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Review

Unravelling tumour heterogeneity using next-generation imaging: radiomics, radiogenomics, and habitat imaging

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Tumour heterogeneity in cancers has been observed at the histological and genetic levels, and increased levels of intra-tumour genetic heterogeneity have been reported to be associated with adverse clinical outcomes. This review provides an overview of radiomics, radiogenomics, and habitat imaging, and examines the use of these newly emergent fields in assessing tumour heterogeneity and its implications. It reviews the potential value of radiomics and radiogenomics in assisting in the diagnosis of cancer disease and determining cancer aggressiveness. This review discusses how radiogenomic analysis can be further used to guide treatment therapy for individual tumours by predicting drug response and potential therapy resistance and examines its role in developing radiomics as biomarkers of oncological outcomes. Lastly, it provides an overview of the obstacles in these emergent fields today including reproducibility, need for validation, imaging analysis standardisation, data sharing and clinical translatability and offers potential solutions to these challenges towards the realisation of precision oncology.

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Introduction

Tumour heterogeneity in cancers has been observed at the histological and genetic levels,^{1–3} and increased levels

of intra-tumour genetic heterogeneity^{4–8} have been reported to be associated with adverse clinical outcomes.^{9,10} In oncological imaging, phenotypic heterogeneity between and within tumours of a given patient is readily apparent and various imaging features are routinely described subjectively in radiology reports.¹¹ Recently, however, imaging research has focused increasingly on the newly emergent field of radiomics, which is defined as a high-throughput process in which a large number of shape, edge, and

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texture metrics are extracted and quantified objectively and in a reproducible form^{12–14} (Fig 1). These quantitative metrics can provide important insights into tumour phenotype and as well as the interaction of the tumour with its microenvironment, defined as “habitat imaging”.^{11,15} In the effort to delineate the biological and clinical implications of these new quantitative metrics, radiomic metrics obtained from magnetic resonance imaging (MRI), including diffusion-weighted (DW) and dynamic-contrast-enhanced (DCE) MRI sequences, computed tomography (CT), and combined 2-[¹⁸F]-fluoro-2-deoxy-D-glucose (FDG) positron-emission tomography (PET)/CT have been further correlated with genomics data, a process defined as radiogenomics.¹⁶ Radiogenomics and outcome data can be meaningfully mined with the goal of developing robust biomarkers that may potentially aid cancer diagnosis, improve assessment of treatment response, and better predict patient outcome.

Potential value of radiomics and radiogenomics

To date, several studies have focused on the correlation and integration of radiomics with genomics (defined as the systematic study of the complete DNA sequences [genome] of organisms¹⁷) and proteomics (defined as the systematic study of the complete complement of proteins [proteome] of organisms¹⁸) data, and their results continue to support the notion that radiomic metrics may perform relatively well as surrogates of molecular alterations and protein expression found in tissue samples.

Radiomics approaches for assisting in diagnosis and assessment of cancer aggressiveness

Studies using radiomic analyses have shown that radiomic metrics are capable of distinguishing between benign and malignant tissue and aiding in the assessment of cancer aggressiveness in a variety of clinical settings. Analysis of heterogeneity in enhancement patterns, found on perfusion deficits, which ultimately lead to different micro-environmental selection pressures,¹¹ has been performed with promising findings. For example, grey level co-occurrence matrix analyses of DCE images distinguished between benign and malignant breast lesions with very high diagnostic accuracy.¹⁹ Texture analysis of DCE images using a structured fractal-based approach improved differentiation between low- and high-grade gliomas by orders of magnitude²⁰; however, texture analysis is not limited to enhancement patterns. Measures of heterogeneity in T1-weighted (W), T2W, and DW MRI images can reveal differences in cellular density in tumours, which in turn can be matched to histological findings and aid in distinguishing malignant versus benign soft-tissue masses.²¹ Similarly, analysis of prostate MRI using T2W and DWI sequences has been successful in discriminating prostate cancer from benign prostate tissue and in providing information on prostate cancer aggressiveness using Gleason scores.⁵ In this study, five textural features (entropy, inertia, energy, correlation, and homogeneity) on apparent diffusion coefficient (ADC) maps and two textural features (inertia and correlation) on T2W imaging were significantly different between prostate tumours and benign prostate tissue.⁵

