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REVIEW AND PERSPECTIVES

Tumor regression grading of gastrointestinal cancers after neoadjuvant therapy

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Abstract Neoadjuvant therapy has been successfully introduced in the treatment of locally advanced gastrointestinal malignancies, particularly esophageal, gastric, and rectal cancers. The effects of preoperative chemo- or radiochemotherapy can be determined by histopathological investigation of the resection specimen following this treatment. Frequent histological findings after neoadjuvant therapy include various amounts of residual tumor, inflammation, resorptive changes with infiltrates of foamy histiocytes, foreign body reactions, and scarry fibrosis. Several tumor regression grading (TRG) systems, which aim to categorize the amount of regressive changes after cytotoxic treatment in primary tumor sites, have been proposed for gastroesophageal and rectal carcinomas. These systems primarily refer to the amount of therapy-induced fibrosis in relation to the residual tumor (e.g., the Mandard, Dworak, or AJCC systems) or the estimated percentage of residual tumor in relation to the previous tumor site (e.g., the Becker, Rödel, or Rectal Cancer Regression Grading systems). TRGs provide valuable prognostic information, as in most cases, complete or subtotal tumor regression after neoadjuvant treatment is associated with better patient outcomes. This review describes the typical histopathological findings after neoadjuvant treatment, discusses the most commonly used TRG systems for gastroesophageal and rectal carcinomas, addresses the limitations and critical issues of tumor regression grading in these tumors, and describes the clinical impact of TRG.

Keywords Tumor regression grading · Histopathology · Gastric cancer · Esophageal cancer · Rectal cancer · Neoadjuvant therapy

Introduction

Multimodal treatment has been successfully introduced in the therapy of gastrointestinal malignancies, particularly esophageal, gastric, and rectal carcinomas. Preoperative/neoadjuvant treatment, typically chemo- or radiochemotherapy, has been associated with survival benefit for patients compared to surgery alone in locally advanced gastrointestinal cancers. Therefore, a combination of neoadjuvant therapy followed by surgery with or without subsequent adjuvant treatment currently represents the standard approach for these tumors [1–9]. The effects of neoadjuvant therapy on the primary site of the tumor can be assessed by macroscopic and, particularly, histopathological investigation of the resected specimens. These analyses may show marked variations among tumors, even within the same type of cancer [10–13]. Several tumor regression grading (TRG) systems for esophageal, gastric, and rectal carcinomas have been proposed in past decades, which categorize the degree of regressive changes after neoadjuvant treatment [12, 14–19]. It has been shown that TRGs provide highly valuable prognostic information, as in most cases, complete or subtotal tumor regression after neoadjuvant treatment is associated with better patient outcomes. For other gastrointestinal malignancies, such as colon carcinomas, anal carcinomas, or mesenchymal tumors, TRGs have not been developed, since neoadjuvant therapeutic concepts have not entered routine clinical treatment.

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In the following, we describe the characteristic histopathological findings observed after neoadjuvant therapy, summarize the concepts of tumor regression grading, present examples of some commonly used tumor regression grading systems for esophageal, gastric, and rectal cancers, discuss their limitations and critical issues, and describe the clinical impact of TRGs for gastrointestinal carcinomas.

Histopathological findings after neoadjuvant therapy

The first step of the pathological work-up is the macroscopic assessment of the resection specimens of the tumor. Here, an initial rough estimation of tumor regression is feasible, but it is of even greater importance to estimate the extent of the so-called tumor bed (the previous site of the tumor) to assure proper embedding for accurate histological investigation (Fig. 1). By histology, tumor regression after neoadjuvant therapy basically represents subacute to subchronic inflammation following cytotoxic effects that occur a few to several weeks beforehand. In most cases, the tumors are resected after a certain delay following the completion of the last cycle of preoperative treatment.

