Validating imaging biomarkers as disease-relevant

The development of imaging technology for the quantification of optical biomarkers of pathological processes should involve the validation of the biomarkers' biological accuracy.

Optical modalities for molecular imaging — from microscopy to whole-body imaging, using contrast enhancers, or endogenous chromophores or scatterers — enable the visualization of a wealth of disease-relevant molecular information across length and time scales. They are thus particularly relevant to precision medicine, which links data from biomarkers of physiology or disease (such as blood-vessel dilation for tissue inflammation, hypoxia and glycolysis for cellular metabolism, and intracellular calcium signalling for neuronal activity) with genetic or environmental factors of an individual or group. However, for developments in molecular imaging to eventually be useful in medicine, the biomarkers measured need to be biologically accurate and disease-relevant.

Improving biological accuracy is distinct from boosting technical accuracy (that is, the minimization of both random and systematic errors). The biological accuracy of a biomarker refers instead to the closeness of the measured values to naturally occurring physiological dynamics, and to whether these fluctuations are relevant to disease. Yet, because disease-relevant biomarkers can be difficult to standardize, not least because in some cases they are only a proxy (sometimes a notably imperfect one) for true pathological features (for example, serum prostate-specific antigen is used as a proxy for prostate size), clinically relevant developments in imaging may follow a tortuous path towards eventual implementation in healthcare.

For example, levels of blood oxygenation, which can be measured optically, can be an indicator of inflammation or neoangiogenesis; but, because there is no quantitative standard relating a precise value in oxygen saturation or blood volume to a state of inflammation, the measurements are subject to interpretation. In oncology, the expression of a particular protein or the presence of a specific metabolite can be used to identify a genomic signature; yet the numerical values can vary significantly between patients, thereby limiting the measurement's usefulness (in such cases, the use of multiple disease-relevant biomarkers can be helpful). It is therefore highly desirable for imaging biomarkers of disease to be representative of a clinically relevant 'ground truth'.

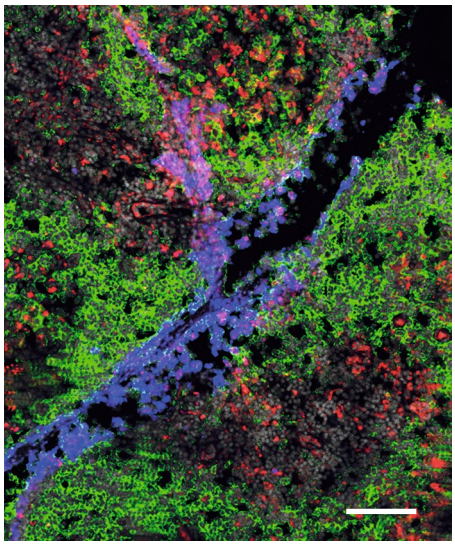

Fig. 1 | Stained sections of a lymph node from a macaque, showing that an injected mRNA vaccine (mRNA in blue; expressed vaccine protein in red) preferentially locates in the periphery of germinal centres (green). Scale bars, 100 μm. Figure reproduced from Lindsay, K. E. et al. https://doi.org/10.1038/s41551-019-0378-3 (2019), Springer Nature Ltd.

Moreover, the biological validation of a new biomarker, or of a new way to measure an existing biomarker, is only one aspect of the road to clinical implementation, as discussed by Sarah Bohndiek and co-authors in a Perspective included in this issue. The authors also highlight the lack of standardization (for comparing imaging devices, biomarkers, and imaging and diagnostic performance), of defined safe and effective illumination levels for many optical-imaging modalities and tissue types and of ex vivo tissue models for validation, as well as difficulties in carrying out human trials that are representative of the target population and the clinical environment. These all create barriers (which can be amplified by feedback loops between them) to the translation of optical-imaging technologies and biomarkers.

This issue also highlights developments in imaging technologies that include biological validation of the accuracy of a measured biomarker. In one study, Philip Santangelo and co-authors labelled a model messenger RNA vaccine with a probe for both positron-emission-tomography–computed-tomography and near-infrared imaging to quantitatively monitor the early trafficking of the vaccine to draining lymph nodes after intramuscular injection in non-human primates. They used near-infrared fluorescence (as well as flow cytometry) to validate, from tissues extracted from sacrificed animals, the distribution of the vaccine components (Fig. 1) and the cell types that take them up. As noted by Sebastian Ols and Karin Loré in an associated News & Views, "much better mechanistic understanding of vaccine dynamics after administration is [...] needed to select safe formulations with the capacity to elicit stronger immunity".

In another study, Wei Min and colleagues observed heterogeneity in how tissues and even single cells metabolize glucose — of high relevance in cancer progression — by using Raman spectroscopy and stimulated Raman scattering to trace deuterated glucose in living mice. They validated their quantitative measurements of Raman intensity by quantifying the turnover of specific metabolic products and the differential utilization of glucose metabolism, by comparing metabolic activity under different conditions, and also via nuclear-magnetic-resonance spectra of tissue lipid extracts. Such multiplexing optical imaging of glucose metabolites may in future uncover new metabolic biomarkers of disease or be used for the metabolic phenotyping of biopsied tissues from patients.

The intratumoral metabolic heterogeneity of patient tissues can also be determined via photoacoustic microscopy, by measuring the oxygen-consumption rates of single cells taken from the tissue after it is homogenized into a single-cell suspension and the cells deposited in microwell arrays, as demonstrated by Lihong Wang and co-authors for tissues from breast-cancer patients. As noted by Melissa Skala and co-authors in an accompanying News & Views, the microwell system, which does not require exogenous labels, allows for the measurement of cell responses to multiple perturbations.

Because ultrasound scattering is orders-of-magnitude weaker than the scattering

of light, photoacoustic technology enables the imaging of dynamic changes in cells in vivo at larger depths than is possible with purely optical microscopy. An Article by Shy Shoham, Daniel Razansky and colleagues shows that, in anaesthetized mice expressing a fluorescent, genetically encoded calcium indicator, high-resolution whole-brain snapshots of neuronal activity (in response to electrical stimuli of the mouse's hind paw) can be rapidly obtained, and that these measurements are sufficiently sensitive to be distinguished from the strong background absorption of the photoacoustic signal by haemoglobin. The researchers validated the measurement of neuronal calcium dynamics with simultaneously acquired wide-field fluorescence. As highlighted by Alessio Andreoni and Lin Tian in a News & Views, "optoacoustic imaging may continue to bridge the gap between microscopic and macroscopic scales for the investigation of brain function in rodent models".

In fact, as discussed by Vasilis Ntziachristos and colleagues in a Review Article on optoacoustic mesoscopy, implementations of this technology can fill a performance gap between microscopy and macroscopy (in mesoscopy, fields of view and volumes can be much larger than in microscopy yet can be imaged at comparable scan times). This enables the non-invasive imaging of disease-relevant biomarkers (in particular for skin diseases, in endoscopy and in intravascular imaging) at high resolution and increasingly quantitatively over clinically relevant areas and volumes without the use of exogenous labels.

As exemplified by research highlighted in this issue, the development of optical-imaging technologies does not necessarily have to be geared towards testing the potential utility of a clinically relevant biomarker; it also helps broaden the range of biomarkers that are disease-relevant and could become clinically useful. Imaging modalities that bridge across length scales can also help determine the degree by which a biomarker can vary (spatially and temporally), and hence aid its interpretation. Yet validation of the biological accuracy of disease-relevant biomarkers will be necessary for developments in optical imaging to become meaningful contributors to precision medicine. ❐

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