Typical Radiomics Analysis Workflow

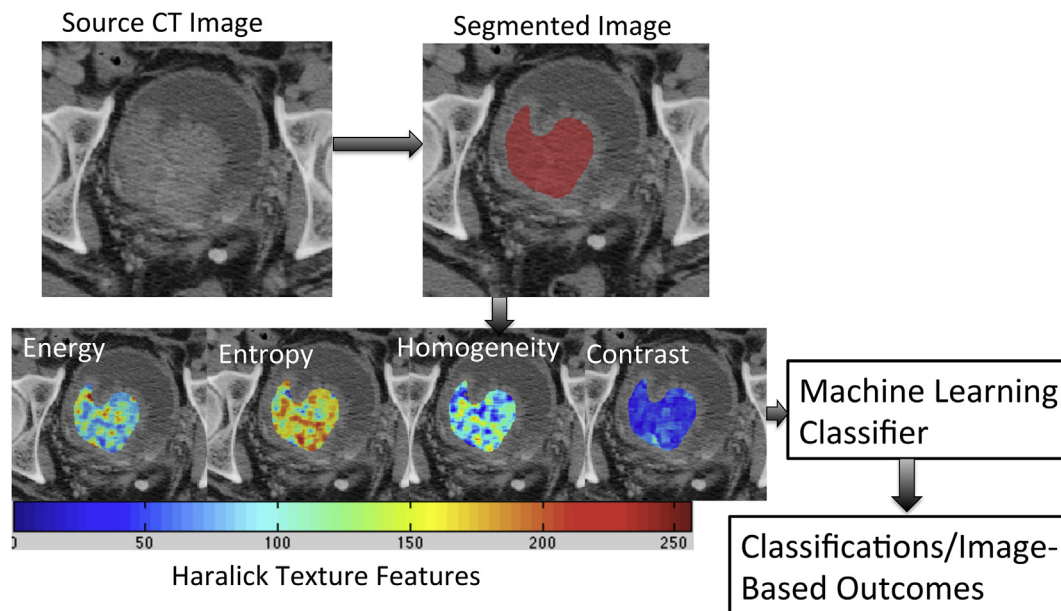


Figure 1 Radiomics analysis workflow. Radiomics-based analysis starts with segmentation of the structure(s) of interest, in this case, bladder cancer. Various texture features including Haralick textures are generated. A machine-learning classifier is trained using features generated from several images. Classification of various measures is then performed on never-seen-before images.

Using textural features, the same group also reported 90% accuracy for discriminating Gleason score of six tumours (which are usually considered “low risk”) from those with a Gleason score of ≥ 7 .⁵ Similarly, a study by Fehr *et al.*²² used texture features derived from ADC and T2W MRI to obtain classification of Gleason patterns with $>92\%$ accuracy (Fig 2).

Associations between radiomics, radiogenomics, and patient outcomes

Radiomics has the potential to identify imaging phenotypes of prognostic value by exploring intra-tumour heterogeneity features. For example, Grove *et al.*⁸ assessed spiculation and entropy gradients in patients with lung cancer to find that these measures were strong prognostic indicators in patients with early stage lung cancer. Another study by Aerts *et al.*²³ demonstrated that a radiomic signature (size, shape, texture, and wavelets) was predictive of outcome in independent cohorts of patients with lung cancer. Additionally, this same signature could be applied to patients with head and neck cancer with equivalent prognostic power.²³ The presence of different habitats can be a source of radiomic features due to their distinct volumes, each with a specific combination of flow, cell density, necrosis, and oedema¹² (Fig 3). Habitat distribution in patients with glioblastoma multiforme has enabled us to discriminate between cancers that progress quickly and those that are more indolent.²⁴

Texture analysis can also be applied to PET. A study by Nair *et al.*²⁵ demonstrated correlation of PET imaging features with gene expression, which led to an association of metagene clusters to imaging features and yielded good prognostic models in patients with resected non-small cell lung cancer.