In cases of complete tumor regression, malignant cells are destroyed by cytotoxic treatment and/or subsequent inflammatory reaction, and the tumor is replaced by fibrous or fibro-inflammatory granulation tissue. Residual tumor, in contrast, may be abundant or only comprise small single cells, clusters resembling tumor buds, or tumor cell groups. Several patterns of tumor regression can be observed: tumors may show shrinkage or fragmentation [20, 21]. Tumor regression may also follow a centrifugal pattern, where the residual tumor can be observed in the superficial or deep periphery of the previous tumor site only and not in the tumor center [12]. Resorptive changes comprise a histiocytic reaction with foamy or occasionally hemosiderin-laden macrophages, cholesterol clefts and foreign body reactions, and dystrophic calcifications [10–12, 22–24]. The presence of mucinous changes, occasionally with abundant and acellular mucin lakes, can be frequently observed in adenocarcinomas treated with neoadjuvant therapy [12, 24, 25]. This acellular mucin should not be considered a viable residual tumor [10]. Notably, the presence of foamy histiocytes and a central fibrosis pattern of regression were most specific for therapy-induced regression in a study comparing treated with treatment-naïve tumors [12] (Fig. 2). Stromal changes, such as general fibrosis, inflammation, or granulating changes following endogenous tumor necrosis, can also be observed in untreated carcinomas.

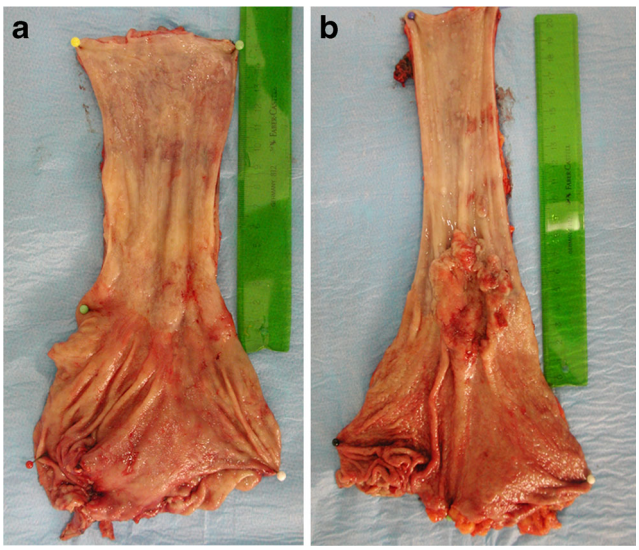

Fig. 1 Macroscopic images of adenocarcinomas of the distal esophagus with **a** macroscopic significant regression and **b** no macroscopic significant regression following neoadjuvant chemotherapy

On a cellular level, residual tumor cells can show characteristic wide eosinophilic cytoplasm with vacuolization or oncocytic differentiation. Nuclear atypia, including hyperchromasia, pyknosis, karyorrhexis, or the formation of large, bizarre nuclei, are frequent findings. Giant cells may also be present. In contrast to apoptotic figures, mitoses are rarely observed. These changes may be observed in a rather localized manner, and histologically unremarkable areas of cancer infiltrates can be observed immediately adjacent to atypical tumor glands or cells with significant cytopathological changes [10, 12, 22]. Both tumoral and non-tumoral stroma show significant alterations, with the characteristic finding of bizarre stromal fibroblasts. Vascular changes, such as myxohyaline intimal proliferation in vessels, often with highly atypical reactive endothelial cells, telangiectasia, organizing thrombi, and obliterating endarteritis, have been frequently observed. In non-neoplastic adjacent tissue, treatment-associated changes, such as edema and inflammation, have also been observed. Alterations of non-neoplastic epithelia may be similar to changes in tumor cells, with nuclear pleomorphism, condensed chromatin, and eosinophilia. Such alterations of non-neoplastic tissues may occasionally appear worrisome and may cause difficulties in the discrimination from cancer. An important pitfall, for example, is atrophy and metaplastic changes of non-neoplastic gland structures of the esophagus and stomach [10, 12, 22] (Fig. 2).

Classification of tumor regression

Tumor regression grading (TRG) systems aim to categorize the amount of regressive changes after cytotoxic treatment to demonstrate potential prognostic information based on objectively determinable histopathological findings. Currently, these strategies refer to regressive changes observed at the

