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Computational pathology and biomarker discovery in the phase II ICARUS-LUNG01 study

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COMPUTATIONAL PATHOLOGY AND TRANSLATIONAL BIOMARKER DISCOVERY

Precision oncology has evolved from single-biomarker treatment selection toward multidimensional patient stratification that integrates molecular profiles, targeted therapies, immunotherapy, and individualized treatment strategies[1,2,3]. Although individual biomarkers remain clinically important, they may be insufficient to capture the spatial, cellular, and mechanistic heterogeneity that shapes treatment response. The integration of multiomics profiling, spatial analysis, and machine learning is reshaping biomarker discovery by enabling a more comprehensive and mechanistically informed framework[4,5,6]. The study presented by Planchard *et al.*, the Phase II ICARUS-LUNG01 study, illustrates this transition by combining the clinical evaluation of datopotamab deruxtecan in advanced non-small cell lung cancer (NSCLC) with computational pathology, spatial modelling, and multimodal molecular characterization to investigate mechanisms of response and resistance beyond conventional trophoblast cell surface antigen-2 (TROP2) assessment[7].

KEY INSIGHTS AND MAIN CLINICAL SIGNALS FROM THE PHASE II ICARUS-LUNG01 STUDY

The Phase II ICARUS-LUNG01 study[7] enrolled 100 pretreated patients with advanced NSCLC and reported an objective response rate of 26.0%, median progression-free survival of 3.6 months, and median overall survival of 11.9 months, with greater benefit in non-squamous tumors. The principal translational finding was that response to Dato-DXd was not captured by conventional TROP2 immunohistochemistry alone. Although conventional TROP2 expression was not significantly associated with objective response, machine learning-based computational pathology indicated that the cytoplasmic distribution of TROP2 may provide a more informative measure of drug activity. However, the operational definition, quantitative threshold, and reproducibility of cytoplasmic TROP2 require clearer reporting, including the number of patients and valid tissue samples contributing to each biomarker analysis. It is also important to establish whether

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model development and evaluation used strictly separated datasets, whether correction for multiple testing was applied, and how robust the signal is to fixation conditions, antibody selection, section quality, image acquisition, and segmentation algorithms. By integrating histopathology, immunohistochemistry, whole-exome sequencing, bulk RNA-sequencing, imaging mass cytometry, and spatial transcriptomics with machine learning-based image and spatial analyses, the study linked cytoplasmic TROP2 patterns to greater antibody-drug conjugate internalization and stronger DNA damage response in preclinical models. Early activation of DNA repair pathways in on-treatment samples was associated with non-response, whereas responders showed enrichment of immune-related pathways and immune cell phenotypes in the tumor microenvironment[7]. These associations are biologically plausible but should not yet be interpreted as Dato-DXd-specific predictive effects. The study further demonstrates how machine learning can extend pathology beyond descriptive assessment by identifying spatial and subcellular biomarker patterns associated with therapeutic response. Together, these findings support the results reported by Planchard *et al.* as both a positive phase II signal for Dato-DXd and a proof of concept for multimodal biomarker discovery beyond single-marker approaches[7].

LIMITATIONS AND TRANSLATIONAL READINESS

Despite these strengths, the translational findings presented by Planchard *et al.* should still be considered exploratory[7]. The study was a single-arm phase II trial with a modest sample size, and several key analyses were performed in smaller selected subsets, which may limit statistical power and the stability of some associations. The absence of a non-Dato-DXd control group is particularly important: associations involving DNA repair pathways, Schlafen11 (SLFN11), immune infiltration, or cytoplasmic TROP2 cannot distinguish treatment-specific predictive biomarkers from general prognostic factors. Demonstration of predictive value will require a treatment-by-biomarker interaction in a randomized or otherwise appropriately controlled cohort. Additional limitations arise from the machine learning-based computational pathology workflow. The machine learning-derived biomarkers proposed in this study may themselves be sensitive to variability in staining, image quality, tissue sampling, cohort composition, and analytical choices, because they depend on image processing, segmentation, feature extraction, and spatial modeling steps. Transparent reporting of model training, internal testing, prespecified thresholds, missing-sample handling, multiple-testing control, and independent validation is therefore essential before clinical translation. Moreover, given the limited amount of data available for several translational analyses, conclusions drawn from these computational signatures should be interpreted with appropriate caution. A central methodological concern is the marked imbalance in the response-evaluable imaging cohort, which comprised 17 responders and 50 non-responders. In a dataset of this size and asymmetry, high-capacity learning algorithms may preferentially minimize average error by capturing majority-class or cohort-specific patterns rather than reproducible response biology. This increases the risks of overfitting, unstable feature selection, optimistic performance estimates, and poor calibration in external populations. Future development should therefore use patient-level separation of training and testing data, nested cross-validation with preprocessing and feature selection confined to each training fold, imbalance-aware loss functions or sampling strategies, uncertainty and calibration analyses, and independent multicenter validation. Performance should be reported using class-sensitive measures, including sensitivity, specificity, balanced accuracy, precision-recall curves, and confidence intervals, rather than accuracy alone. The immune-related pathway enrichment inferred from bulk RNA sequencing is also vulnerable to variation in cellular composition, tumor purity, sample quality, and technical batch effects. Because bulk expression profiles cannot reliably assign signals to specific cell populations and may confound correlation with causation, differential-expression and pathway analyses should be interpreted as hypothesis-generating unless corroborated by orthogonal spatial or single-cell evidence[8]. Further investigation is therefore required to establish reproducibility, external validity, and

