH1, CSF3R, CD86, ITGAM, CSF1R, CD163, TNFRSF9, HAVCR2, CSF2RA, IL21R, CDH5, SPN, IFNAR2, CX3CL1, PTAFR, FLT4, CASR, CXADR, LPL, and ITGB4) emerged as high-priority therapeutic targets, with 23% (KDR, CSF3R, CSF1R, CSF2RA, IFNAR2, and FLT4) currently under investigation in ongoing OS clinical trials, underscoring CSAT's accuracy and adaptability. Moreover, external datasets confirmed the heightened expression of selected uiTAAs in OS patients and cell lines. Notably, multivariate analysis showcased a significant correlation of all 153 uiTAAs with OS.

DISCUSSION: These discoveries hold implications for enhancing comprehension and treatment strategies in OS and potentially other malignancies.

SIGNIFICANCE/CLINICAL RELEVANCE: Our CSAT system effectively identifies potential surface uiTAAs, offering prospects as OS diagnostic markers and for immunotherapeutic interventions.

## REFERENCES: NA

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## IMAGES AND TABLES:

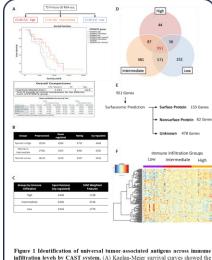
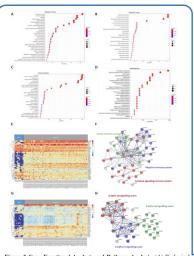


Figure 1 Identification of universal tumor-associated antigens across immune infiltration levels by CAST system. (A) Raplan-Meier survival curves showed the median overall survival was negatively correlated with immune infiltration levels in OS patients with p-value = 0.019. (B) DEG analysis was conducted in 3 separate groups by immune infiltration levels, upregulated genes were selected to proceed to SYM binary classification. (C) Linear SYM was conducted by 3 groups, the highest weighted features were extracted after SVM voting. (D) A Venn diagram was infiltration group. (E) Surfaceonic prediction was conducted, the results showed 133 genes that were identified as surface proteins. (F) The heaturap of 133 selected genes through CSAT system, and showed similar expression trend among different immune infiltration groups (log2 transformed gene expression presentation).



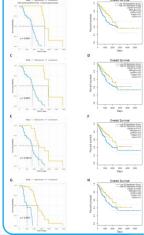


Figure 2 Gene Functional Analysis and Pathway Analysis. (A) Biological process by GO analysis with 153 selected surface protein showed highest enrichment in "response to stimulus", "cell communication", and "signaling" which signified the genes were responsible signaling received activity". In a "transmention by 30 selected surface proteins showed highest enrichment in "signaling receptor activity", unlockular transducer activity", and "transmentorane signaling receptor activity", which signified the genes have receptor-like nature. (C) Cellular components of on analysis with 153 selected surface proteins showed highest enrichment in "membrane", "intrinsic component of membrane", and "membrane part", which signified the genes were located on the cell membrane and highlighted the success through surfaceoning prediction for selected 38 gene hits by Reaction pathway analysis for signal transduction pathway showed give expression difference between normal human osteoblast data to primary-OS data. (H) STRING analysis of the selected 37 gene hits by Reaction pathway analysis for signal transduction pathway showed literature research evidence in protein-protein interactions. Figure 2 Gene Functional Analysis and Pathway Analysis. (A) Biological process

Figure-3 Multivarate survival analysis.

(A) The Kaplan-Meier curve of 153 utTAAs as gene markers for primary OS patients (N = 73) with statistical significance (p-value < 0.0001). (B) The Kaplan-Meier curve of 153 utTAAs as gene markers for SARCOMA dataset (N = 262) with significance (p = 0.026), data shown with median cutoffs, results obtained from GFPIA2\*. (C) The Kaplan-Meier curve of 46 utTAAs under immune system identified by Reactome database for primary OS patients (N=73) with statistical significance (p-value identified by Reactome database for Pin Kaplan-Meier curve of 46 utTAAs under immune system identified by Reactome database for primary OS patients with significance (p = 0.026), data shown with median cutoffs, results obtained from GFPIA2\*. (E) The Kaplan-Meier curve of 22 utTAAs under adaptive immune system identified by Reactome database for primary OS patients (N=73) with statistical significance (Po Reactome database for primary OS patients (N=73) with statistical significance (c) when Pin (N=74) with the primary OS patients (N=74) with statistical significance (c) when Pin (N=74) with the primary OS patients (N=74) with statistical significance (c) when Pin (N=74) with the primary OS patients (N=74) with the database for primary OS patients (N=73) with statistical significance (p-value = 0.00013). (F) The Kaplan-Meier curve of 22 uiTAAs under adaptive immune stem identified by Reactome database for SARCOMA dataset (N = 262) with significance (p = 0.013), data shown with median cutoffs, results obtained from GEPIA2\*. (G) The Kaplan-Meier curve of 22 uiTAAs under cytokine signaling in immune system identified by Reactome database for primary OS patients (N=73) with statistical significance (p-value < 0.0001). (H) The Kaplan-Meier curve of 22 uiTAAs under Kaplan-Meier curve of 22 uiTAAs under cytokine signaling in immune system identified by Reactome database for SARCOMA dataset (N = 2.62) with significance (p = 0.026), data shown with median cutoffs, results obtained from GEPIA2\* "NOTE: GEPIA2 is a web tool for Kaplan-Meier survival curve plots with multi-gene signatures (http://gepia2.cancer-pku.cn/#survival).

Figure3 Multivarate survival analysis.