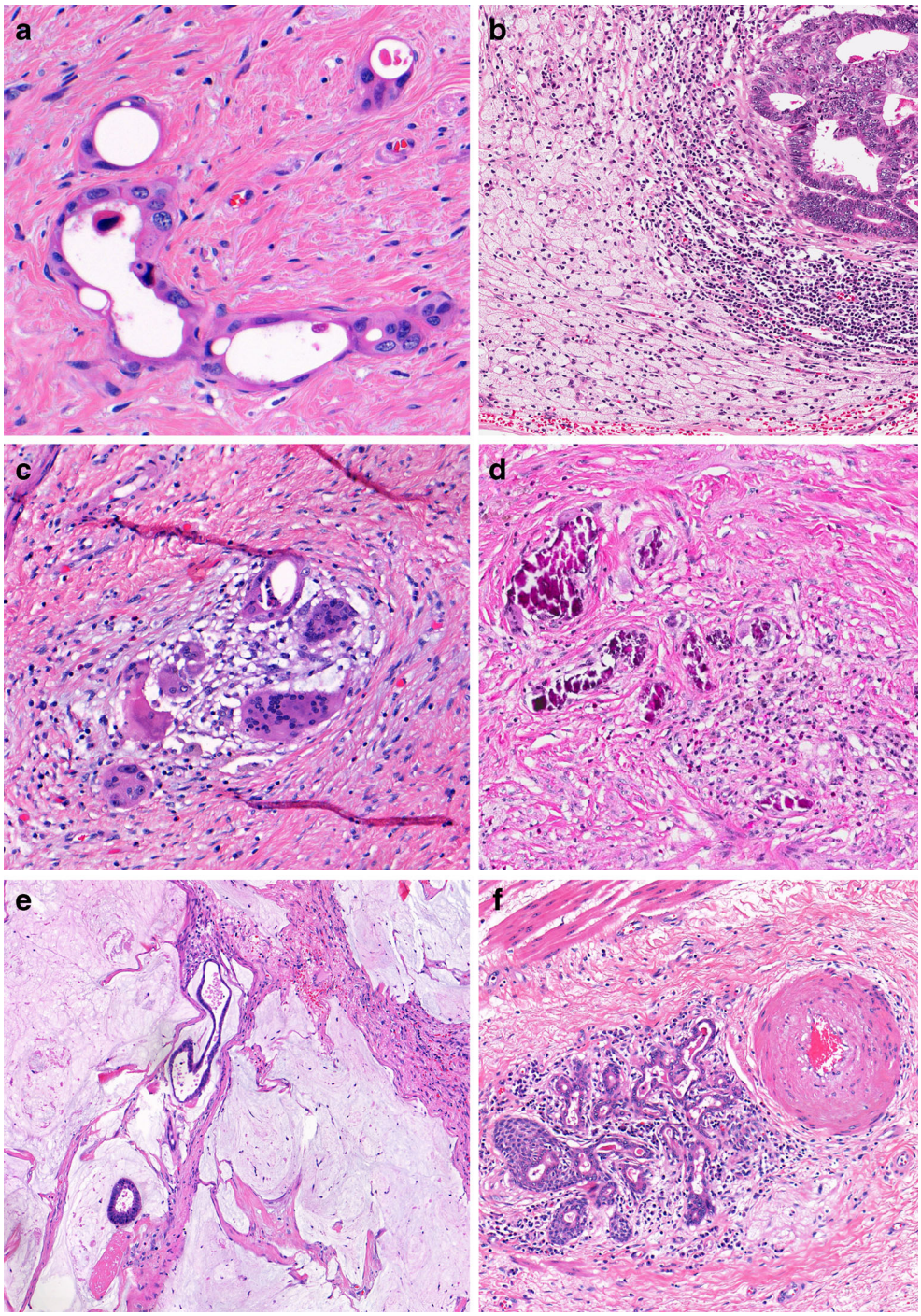

Fig. 2 Histologic findings of tumors treated by neoadjuvant (radio) chemotherapy **a** cytologic atypia **b** foamy histiocytes (adjacent to residual tumor) **c** foreign body reaction **d** calcifications and inflammation **e** acellular mucin (note scarce residual tumor) **f** obliterated vessel and regressive changes in non-neoplastic esophageal glands.

primary site of the tumor and not to findings in lymph nodes or distant metastases. On a descriptive level, tumors may show complete regression or various amounts of residual tumor: a few scattered residual tumor cells or groups within areas of scarry fibrosis, inflammation, or resorption, which can be described as "subtotal regression"; more residual tumor cells or groups, which can be described as "partial regression"; and a significant amount of tumor with or without signs of regressive changes, which can be labeled as "no significant regression". As stated above, many histopathologically detectable alterations and findings are only infrequently observed and are not entirely specific for tumor regression after cytotoxic treatments. Therefore, the regression grading systems primarily refer to single, better reproducible parameters. Two concepts to classify the degree of tumor regression can be differentiated: estimation of the amount of residual tumor, which is typically stated as a percentage; and the estimation of the relation between residual tumor and regressive fibrosis,

