Imaging-based characterization of tumoral heterogeneity for personalized cancer treatment
Charakterisierung der Tumorheterogenität mittels bildgebender Verfahren zur personalisierten Krebsbehandlung

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ABSTRACT
With personalized tumor therapy, understanding and addressing the heterogeneity of malignant tumors is becoming increasingly important. Heterogeneity can be found within one lesion (intralesional) and between several lesions (interlesional). The heterogeneous tumor cells may show a different response to treatment due to their biology, which in turn influences the outcome of the affected patients and the choice of therapeutic agents. Therefore, both intra- and interlesional heterogeneity should be addressed at the diagnostic stage. While genetic and biological heterogeneity are important parameters in molecular tumor characterization and in histopathology, they are not yet addressed routinely in medical imaging. This article summarizes the recently established markers for tumor heterogeneity in imaging as well as heterogeneous/mixed response to therapy. Furthermore, a look at emerging markers is given. The ultimate goal of this overview is to provide comprehensive understanding of tumor heterogeneity and its implications for radiology and for communication with interdisciplinary teams in oncology.

Key points:
▪ Tumor heterogeneity can be described within one lesion (intralesional) or between several lesions (interlesional).
▪ The heterogeneous biology of tumor cells can lead to a mixed therapeutic response and should be addressed in diagnostics and the therapeutic regime.
▪ Quantitative image diagnostics can be enhanced using AI, improved histopathological methods, and liquid profiling in the future.

ZUSAMMENFASSUNG
Im Rahmen der personalisierten Tumorthherapie wird es immer bedeutender, die Heterogenität von bösartigen Tumoren zu verstehen und zu berücksichtigen. Diese kann innerhalb einer Läsion (intralesional) und zwischen mehreren Tumorläsionen auftreten, die aus einem primären Tumor hervorgehen (interlesional). Die heterogenen Tumorzellen können aufgrund ihrer Biologie unterschiedliche Reaktionen auf verschiedene Behandlungen zeigen, was wiederum das Outcome der betroffenen Patienten und die Wahl der Therapie beeinflusst. Daher sollten sowohl intra- als auch interlesionale Heterogenität in der Diagnostik berücksichtigt werden. Während genetische und biologische Heterogenität wichtige Parameter in der molekularen Tumorcharakterisierung und in der Histopathologie sind, werfen sie in der medizinischen Bildgebung noch nicht routinemäßig berücksichtigt. Dieser Artikel fasst die etablierten Marker für Tumorheterogenität in der Bildgebung sowie für heterogenes/gemischtes Therapieansprechen zusammen. Darüber hinaus wird ein Ausblick über aufkommende Marker gegeben. Ziel dieser Übersichtsarbeit ist es, ein umfassendes Verständnis der Heterogenität von Tumoren und ihrer Auswir-
kungen auf die Radiologie und die interdisziplinäre Kommunikation in der Onkologie zu vermitteln.

Kernaussagen:
- Tumorheterogenität kann innerhalb einer Läsion (intraleisonal) oder zwischen mehreren Läsionen (interlesional) beschrieben werden.
- Die heterogene Biologie von Tumorzellen kann zu einer gemischten therapeutischen Reaktion führen und sollte sowohl bei Diagnose als auch Therapie berücksichtigt werden.
- Die quantitative Bilddiagnostik kann in Zukunft durch den Einsatz vonKI, verbesserten histopathologischen Methoden und Liquid Profiling ergänzt werden.