Finally, correlation of radiogenomic data may enable us to make a decision about where to biopsy, when necessary. Quantitative analysis of regionally distinct radiomic features has the potential to inform precisely about the best biopsy sites by identifying the locations within complex

tumours that are most likely to contain important diagnostic, prognostic, or predictive information. In fact PET, after overlaying functional information on CT or MRI images, has been employed to guide biopsies in the abdomen and in patients with bone disease, demonstrating the potential of radiomics to enable better-informed decisions about ideal biopsy sites.^{26,27}

Improving evaluation of treatment response

Radiogenomic analysis can be used to guide therapy for treatment of individual tumours by predicting drug response and potential therapy resistance. While Kuo *et al.*²⁸ were the first to explore specific hepatocellular carcinoma imaging phenotypes that correlated with doxorubicin drug response in 2007,²⁸ more recently, a study in women who completed treatment for locally advanced breast cancer suggested that texture analysis of DCE MRI can enable us to predict response to neoadjuvant chemotherapy before its initiation.²⁹ Understanding the spatial and temporal phenotypic, physiological, and genetic heterogeneity of most solid tumours has led to the realisation that most chemotherapy responses are not durable and that targeted therapies are necessary to improve outcomes.^{6,30} Furthermore, inter- and intra-tumour phenotypic heterogeneity are associated with treatment failure and therapy resistance.¹¹ For example, in a study of malignant gliomas using fused MRI sequences, the regions of tumour that were poorly perfused on post-T1W contrast-enhanced images may exhibit areas of low or high water content on T2W images and low or high diffusion on DWI. Thus, high or low cell densities can coexist in poorly perfused volumes, creating perfusion–diffusion mismatches. Poorly perfused regions with high cell density are concerning because they can represent cell populations that are adapted to live in micro-environmental conditions associated with poor perfusion. The associated hypoxia, acidosis, and nutrient deprivation have been suggested to select for cells that are resistant to apoptosis, and subsequently, are likely to be resistant to therapy.^{31,32}

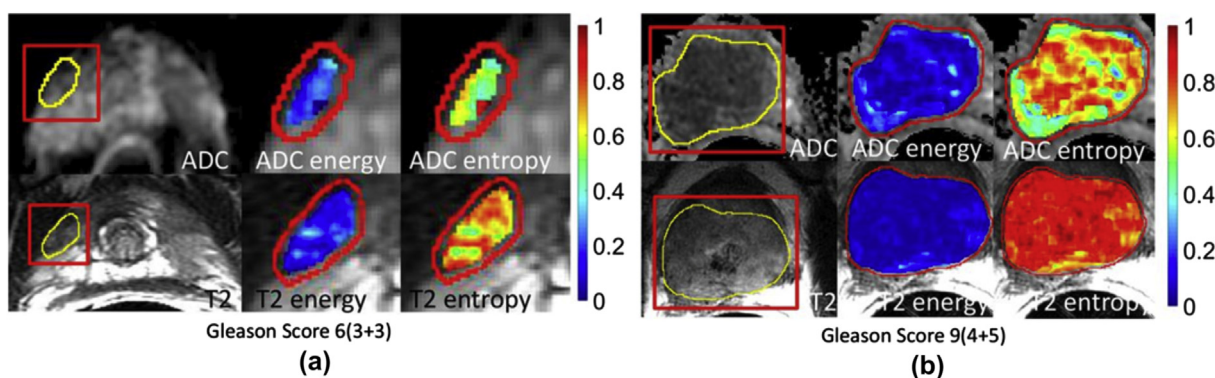


Figure 2 Example of texture analysis on ADC map and T2W image of prostate cancer. Energy and entropy values are overlaid on the tumour on ADC map (the top row) and T2W image (the bottom row). The texture features differ between a tumour of Gleason score (GS) 6 (3+3) (a) and a tumour of GS 9 (4+5) (b). Fehr *et al.*²² reported that texture analysis together with machine learning had distinguished GS6 (3+3) versus GS ≥ 7 and GS 7 (3+4) versus GS (4+3) with high accuracy: 93% and 92%, respectively. Reprinted with permission from “Automatic classification of prostate cancer Gleason scores from multiparametric magnetic resonance images,” by Fehr *et al.*,²² *Proc Natl Acad Sci U.S.A.*, 2015 Nov 17; 112 (46):E6265–E6273.