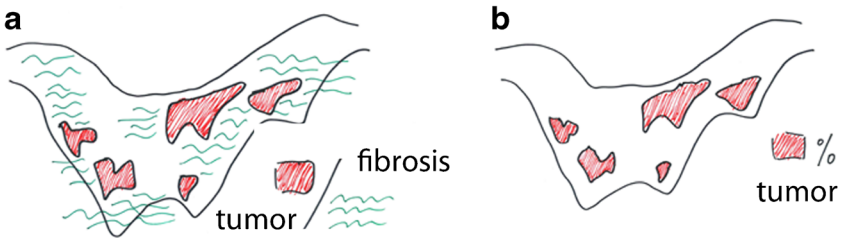

Fig. 3 Principles of the assessment of tumor regression grading: **a** tumor/fibrosis relation **b** tumor in %

which is typically based on description (Fig. 3). In the following, some examples of TRGs which are widely used in diagnostic practice and well known to pathologists and clinicians are presented. The tumor regression grading systems according to Mandard [15], Dworak [14] or the American Joint Committee on Cancer/College of American Pathologists (AJCC/CAP) [26] are examples for TRGs that refer to the relation of tumor/fibrosis or use descriptions. The Becker system [12], the Rödel system [17], and the TRGs of the Japanese Gastric Cancer Association [27], and the Rectal Cancer Regression Grading System of the Royal Association of Pathologists [28] use the percentage of residual tumor as a reference for regression grading. An overview concerning the various TRGs and their relation to descriptive labeling is provided in Table 1.

TRG according to Mandard

The Mandard classification system, published in 1994, was first applied for the categorization of tumor regression in esophageal squamous cell carcinomas following neoadjuvant treatment with cisplatin and radiotherapy [15]. The initial study comprised 93 resected specimens. The macroscopic division into three distinct groups (obvious residual tumor with ulceration/fungating/infiltrative features; apparent tumor regression and scarring; and doubtful cases) is not used in diagnostic practice. Using histology, the cases were separated into two groups with or without regressive changes, the latter including cytological alterations and stromal changes. Tumor regression was classified into five histological categories based on the ratio of vital tumor tissue to fibrosis: TRG 1 = complete regression, i.e., fibrosis without detectable tissue of tumor; TRG 2 = fibrosis with scattered tumor cells; TRG 3 = fibrosis and tumor cells with preponderance of fibrosis; TRG 4 = fibrosis and tumor cells with preponderance of tumor cells; and TRG 5 = tumor tissue without changes of regression. In this study, 44% of the cases were TRG 1–2, 20% of the cases were TRG 3, and 33% of the cases were TRG 4–5. TRG is correlated with disease-free survival (DFS) and was also an independent predictor of DFS in multivariate analysis when collapsing TRG 1–3 vs. TRG 4–5. Since then, the Mandard system has been frequently applied to each type of gastrointestinal carcinoma after neoadjuvant treatment and is one of the most widely used TRG systems [10].

AJCC/CAP

The AJCC/CAP uses a four-tiered grading system that is also recommended for rectal cancer in the AJCC TNM staging system [26]. There is no description concerning the macroscopic work-up. The four TRGs are as follows: TRG 0 = no residual tumor cells; TRG 1 = single cells or small groups of cells; TRG 2 = residual cancer with desmoplastic response; and TRG 3 = minimal evidence of tumor response. After its initial proposal in 2010, the AJCC/CAP system has successfully been applied on larger cohorts of rectal cancers [29, 30].