cross-platform robustness across broader clinical settings. Phase II ICARUS-LUNG01 study[7] should therefore be viewed as a hypothesis-generating study that identifies promising biological axes for future development rather than a finalized framework for patient selection. Its main translational contribution is to suggest that clinically useful stratification for Dato-DXd may ultimately require composite biomarkers integrating target localization, internalization-related biology, resistance pathways, and immune context. The next stage should comprise validation in larger, preferably randomized cohorts, alongside technical harmonization of the computational workflow.

A ROADMAP FOR COMPOSITE BIOMARKER DEVELOPMENT

A feasible roadmap should progress from analytically defined components to a prespecified clinical classifier. Standardized digital pathology should first quantify membrane and cytoplasmic TROP2, spatial heterogeneity, tissue quality, and measurement uncertainty using locked segmentation and quality-control procedures. These features should then be integrated with orthogonal measurements of internalization and payload response, including targeted DNA or RNA sequencing, proteomics, DNA-damage and repair markers, and spatially resolved immune profiling. Longitudinal sampling at baseline, an early on-treatment time point, and progression could help distinguish pre-existing sensitivity from adaptive resistance, while circulating tumor DNA or other minimally invasive assays may reduce dependence on repeated biopsies. AI-assisted analytical agents may support data harmonization, multimodal feature extraction, and traceable quality control, but should not replace prespecified statistical analysis or independent validation. Functional organoid or gene-editing studies may test the causality of candidate mechanisms, whereas nanomaterial-based sensors or delivery models remain exploratory tools rather than immediate clinical requirements. The final signature should be reduced to the smallest reproducible panel that adds value beyond established clinical covariates and should be prospectively validated using a locked assay, threshold, and analysis plan.

TROP2 BIOMARKERS ACROSS ANTIBODY-DRUG CONJUGATE PLATFORMS

The potential relevance of cytoplasmic TROP2 in the Phase II ICARUS-LUNG01 study[7] should also be interpreted in the context of other TROP2-directed antibody-drug conjugates (ADCs). In ASCENT, sacituzumab govitecan produced numerically greater benefit in tumors with high or intermediate TROP2 expression, whereas the small low-expression subgroup prevented definitive conclusions; subsequent analyses nevertheless supported activity across expression quartiles[9,10]. In TROPiCS-02, benefit in pretreated hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR-positive/HER2-negative) metastatic breast cancer was observed across assessed TROP2-expression groups, without establishing a mandatory treatment-selection threshold[11]. Sacituzumab govitecan has an average drug-to-antibody ratio of approximately 8 and a hydrolysable linker that facilitates extracellular release of SN-38 and bystander killing[12]. Dato-DXd has an average drug-to-antibody ratio of approximately 4 and requires TROP2 binding, internalization, intracellular trafficking, linker cleavage, and release of the DXd payload[13]. These pharmacological differences provide a plausible rationale for investigating whether subcellular TROP2 localization is particularly informative for Dato-DXd. However, this remains a mechanistic hypothesis rather than a demonstrated cross-trial difference, because both ADCs may generate bystander effects and the trials involved different tumor types, assays, thresholds, populations, and designs. The predictive utility of TROP2 should therefore not be assumed to transfer across tumor types or ADC formats; it may depend jointly on antigen distribution, antibody properties, linker stability, drug-to-antibody ratio, payload permeability, internalization, and tissue context.