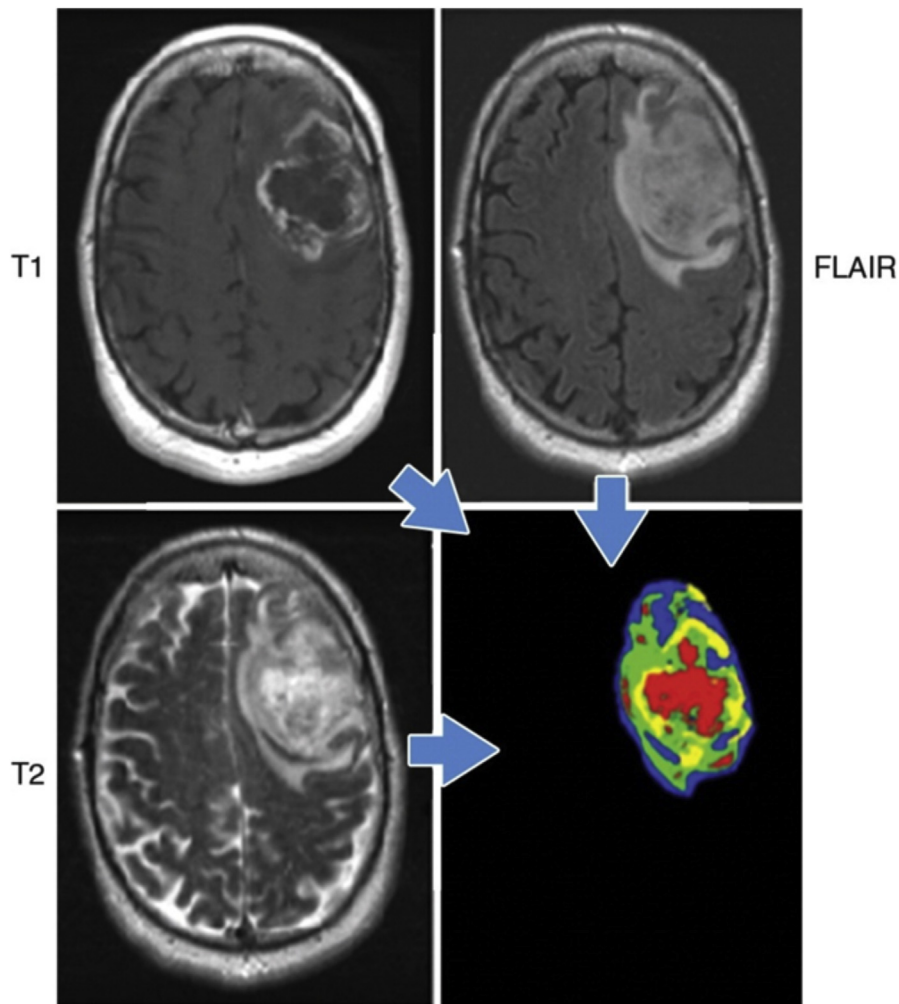


Figure 3 Example of habitat imaging in a patient with glioblastoma multiforme. Habitat imaging (lower right) is obtained by the combination of contrast-enhanced T1W, T2W, and fluid-attenuated inversion recovery (FLAIR) images. Each voxel of a tumour is assigned a specific colour depending on the combination of signal intensity (high/low) of these sequences, e.g., the red voxel is low on T1W images, and high on T2W and FLAIR images in this case. The clusters of voxels with specific colours yield regions that reflect different physiologic microenvironments, called habitats. This regional analysis would help the deeper understanding of tumour heterogeneity. Figure provided courtesy of R. A. Getenby.