Zitierweise

Background
Although there have been significant advancements in cancer treatment in the last few years, there is still a need for improvement. This is especially true in the context of personalized cancer treatment, which takes the variability of tumoral biology among patients with the same tumor entities into account and addresses it with corresponding targeted treatment approaches. The underlying reason for different responses to (conventional) treatments is the heterogeneity of their neoplastic biology [1]. Additionally, tumor biology not only differs between patients but there is also heterogeneity within a singular lesion (intraleisonal variability) and between different lesions in one patient (interlesional variability) [2, 3]. These heterogeneities manifest as morphological variations between tumor cells, genetic profiles, and the expression levels of biomarkers [4]. The variability of the tumor cells is caused by genetic heterogeneity (e.g., due to the accumulation of somatic mutations/clonal evolution) as well as non-genetic causes such as changes in the tumor microenvironment [5] (Fig. 1a). Since tumor heterogeneity drives the emergence of resistance, it can have a major impact on patient response to therapy and thus survival [6]. Furthermore, it has been shown that increased heterogeneity of tumor lesions is linked to a worsening of patient survival [7]. Therefore, it is of crucial importance to detect both the interlesional and the intraleisonal tumor heterogeneity and to adapt the targeted (possibly personalized) cancer therapy to it. Despite histopathological or blood-based approaches such as Liquid Profiling (LP), modern imaging modalities and quantitative image analysis are promising devices for detecting tumor heterogeneity [8]. Since heterogeneity in or between tumoral lesions is not yet sufficiently considered in the current clinical routine, there is a need for integration of these methods, which can recognize and take heterogeneity factors into account. This review aims to give an overview of potential imaging markers for intraleisonal, interlesional, and response-associated tumor heterogeneity (Fig. 1b).

Imaging markers for intraleisonal heterogeneity

Traditionally, certain descriptors for specific tumoral lesions have been introduced to achieve better characterization of lesions in imaging. For example, the classification of lesion size and solid and subsolid characteristics according to the Fleischner guidelines is routinely used in the classification of incidental lung nodules [9]. Also, visual contrast enhancement, lesion size, and magnetic resonance signal (MR signal) characteristics have, as a result, been implemented in a wide variety of structured reporting schemes in oncologic imaging [10, 11]. While the multiparametric assessment of (singular) lesions has become firmly established in the clinical routine, the tumoral heterogeneity within a specific lesion is not assessed routinely in imaging.

In this context, intraleisonal heterogeneity is defined as the diversity of the cellular composition of a tumoral lesion, which may be assessed by imaging. For this approach, the estimation of diffusion and apparent diffusion coefficient (ADC) parametric maps on magnetic resonance imaging (MRI) are especially promising, as they allow an estimation of diffusion rates within a lesion [12]. These may vary based on the cellularity and biological properties of the local tumor environment [13, 14]. In soft tissue sarcoma, a correlation of lower ADC values with G2/3 tumor grade based on multiple intraoperative biopsies has been described [15]. For Prostate Cancer Gleason Score estimation from multiparametric MRI, an extraction of the radiomics features energy and entropy from ADC and T2 could achieve a noninvasive estimation of the underlying histology with an accuracy of up to 93% [16]. In patients

ABBREVIATIONS

- ADC: Apparent diffusion coefficient
- CT: Computed tomography
- ctDNA: Circulating tumor deoxyribonucleic acid
- DWI: Diffusion-weighted imaging
- FABPI: Fibroblast activation protein inhibitors
- FDG: Fluoro-2-deoxy-d-glucose
- HCC: Hepatocellular carcinoma
- LP: Liquid profiling
- MRI: Magnetic resonance imaging
- RECIST: Response Evaluation Criteria In Solid Tumors
- PET/CT: Positron emission tomography computed tomography
- PSMA: Prostate-specific membrane antigen
- SUV: Standard uptake value

Fig. 1a ▶ Haag F et al. Imaging-based characterization of... Fortschr Röntgenstr | © 2023. Thieme. All rights reserved.
with lower rectal cancer, the intralesional tumor heterogeneity and therapeutic response can be predicted by diffusion-weighted MRI (DWI) [17]. Also, diagnostic MRI can be used to score heterogeneity in soft tissue sarcoma and identify them as high- or low-grade soft tissue sarcomas [18]. A direct co-registration of DWI and histology in non-small cell lung cancer showed that an estimation of the local spatial tumor cell density can be performed based on DWI data [19]. The feasibility of these methods has also been shown for perfusion MRI approaches, such as Ktrans parameter maps [20]. In a murine model, the association of multiparametric MRI data with histology and tissue biology could be shown for the estimation of malignant potential in breast cancer [21].