TRG according to Dworak

Dworak et al. [14] described their tumor regression grading system for rectal cancers in 1997 based on the findings in 17 patients who received preoperative radiochemotherapy with 5-FU/50 Gy. Different approaches for sampling the tumor tissue were used according to the macroscopic appearance. In cases where no visible tumor tissue was present, the whole fibrotic scar was embedded. In cases where macroscopically obvious tumors were observed, a minimum of four tissue blocks and an additional large tumor area block was investigated. Lower tumor regression grades describe lower degrees of regression, in contrast to the Mandard or AJCC systems: TRG 0 = no regression; TRG 1 = dominant tumor mass with obvious fibrosis and/or vasculopathy; TRG 2 = dominantly fibrotic changes with few tumor cells or groups, which are easy to detect; TRG 3 = very few tumor cells, which are difficult to detect, in fibrotic tissue with/without mucous substance; TRG 4 = only fibrotic mass without tumor cells, i.e., complete regression.

TRG according to Becker

In 2003, Becker et al. [12] proposed a grading system for locally advanced gastric carcinomas treated by neoadjuvant chemotherapy with etoposide, doxorubicin, and cisplatin. Thirty-six gastric carcinoma cases were investigated and compared to cases treated with surgery alone. For histopathological analysis, the entire macroscopically identifiable residual "tumor beds" (previous site of the tumor) were embedded and analyzed. Tumor regression grading was based on the estimation of the percentage of vital tumor tissue in relation

Table 1 Examples for tumor regression grading systems AJCC = American Joint Committee on Cancer; JSED = Japanese Society of Esophageal Diseases; JGCA = Japanese Gastric Cancer Association; and Cologne = Cologne Regression Grading System

Examples for tumor regression grading systems—complete regression = lowest category of TRG					Examples for tumor regression grading system—no regression = lowest category of TRG			
Descriptive	Mandard	AJCC	Becker	JGCA/JSED	Rödel	Dworak	Cologne	Descriptive
Complete	TRG 1	TRG 0	TRG 1a	TRG 1	TRG 0	TRG 0	TRG 1	No regression
	No residual cancer cells	No residual tumor cells	Complete regression	0% residual tumor	No regression	No regression	> 50% Vital residual tumor cells	
Subtotal	TRG 2	TRG 1	TRG 1b	TRG 2	TRG 1	TRG 1		
	Rare cancer cells	Single cell or small group of cells	< 10% residual tumor	1–33% residual tumor	Fibrosis < 25% of tumor mass	Predominantly tumor with significant fibrosis and/or vasculopathy		
Partial	TRG 3	TRG 2	TRG 2	TRG 3	TRG 2	TRG 2	TRG 2	Partial
	Fibrosis outgrowing residual cancer	Residual cancer with desmoplastic response	10–50% residual tumor	34–66% residual tumor	Fibrosis 25–50% of tumor mass	Predominantly fibrosis with scattered tumor cells (easy to find)	10–50% vital residual tumor cells	
No regression	TRG 4	TRG 3	TRG 3	TRG 4	TRG 3	TRG 3	TRG 3	Subtotal
	Residual cancer outgrowing fibrosis	Minimal evidence of tumor response	> 50% residual tumor	67–100% residual tumor	Fibrosis > 50% of tumor mass	Only scattered tumor cells (difficult to find microscopically) in the space of fibrosis with/without acellular mucin	< 10% Vital residual tumor cells	
	TRG 5 Absence of regressive change				TRG 4 Complete regression	TRG 4 No vital tumor cells detectable	TRG 4 Complete regression	Complete

to the tumor bed and divided into four grades: TRG 1a = complete tumor regression without residual tumor; TRG 1b = < 10% residual tumor per tumor bed, corresponding to a subtotal tumor regression; TRG 2 = 10–50% residual tumor, i.e., partial regression; and TRG 3 = > 50% residual tumor cells with or without signs of treatment effect the tumor regression (Fig. 4). In this initial study, no patient had complete tumor regression, 4 patients showed TRG 1b, 9 patients showed TRG 2, and 23 patients showed TRG 3. The TRG correlated significantly with survival, besides tumor size and lymphatic vessel invasion. The Becker system was successfully applied in other gastrointestinal malignancies following neoadjuvant chemo- or radiochemotherapy [31, 32]. Subsequently, the same group confirmed TRG as an independent prognostic factor for gastric cancer in an expanded cohort of 480 gastric carcinomas [33]. Notably, the regression grading to Schneider for esophageal cancer ("Cologne system") [16] and a TRG proposal from the MD Anderson Cancer Center [6] are 4-step grading systems based on the percentage of viable tumor cells using the same cutoff values as the Becker system. The feasibility of the percentage approach could also be confirmed through the digital analysis of resected specimens of gastric cancers following neoadjuvant chemotherapy [34].