Developing radiomics as biomarkers of oncological outcomes

The field of biomarker discovery and validation has evolved rapidly over the past few years with the emergence of precision medicine, aiming to tailor medical care to the individual by taking into account the variability in genes, environment, and lifestyle. Biomarkers must accurately reflect the underlying molecular cancerous machinery^{33,34} and are measured by assays often requiring tissue analysis obtained through surgery or biopsy. The limitations of this approach include its invasive nature and the fact that samples are often obtained from only a portion of a generally heterogeneous lesion and cannot completely represent the lesion's anatomical, functional, and pathological properties.³⁵ Various groups have proposed that image-derived indices may reliably identify important subregions non-invasively and create predictive imaging biomarkers.³⁶ These imaging biomarkers could then act as non-invasive measurements of functional and physiological processes

in vivo and provide information on treatment planning, prognosis, and therapy response.³⁵ For example, O'Connor *et al.*³⁷ predicted colorectal cancer liver metastasis shrinkage following anti-angiogenic and cytotoxic therapy by using heterogeneity of tumour vascular enhancement as a prognostic/predictive biomarker; however, such an endeavour has proven to be difficult in radiogenomics, making implementation of predictive biomarkers more difficult than expected.³⁸ In fact, only a few single genes, transcriptomic, epigenetic or structural genomic alterations in tumours have been discovered that could serve as clinically implementable biomarkers. For example, in breast cancer, only a few prognostic gene signatures and three traditional single gene predictive markers, namely oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), are in routine clinical use.^{13,39} The reasons behind the limited prognostic/predictive value of many proposed biomarkers are manifold, and include intra- and inter-tumour genetic heterogeneity; technical issues including reproducibility

and reliability of the biomarker assay; and retrospective, small and often clinically heterogeneous patient cohorts.³⁴

Despite these challenges, given the potential usefulness of imaging biomarkers, the National Cancer Institute (NCI) in the United States has established the Quantitative Imaging Network, which promotes the development of imaging methods, protocols, and software tools.¹⁹ Although a lot of work remains to be done, a multidisciplinary approach that combines phenotype and genetics is an important strategy for advancing precision medicine.

Challenges and future directions

Radiogenomics is still in its early phases and its implementation requires completion of a series of steps to make it usable in daily clinical practice. First, it requires standardisation of imaging protocols, including image acquisition and post-processing, as well as robust segmentation algorithms that require minimal operator input.¹³ As with any study, a radiogenomics study must be validated against a set of independent data, to ensure its reproducibility. Radiomic analyses require large image datasets with the expectation that large numbers may be able to overcome some of the inherent heterogeneities in clinical imaging. Hence, the need for informatics databases that allow for image data sharing along with medical and genetic data across sites becomes very important. Incorporating detailed clinical and patient risk factor data into radiomics is essential in clinical translation and development of biomarkers. Currently, the most important goal is optimisation of each of these steps, with a future plan to gradually harmonise and standardise the entire process.¹³

Reproducibility

Radiomics has remarkable potential to accelerate precision medicine; however, it faces challenges common to biomarker development including suboptimal study designs, high technical complexity, data overfitting, incomplete result reporting, and the presence of many confounding variables, especially when dealing with retrospective data, which makes it difficult to ensure reproducibility.¹² For example, in radiomics, a large number of imaging features may be computed, which increases the risk of extracted features becoming higher than the number of samples in a study, reducing its power and increasing the probability of overfitting the data.¹² Dimensionality reduction offers a solution to this particular issue by selection of task-specific features or by combining the original features to obtain a new set of features by using methods, such as principal component analysis.¹³ Furthermore, although some standardised tools for genomic profiling exist, they are not universally accepted and applied across different institutions.¹²

The issue of reproducibility extends beyond radiomics to biomedical research in general. A 2009 analysis of biomedical research reports found that at least 50% of studies were too poor, insufficient, or incomplete to be usable.⁴⁰ Furthermore, when scientists at Amgen (Thousand Oaks,

CA, USA) tried to replicate 53 landmark studies in the basic science of cancer, they were able to reproduce the original results of just six.⁴¹ In order to address this issue, editors from more than 30 high-impact-factor biomedical journals have imposed common standards for statistical testing and to improve access to raw data.⁴² Furthermore, reporting guidelines have been developed by many organisations, such as the Equator network, which focus on improving health research quality and transparency.⁴³ Development of such standard guidelines provides a roadmap to navigate the complex issues inherent to radiomics, particularly acquisition and analysis of high-dimensional data¹²; however, at present, adherence to these guidelines is not mandatory.

