

Integrating Molecular Imaging with Spatial Omics to Map Drug Action in Solid Tumors through Surgical-Window Trials

Authors: Guolan Lu¹, Garry P. Nolan², Eben L. Rosenthal³

Affiliations: ¹Department of Urology, Stanford University School of Medicine, Stanford, CA, USA; ²Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA; ³Department of Otolaryngology, Stanford University School of Medicine, Stanford, CA, USA;

Background: Antibody-based therapies have transformed cancer treatment, yet limited tumor penetration and heterogeneous target engagement constrain their efficacy in solid tumors. Plasma pharmacokinetics and whole-body imaging cannot resolve these processes at cellular resolution within human tumors. We developed an integrated framework combining molecular imaging of fluorescently labeled antibodies with high-plex spatial proteomics to quantify drug distribution and target engagement in the context of the tumor microenvironment.

Methods: Patients with head and neck or pancreatic cancer received intravenous panitumumab-IRDye800CW, a near-infrared fluorescent antibody targeting epidermal growth factor receptor (EGFR), before tumor resection in first-in-human surgical-window trials. We conjugated and validated a DNA-barcoded antibody panel comprising more than 50 protein markers to profile tumor cells, immune cells, cancer-associated fibroblasts, and extracellular matrix proteins using co-detection by indexing (CODEX). Drug fluorescence imaging and CODEX imaging were performed on the same formalin-fixed, paraffin-embedded tissue sections. An integrated computational workflow registered the imaging modalities and quantified drug signal, target abundance, stromal composition, and cellular neighborhoods at single-cell resolution.

Results: This single-cell spatial pharmacology framework identified the cell types bound by the infused antibody and quantified drug-target engagement and penetration depth within human tumors. Integration of drug localization with cellular and stromal maps resolved heterogeneous antibody distribution across distinct tissue microenvironments. Across head and neck and pancreatic cancers, the analysis revealed conserved stromal niches enriched in specific cancer-associated fibroblast populations and extracellular matrix proteins that were associated with restricted antibody penetration. These findings linked intratumoral drug distribution to defined spatial features of the tumor microenvironment.

Conclusions: We established a framework for mapping in vivo drug-target-microenvironment interactions in human tumors. This approach provides a foundation for identifying barriers to drug delivery, discovering candidate predictive biomarkers, and informing strategies to advance the development of antibody therapeutics. Future studies could extend the framework to prostate cancer and other therapeutic antibodies, including immune checkpoint inhibitors.

Funding Acknowledgements: NIH K99CA267171 and NIH R00CA267171; NIH R01CA190306; NIH R01CA273035.

Conflicts of Interest Disclosure Statement: The author declares no conflicts of interest. G.P.N. has equity in Akoya Biosciences and G.P.N is a scientific advisory board member of Akoya Biosciences. Akoya Biosciences makes reagents and instruments that are dependent on licenses from Stanford University. Stanford University has been granted US patent 9909167, which covers some aspects of the technology described in this paper. The other authors declare no competing interests.