Japanese Society for Esophageal Disease/Japanese Gastric Cancer Association

A large number of other regression grading systems use the principle of the estimation of residual tumor for classification of TRGs: the Japanese Society for Esophageal Disease [27] and the Japanese Gastric Cancer Association [35] designate tumor regression as a continuous variable and categorize these tumors into 4 groups using cutoff values of 0, 1–33, 34–66, and 67–100% residual tumor.

Rectal Cancer Grading System of the Royal College of Pathologists

The British Royal College of Pathologists describes an initial five-tiered and subsequently modified grading system with the following cutoff values: 0–5, 5–50, and > 50% residual tumor [28].

TRG according to Rödel

Rödel et al. [17] use a 5-tiered TRG system for rectal cancer, ranging from grade 0 (no regression) to grade 4 (complete regression) in < 25, 25–50, and > 50% increments, which describe the degree of tumor regression but not the amount of residual tumor. In an initial study from 2005, complete regression was observed in 10% of the patients, and an intermediate pathologic response (TRG 2

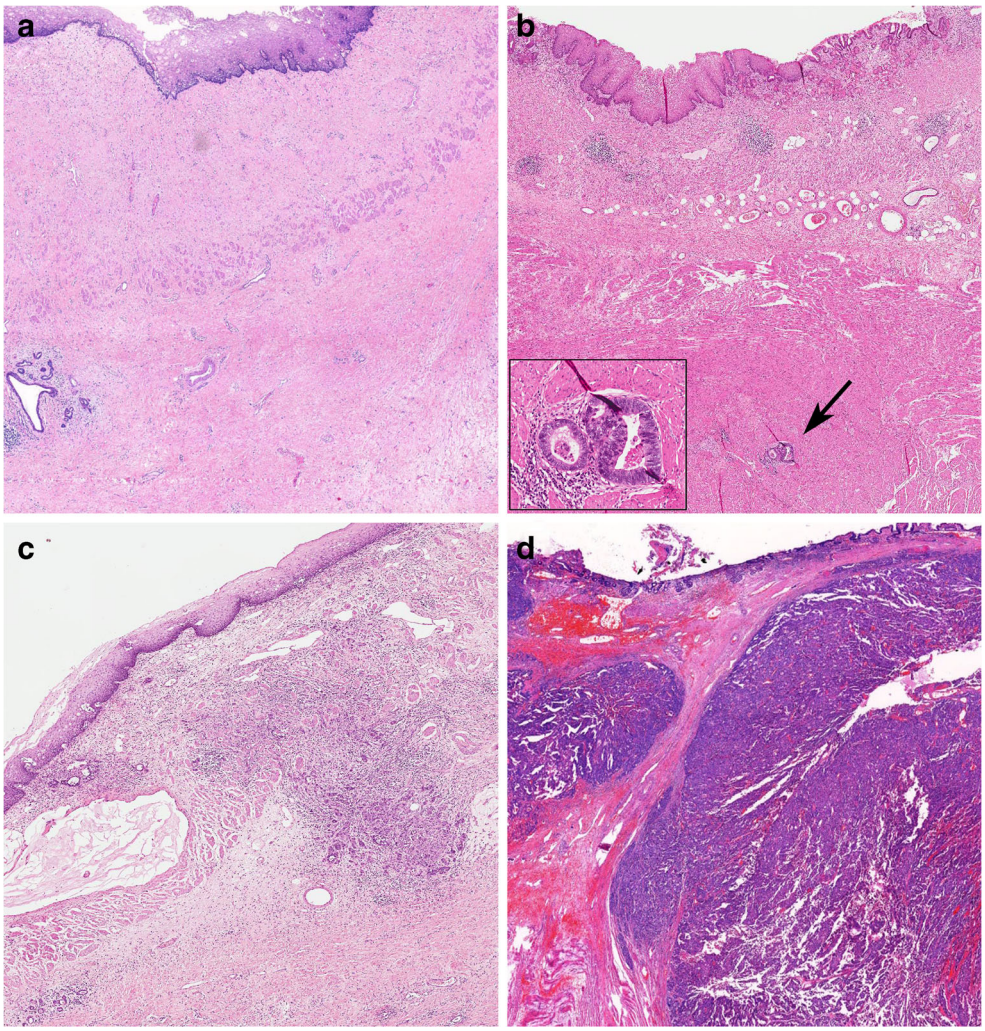

Fig. 4 Examples of tumor regression grades according to Becker. **a** TRG 1a complete regression (equivalent to TRG 1 according to Mandard and TRG 4 according to Dworak). Note pre-existing non-neoplastic esophageal glands within the tumor bed. **b** TRG 1b < 10% residual tumor (equivalent to Mandard TRG 2, and Dworak TRG 3). The arrow indicates a small nest of residual tumor which is magnified in the insert. **c** TRG 2 10–50% residual tumor (equivalent to Mandard TRG 3, but TRG 2 could also be possible since there is no strict definition of "scattered tumor cells" and "preponderance of fibrosis"); in the Dworak system TRG 2 would be applied: the term "scattered tumor cells" here is by complemented by "histologically easy to find". **d** TRG 3 > 50% residual tumor (equivalent to Mandard TRG 4 or 5 and Dworak TRG 0 or 1, since fibrotic strands which could be preexisting desmoplasia or regression can be seen)

and 3) was observed in 65% of the patients. Complete and intermediate pathological response were associated with prolonged disease-free survival after preoperative radiochemotherapy and surgery. This finding could be confirmed in a follow-up study, where residual lymph node metastasis and TRG were the only independent prognostic factors for the incidence of distant metastasis and for disease-free survival [36]. In 2007, Rizk et al. use a similar approach in a study on esophageal cancer following neoadjuvant chemoradiation [18]. Here, the degree of tumor regression, instead of residual tumor, was determined in the following six grades: 0–20% regression; 20–50% regression; 50–80% regression; 80–90% regression; 90–99% regression; and 100% regression. Similar to other TRG systems, high degrees of tumor regression were associated with improved outcome.