Need for validation

Validation with prospectively collected independent cohorts, ideally in the setting of clinical trials, is the reference standard for verifying an identified statistical association or biomarker.^{34,44} As with any biomarker study, a retrospective radiomics investigation must be validated against a completely independent dataset, preferably from a different institution.¹² In a thorough review by Bai *et al.*,³⁵ a validation dataset was used in only eight out of 27 studies. One problem that prevents retrospective validation studies is the availability of publicly available radiomics data, which creates the need for shared databases that can be used as validation sets. The Cancer Genome Atlas (TCGA) has generated comprehensive multidimensional genomic data of more than 30 types of cancer, which together with the clinical annotations are available to the public.⁴⁵ The Cancer Imaging Archive (TCIA)⁴⁶ is another publicly available resource that contains imaging corresponding to the patients in the TCGA database and can be used as valuable sources for both hypothesis-generating and validation purposes.

Sample size

Sample size determines the power of predictive classifier models used in radiomics and other biomedical studies. According to Gillies *et al.*,¹² 10 patients are needed for each feature in a model based on binary classifiers; however, the need for accommodation of additional clinical or genomic covariates demands a large sample size. Additionally, as few as 100 patients can be used in radiomics studies; however, datasets with larger samples provide more power.¹² Furthermore, the presence of heterogeneity in clinical imaging requires a large enough dataset to overcome such inherent differences.¹³ Acquiring a sufficiently sized sample set requires large databases and data sharing capability across sites, which already exists in form of various online repositories that host image and clinical data, as described above.¹³

Standardisation of imaging analysis

Image acquisition parameters vary widely in routine clinical practice. For example, patient positioning, pixel or matrix size, section thickness, variable reconstruction

algorithms, and washout period in the case of PET imaging vary widely in different institutions.¹³ As such, comparing results obtained across different machines becomes challenging and allows room for error. Furthermore, varying image parameters makes it more difficult to identify large numbers of image data examples with similar clinical parameters such as disease stage.¹³ For example, Kumar *et al.*¹³ showed the effect of the large variation of section thickness and pixel size in a study that aimed to develop prediction models by using image features to classify non-small cell cancer tumours. The large variation in this scenario and when performing radiomic analysis in general affects the information being extracted by image feature algorithms and subsequently classifier performance.¹³ As a result, there is an indisputable need for standardisation of imaging analysis and further research remains to be done in this area.

Image segmentation challenges

One of the most critical steps in feature data analyses is the segmentation of images into volumes of interest (VOI), which presents a variety of challenges. Many tumours have indistinct borders, which make reproducibility of their delineation an important issue.^{47,48} Manual segmentation is often performed; however, it suffers from high inter- and intra-reader variability and is labour intensive, and hence, it is not feasible for examining the large datasets required in radiomics.¹³ Automatic and semi-automatic segmentation algorithms have been developed; however, there is no universal segmentation algorithm that can work for all medical image applications.¹³ Furthermore, even when using automatic or semi-automatic segmentation, the VOI will be different even when using the same algorithm performed multiple times with different initialisations.¹³ Thus, it becomes essential to develop agreed-upon metrics to evaluate segmentation algorithms. Currently, a consensus is emerging that computer-aided edge detection followed by manual adjustment may result in optimal reproducible segmentation.¹²

Adequacy of the reference standards

Histopathology and results of genomic testing are often used as standards of reference; however, only few studies have explored the spatial relationship between imaging, genomics, and histopathology.¹⁵ Although there is a need for large prospective studies, a few issues arise when attempting to integrate imaging, genomic, and histopathological data. These studies must evaluate large and complex data across a range of biologically different scales.^{19,49} One problem is that CT, MRI, or PET voxels are usually non-isotropic, where section thickness exceeds in-plane resolution. Hence, compared with genomic and histopathology biomarkers, this represents many orders of magnitude difference in scale,⁴⁹ making it challenging to validate image heterogeneity biomarkers against histopathology. Furthermore, it is unclear whether imaging, genomics, and histopathology show spatial correspondence because they measure the same biology in different ways or alternatively

measure different biology, providing complimentary data.¹⁵ If the last statement is true, this would open up new multidisciplinary strategies for advancing precision medicine by bringing imaging phenotype and genotype together, rather than assessing genomics in isolation.¹⁵