SAFETY-RELATED BIOMARKERS AND CLINICAL UTILITY

Patient selection should balance the probability of therapeutic benefit against susceptibility to serious toxicity. In the Phase II ICARUS-LUNG01 study[7], stomatitis affected approximately half of treated patients, while interstitial lung disease or pneumonitis, although less frequent, included fatal events[7]. Biomarker

development should therefore extend beyond tumor response. For stomatitis, prospective models could integrate baseline oral health, previous mucosal toxicity, nutritional status, salivary or inflammatory markers, pharmacokinetic exposure, and early patient-reported symptoms. For interstitial lung disease or pneumonitis, candidate risk models could assess pre-existing lung abnormalities on computed tomography, pulmonary function, smoking and treatment history, concomitant medication, circulating epithelial-injury or inflammatory markers, oxygen saturation, and early radiomic changes. These variables remain candidate predictors rather than established biomarkers. Because severe events are too infrequent for reliable modeling within Phase II ICARUS-LUNG01 alone, validation will require pooled individual-patient data, standardized event adjudication, harmonized imaging and biospecimen collection, and prospective evaluation across Dato-DXd trials. A clinically useful composite framework should ultimately estimate both expected efficacy and toxicity risk, thereby supporting treatment choice, monitoring intensity, and early intervention.

DECLARATIONS

Authors' contributions

Contributed to the conception, writing, and revision of the commentary: Barbosa MI, Rodrigues PM

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

During the preparation of this manuscript, the AI tool Microsoft 365 Copilot (version GPT 5.5, released 2026-05-07) was used solely for language editing. The tool did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. All authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Rulten SL, Grose RP, Gatz SA, Jones JL, Cameron AJM. The future of precision oncology. *Int J Mol Sci.* 2023;24:12613. DOI PubMed PMC
2. Chang TG, Park S, Schäffer AA, Jiang P, Ruppin E. Hallmarks of artificial intelligence contributions to precision oncology. *Nat Cancer.* 2025;6:417-31. DOI PubMed PMC
3. Sonkin D, Thomas A, Teicher BA. Cancer treatments: past, present, and future. *Cancer Genet.* 2024;286-7:18-24. DOI PubMed PMC
4. Srivastava R. Applications of artificial intelligence multiomics in precision oncology. *J Cancer Res Clin Oncol.* 2023;149:503-10. DOI PubMed PMC
5. Azuaje F. Artificial intelligence for precision oncology: beyond patient stratification. *NPJ Precis Oncol.* 2019;3:6. DOI PubMed PMC
6. Narote S, Desai SA, Patel VP, Deshmukh R, Raut N, Dapse S. Identification of new immune target and signaling for cancer immunotherapy. *Cancer Genet.* 2025;294-5:57-75. DOI PubMed

7. Planchard D, Cozic N, Mosele MF, et al. Efficacy, safety, and biomarker analysis of datopotamab deruxtecan in advanced non-small cell lung cancer: ICARUS-LUNG01 phase 2 study. *Cancer Cell.* 2026;44:1147-59.e6. DOI PubMed
8. Liu H, Li Y, Karsidag M, Tu T, Wang P. Technical and biological biases in bulk transcriptomic data mining for cancer research. *J Cancer.* 2025;16:34-43. DOI PubMed PMC
9. Bardia A, Tolaney SM, Punie K, et al. Biomarker analyses in the phase III ASCENT study of sacituzumab govitecan versus chemotherapy in patients with metastatic triple-negative breast cancer. *Ann Oncol.* 2021;32:1148-56. DOI PubMed
10. Bardia A, Rugo HS, Tolaney SM, et al. Final results from the randomized phase III ASCENT Clinical Trial in metastatic triple-negative breast cancer and association of outcomes by human epidermal growth factor receptor 2 and trophoblast cell surface antigen 2 expression. *J Clin Oncol.* 2024;42:1738-44. DOI PubMed PMC
11. Rugo HS, Bardia A, Marmé F, et al. Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPiCS-02): a randomised, open-label, multicentre, phase 3 trial. *Lancet.* 2023;402:1423-33. DOI PubMed
12. Tolaney SM, Cardillo TM, Chou CC, Dornan C, Faris M. The mode of action and clinical outcomes of sacituzumab govitecan in solid tumors. *Clin Cancer Res.* 2025;31:1390-9. DOI PubMed PMC
13. Okajima D, Yasuda S, Maejima T, et al. Datopotamab deruxtecan, a novel TROP2-directed antibody-drug conjugate, demonstrates potent antitumor activity by efficient drug delivery to tumor cells. *Mol Cancer Ther.* 2021;20:2329-40. DOI PubMed PMC