The intralesional differentiation of tumor tissue populations with MRI was identified in a xenograft mouse model of colorectal cancer, which allows for the differentiation of necrotic subpopulations, adipose tissue, and viable tumor. Here, ADC imaging was identified as the dominant parameter for differentiation [22]. For hepatocellular carcinoma, quantitative parameter extraction from multiparametric MRI including histogram analysis could estimate a high degree of heterogeneity within hepatocellular carcinoma (HCC) lesions [23]. Furthermore, quantitative feature extraction and visualization may help reveal novel intralesional patterns. In this context, radiomics is a good example of the unrevealed possibilities in conventional imaging such as MRI or CT. It describes...
the abstraction of parameters from diagnostic images, which are not recognizable to the human eye [24, 25]. The potential of radiomics for the detection of tumor heterogeneity has been demonstrated several times for different tumor entities such as breast cancer [26–28] or hepatocellular carcinoma [29–31].

**Fig. 2** shows the example of a patient with sarcoma. In the shown case, radiomics feature mapping indicates intralesional heterogeneity and may potentially help to differentiate between subregions within a lesion.

**Fig. 3** Interlesional heterogeneity. Feature extraction and unsupervised clustering approach for identification of lesion clusters on imaging.

**Fig. 4** Example of heterogeneous response. Axial CT scans of a patient suffering cutaneous squamous cell carcinoma. A) Baseline exam with manifestation of a pulmonary metastasis at the right hilus (arrow). B) Follow-up after chemotherapy with mixed response. The pulmonary metastasis regressed, but there is a new cutaneous metastasis at the right thoracic apparatus (*).
mas, rectal cancer and many other malignancies can be detected in intralesional tumor heterogeneity of pancreatic adenocarcinoma [32]. Using 18-F FDG PET/CT metabolic tumor volume and labeled substance provides information about the cell which is taken up by tumor cells. The uptake of the radioactively radioactively labelled 2-[(18)F]fluoro-2-deoxy-d-glucose (FDG), characterization of tumor heterogeneity.

Compared to localized oncologic disease, metastatic disease poses an even more complex challenge in terms of tumoral heterogeneity, because not only biological heterogeneity within the primary lesion but also between metastatic lesions becomes relevant. While this interlesional heterogeneity can be clearly understood as a biological reason for mixed response to treatment, it has not yet been addressed comprehensively in clinical imaging. A study by Siravegna et al. addressed the clonal evolution of cancer foci in metastatic colorectal cancer and investigated the per-lesion heterogeneity in a post-mortem biopsy approach [40]. It shows the importance of a per-lesion investigation of aggressiveness, since per-lesion genetic patterns and evolutionary dynamics were associated with per-lesion response to systemic therapy. In particular, this study showed an association between resistance patterns and lesion response according to Response Evaluation Criteria in Solid Tumors (RECIST).

Despite genetic approaches, the heterogeneity of lesions in imaging is mainly evaluated qualitatively or in terms of response assessments. Parts, associations between mutational patterns in lesions have been described, for example, an association of lower ADC values on MRI [41] and higher standard maximal uptake values (SUV-max) on PET/CT [42] with KRAS mutations in colorectal liver metastases. While a visual classification of lesions in metastatic disease may be partly performed in terms of enhancement or size, the discretization and quantification of lesion texture utilizing the radiomics workflow and/or convolutional neural networks may allow for a more precise classification of lesions (workflow shown in Fig. 3): In the case of metastatic colorectal cancer, radiomics feature extraction and unsupervised hierarchical clustering have been employed to define lesion subtypes (small disseminated, heteroge-
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neous, homogeneous, mixed, very large) [43]. A study by Yousefi et al. utilized cluster analysis to define two radiomics subtypes in non-small cell lung cancer lesions [44] which showed a significant association with EGFR mutation status (p = 0.07), progression-free survival (p = 0.03), and a tendency for overall survival (p = 0.11). Furthermore, the addition of radiomics parameters to circulating tumor deoxyribonucleic acid (ctDNA) and clinical variables resulted in a better model fit (c statistic 0.77 vs. 0.73, p = 0.01) for PFS. In metastatic prostate cancer, the expression of prostate-specific membrane antigen (PSMA) can vary between the different metastases as an expression of intrallesional tumor heterogeneity and due to different gene expressions in the tumor cells. PSMA expression in different lesions can be detected by PET-CT [45]. Heterogeneous expression of PSMA in several lesions has the potential for a severe prognosis [46]. Another example for interlesional heterogeneity in molecular imaging is the so-called flip-flop phenomenon, which can occur in patients suffering from thyroid cancer with multiple lesions. It describes an inverse relation between iodine and glucose utilization (and uptake) in different thyroid cancer lesions according to the degree of differentiation and can lead to a mixed therapy response [47–49]. Given these facts, tumor heterogeneity is a parameter with a high prognostic value and should be monitored in the patient’s follow-up.