Clinical translatability

Radiogenomics is still a relatively new field, and its full potential for clinical translation is yet to be explored; however, many studies have shown early promise. For example, in the research of hepatocellular carcinoma, microscopic venous invasion (MVI) is a sign of poor prognosis.⁵⁰ Imaging techniques have failed to predict MVI and the only way it is diagnosed is by explanted tissue histology.⁵¹ In a study by Segal *et al.*,⁵² 91 genes in the “venous invasion signature” were associated with two predominant imaging traits on CT—the presence of “internal arteries” and absence of “hypodense halos”. Banerjee *et al.*⁵³ again demonstrated that these two imaging biomarkers, along with “tumour–liver difference,” were able to predict histological MVI with high precision. Furthermore, these three features were associated with early disease recurrence and poor overall survival.⁵³ Beyond diagnosis, radiogenomics can have a significant impact on treatment guidance and overall survival.³⁵ In a study of clear cell renal cell carcinoma patients, Jamshidi *et al.*⁵⁴ developed a prognostic multigene signature termed radiogenomic risk score consisting of four CT imaging features (tumour necrosis pattern, transition zone, tumour–parenchyma interaction, and tumour–parenchyma interface) to predict disease-specific survival, independent of disease stage, disease grade, and performance status. The ultimate goal of clinical translation of radiogenomics is to enhance the radiology report to beyond what is typically reported and include genomics data in addition to radiological data, which may have an impact on patient clinical management.

Data sharing

As mentioned above, there is need for the development of integrated publicly available databases where images and the extracted features are linked to clinical and molecular data¹³ to ensure studies of sufficient size for statistical power. Such databases present their own challenges including de-identification, the need for large digital storage space, integration of clinical and molecular data to create a simple work stream, as well as reporting and exporting the data.¹³ Furthermore, as part of a larger network of quantitative imaging sites, we must also be able to exchange data according to an evolving set of standards. Various online repositories that host image data are currently available, such as the online CT image repository, the National Biomedical Image Archive (NBIA), hosted by NCI; however, in addition to images themselves, image annotations, outcome data, acquisition, scanner, and other information should be available. Currently, available clinical image data, which may be used for radiomics study, includes TCIA, TCGA, and others.^{12,13,45,46} Ultimately, the goal is multi-institutional,

national, or international consortia to agree to share data either through centralised or federated networks.¹²

Need for well-designed prospective studies that account for spatial and temporal heterogeneity

Although initial retrospective studies linking imaging phenotype with genotype have shown high prognostic capabilities, they do not provide spatial information, as quantitative imaging features are generated and averaged over the entire tumour, assuming that tumours are heterogeneous but well mixed. This approach ignores the spatial heterogeneity readily apparent on imaging. Subregions of tumour that show distinct imaging features may have different biological behaviour, which may lead to different response to therapy or drive tumour progression and metastatic ability.¹⁵ Indeed, recent genomics work has highlighted the presence of intra-tumour genetic and transcriptomic variation.^{1–3,7,38,55} Nevertheless, little effort if any has been put into integrating imaging, histopathology, and genomics.^{4,56} In a retrospective study of 10 patients with breast cancer, heterogeneous enhancement correlated with genetic subtypes.⁴ Jenkinson *et al.*⁵⁶ found significant differences in ADC values at tumour margins between oligodendroglial tumour genotypes that may reflect underlying tumour biology⁵⁶; however, there is a need for well-designed prospective studies focused on meaningful integration of imaging phenotype and genotype rather than genomics or imaging in isolation.

Important information can be gained by evaluating the quantitative parameters in discrete phenotypic clusters or regions of the tumour by combining perfusion, diffusion, and metabolic maps derived from multiparametric imaging, such as MRI and PET/CT, to create one set of phenotypic heterogeneity maps based on their similarities and differences. This in turn can be used to guide tissue sampling, from both primary tumours and metastatic disease, for detailed histological, immunohistochemical, genetic, epigenetic, and proteomic analysis⁵⁵ with the goal of assessing whether an imaging-based analysis of heterogeneity would be reflective of the underlying pathological and/or genomic heterogeneity of the tumour.

Conclusion

In conclusion, complementary innovations in massive parallel sequencing, proteomics, and imaging that allow spatial and temporal quantification of tumour heterogeneity and its changes during drug treatment will provide the basis for the realisation of precision oncology.

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