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Reply

Reply to Comment on Bel'skaya LV "Metabolic Portrait of Breast Cancer in Mixed Saliva". Frontiers in Bioscience-Landmark. 2026; 31: 47739

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This manuscript responds to the comment entitled "Saliva as a Mirror of Breast Cancer: Promise, Pitfalls and Proof-of-Concept". Our point-by-point responses are presented below.

On the biological plausibility of saliva reflecting systemic metabolic changes caused by non-oral cancer, and the need for parallel analysis of blood, plasma, or tumor tissue.

Recent studies conducted by our group and other research groups have shown that salivary composition is altered in patients with malignancies outside the oral cavity [1,2,3,4,5,6]. Although the mechanisms for changes in salivary composition in oral cancer are well understood, those associated with extraoral malignancies remain unclear [7]. Accumulating evidence may improve our understanding of these mechanisms. At present, however, existing studies should be regarded as providing proof-of-concept evidence, and their findings require further validation.

It is known that various metabolites can enter saliva through salivary gland secretion, blood transfer, gingival fluid, extracellular vesicles, epithelial desquamation, microbiota activity, and local production by oral cells [8]. In non-oral cancers, changes in saliva composition are more often associated not with direct entry from tumor tissue into oral fluid, but with systemic inflammation, metabolic reprogramming, tumor exosomes, cachexia, oxidative stress, and secondary changes in salivary gland function. The oral microbiome also influences salivary composition by contributing to the breakdown of proteins, glycoproteins, amino acids, and lipids; activating the innate immune response; enhancing cytokine production; and potentially increasing epithelial barrier permeability. Therefore, salivary biomarkers should be interpreted as an integral signal of the "tumor-blood-salivary glands-microbiome-oral cavity" axis, and their diagnostic value is most fully realized as part of multi-marker panels after standardization of sample collection, accounting for variability, and clinical validation [8]. Although this remains a hypothesis, we believe it warrants further investigation and validation. Salivary markers may have the greatest practical value for screening at-risk groups, monitoring response to therapy, early detection of adverse dynamics, and personalized assessment of systemic inflammatory stress in cancer patients.

The relationship between the salivary composition, blood, and tumor tissue remains unclear [9]. Most salivary metabolites are not significantly correlated with blood composition, and proteomic studies have shown that most salivary proteins are synthesized in the salivary glands, with only a few entering from the blood or lymph [10]. Exosomes illustrate this process: extracellular vesicles secreted by tumor or tumor-associated cells enter the salivary glands via the general bloodstream or lymph, where they are taken up by acinar cells through membrane fusion or endocytosis. The salivary glands then secrete mixed extracellular vesicles into saliva, originating from both acinar cells and tumors located distant from the salivary glands. This model explains why noncoding RNAs detected in saliva, especially in pathological conditions, differ in composition from those found in other biological fluids, particularly blood [11]. Currently, we believe it is most important to first identify the range of potentially interesting salivary metabolites and only then to describe their possible mechanisms of entry into saliva, as these mechanisms may differ significantly in each case. Undoubtedly, parallel studies involving the saliva-blood-tumor tissue system can provide the necessary information for understanding these mechanisms, as can a more detailed study of the blood-saliva barrier and its functional changes in cancer at distant sites [12].

It should be noted that our study included 787 patients from whom saliva samples were collected. All samples were frozen, and the studies were conducted after histological verification and completion of therapy for all patients. Due to the retrospective design of the study, we were unable to include additional studies of blood or tumor samples. However, this does not mean that similar studies will not be conducted in the future, including by other research groups.

Salivary variability and the need for strict preanalytical control.

Methods for saliva collection and analysis have been established and standardized. This allows us to mitigate the influence of factors such as oral health, microbiome composition, diet, medications, smoking, and circadian fac-

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tors on variability in saliva composition. From a methodological standpoint, saliva analysis requires careful procedures and rigorous quality control, as does the analysis of other biological fluids. Oral health conditions, including active dental caries, oral inflammation (particularly acute periodontitis), and oral mucosal lesions, should be assessed before sample collection because they can influence the oral microbiome and potentially affect sample composition. Therefore, a dental examination before saliva collection is essential for identifying and documenting these potential confounding factors.

Internal contradictions with the authors' previous works.

Regarding the different interpretations of salivary antioxidant defense system parameters, the fact that oxidative stress markers were interpreted as part of the oral defense system in one study does not contradict the fact that activation of the oral defense system occurs against the background of altered metabolism associated with the presence of distant cancer. These are simply different emphases on the same process.

Partial normalization of salivary metabolic parameters following tumor removal and limitations of the biological interpretation of this finding.

The comment regarding changes in saliva composition after tumor surgery is reasonable. We did not observe a complete restoration of saliva composition after surgery due to the short follow-up period, but it was not possible to add additional control points for our patient cohort. We have noted these study limitations.

Distinct metabolic patterns across various breast cancer phenotypes—including human epidermal growth factor receptor 2 (HER2)-high subtypes—and the need for more careful interpretation.

Let me reiterate that this is the first time subtle changes in composition that respond to tumor phenotype in breast cancer or the grade of ovarian cancer (data not published) have been demonstrated. Therefore, it is unsurprising that not all aspects have been fully explored and not all mechanisms are understood; consequently, one should exercise caution regarding possible interpretations. As research in this area advances, these questions are likely to be addressed.

Interpretation of isolated metabolic changes as indicators of oncogenic pathways and risk of erroneous conclusions.

Undoubtedly, linking salivary metabolic changes to oncological pathways requires caution. Currently, there is no unified hypothesis explaining the changes observed in saliva in the context of distant cancer. The observed alterations are not merely a reflection of changes occurring within the tumor tissue itself. In this regard, the blood-saliva barrier—comprising the microvasculature, polarized salivary gland epithelium, tight junction proteins, membrane transporters, and the acinar-ductal system—assumes particular importance. It should be viewed not as a passive barrier between blood and saliva, but as a functional regulator determining how systemic metabolic and inflammatory changes are manifested in saliva. This is especially significant in the case of distant tumors, where the selective transport of amino acids, lipids, ions, and other compounds may serve as an intermediary link between systemic tumor metabolism and the biochemical profile of saliva. Nevertheless, the absence of a single, universally accepted mechanism explaining salivary changes associated with tumors located elsewhere in the body does not deter researchers from actively seeking potential salivary biomarkers for breast cancer and hypothesizing possible links to tumor metabolic pathways [13]. Once metabolites with diagnostic potential have been identified and validated, follow-up experiments can be designed to directly investigate their relationships with specific metabolic pathways. This approach offers a new direction for future research.

Indeed, many questions in this field remain unanswered. Nevertheless, integrating and systematically analyzing accumulating data across the "tumor–blood–salivary glands–microbiome–oral cavity" axis using machine learning algorithms may help identify nonlinear relationships and interactions among features. Although the underlying mechanisms remain incompletely understood, multidimensional molecular data may still be used to develop clinically interpretable diagnostic models. Undoubtedly, further research and validation involving independent cohorts are required before implementation in actual clinical practice.

We are very pleased that this research topic is attracting increasing attention and generating lively, robust discussion.

Author Contributions

LB was responsible for the entire preparation of this manuscript. The author contributed to editorial changes in the manuscript. The author read and approved the final manuscript. The author has participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The author declares no conflicts of interest.

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