### Therapy response assessment

Intra- and interlesional tumor heterogeneity are important determiners of response to therapeutic strategies and patient outcomes [50]. The changing genetic and biological tumor signature is an evolutionary process and is often accelerated by the treatment that is used (example in ▶ Fig.4) [51]. Therefore, on the one hand, the therapy strategy must be adapted accordingly. An example of this approach is the minimally invasive ablation of therapy-resistant liver lesions in patients with multiple cancer lesions, such as in oligometastatic disease with mixed response [52]. On the other hand, it is important to classify the therapy response adequately and supplement existing classifications with the parameters of lesional heterogeneity. An example of an adapted classification for HCC is presented by Zang et al. [53]. The mentioned study suggests determining the expression levels of CD45 and Foxp3 on HCC cells using immunohistochemistry in these patients. Despite molecular parameters, there is also a need to establish noninvasive image parameters. A powerful imaging parameter represents the 3D volumetry of pulmonary metastasis in computed tomography (CT) [54]. Another prognostic marker is CT attenuation of lesions. The mean attenuation of liver lesions was identified as a predictor for therapy response of liver metastasis in colorectal cancer treated with anti-EGFR therapy [55]. Also, CT-based tumor heterogeneity analysis has the potential to predict therapeutic response in patients with pancreatic carcinoma in palliative chemotherapy [56]. A heterogeneous response can also be addressed by MRI. In this context, Lau et al. demonstrated in patients with metastatic melanoma under immune checkpoint therapy that heterogeneity of metastasis and potential therapeutic response can be visualized and assessed by MRI [57]. These data clearly demonstrate that established meth-
ods and imaging devices have the potential to visualize inter- and intratumor heterogeneity and thus a differing response to therapy. In addition to emerging approaches like liquid profiling and integrative diagnostics, it is also crucial to extract the non-human-but machine-readable information of established imaging procedures using quantitative imaging biomarkers.

**Outlook**

In summary, advanced imaging methods (summary given in ▶ **Table 1**) as well as quantitative data analysis approaches (▶ **Table 2**) can be utilized to evaluate tumoral heterogeneity in noninvasive imaging. An overview of the current literature is presented in ▶ **Table 3**.

Although tumoral heterogeneity and heterogeneous response should be evaluated in imaging utilizing these techniques, the optimal predictive value cannot be achieved by imaging alone. A combined approach with other diagnostic modalities such as histology, liquid profiling and molecular pathology enables a comprehensive assessment of cancer biology and the clinical situation. On the one hand, the integration of liquid profiling (LP) information with a corresponding imaging strategy can lead to earlier detection of recurrence, identify the emergence of drug resistance, and quantify minimal residual disease [58, 59]. The potential of LP to detect heterogeneity and therapy resistance was already shown in gastrointestinal cancers [60]. There is evidence that a combination of liquid biomarkers with functional imaging is helpful in the prediction of the outcome of patients suffering from castration-resistant metastatic prostate cancer [61]. Finally, LP has major potential, and it may be a powerful addition to established procedures in routine diagnostics and follow-up examinations in oncologic patients. On the other hand, imaging can guide selection of targets for biopsy to allow for a precise and optimized assessment by histopathology and molecular pathology. Therefore, better assessment of tumoral heterogeneity in diagnostic medicine will support the development of an integrative diagnostic workflow, which has important positive implications along the
whole oncology value chain [62]. Integrative diagnostics refers to the combination and joint interpretation of diagnostic results in the combination of mutual triggering of examinations and more accurate estimation of disease states, resulting in a better, personalized diagnostic strategy and more precise and actionable diagnostic results (▶ Fig. 5) for treatment planning [63].

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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