Critical issues of TRG

One of the most important issues regarding the determination of tumor regression grades is the inter- and intraobserver variability, and a second issue is the lack of standardization. These two factors may contribute to some differences in the prognostic value of TRGs, which is particularly evident for rectal cancers.

In 2012, Chetty et al. [37] presented a study investigating the concordance level among pathologists for tumor regression grading in rectal cancer after neoadjuvant radiochemotherapy. Seventeen expert gastrointestinal pathologists applied the Mandard system, the Dworak system, and a modified Royal College of Pathologists (mRCP) regression grading system on selected slides of ten rectal cancers. Complete consistency between all 17 participants was only observed in one case. Both the Mandard and Dworak grading systems had unsatisfactory inter-observer agreement in this study (kappa values of 0.28 and 0.35, respectively), but the mRCP system, based on the estimation of the percentage of residual tumor, performed only slightly better, with an overall kappa score of 0.38. The authors of this study also provided a questionnaire for the participants, where several issues of tumor regression grading were covered in this and other publications [37, 38]. Additionally, the need for a simple, reproducible regression

grading system with clear criteria was stressed. Moreover, the authors recommended a cumulative or composite score considering all sampled sections of the tumor bed rather than the worst section and a uniform sampling method of these specimens.

Other studies, however, demonstrated good reproducibility of TRGs when comparing the interobserver variability of one or more TRG systems of the same concept (residual tumor percentage or residual tumor/fibrosis), demonstrating kappa values of 0.71 (MD Anderson/Becker system) or 0.84 (simplified three-tiered MD Anderson/Becker system) in esophageal carcinomas [39], or concordance indices of 0.65 (Dworak system), 0.665 (simplified three-tiered Mandard system) and 0.69 (AJCC system) in rectal cancers [40]. The most frequent reasons for disagreement among observers were problems with the precise assessment of the relative amount of fibrosis and the differentiation between regression-induced fibrosis and tumor-induced stromal desmoplasia, which is particularly the case for diffuse type gastric cancers [34]. In contrast, the displacement of the epithelium and the misinterpretation of acellular mucin were only minor points for disagreement [41, 42].

Two studies compared TRG systems of different concepts: Mirza et al. [43] analyzed three tumor regression grading systems (Mandard, Japanese Society for Esophageal Disease, and Becker) for reproducibility and prognostic impact in 36 gastric carcinomas and adenocarcinomas of the gastroesophageal junction after neoadjuvant chemotherapy. Although the sample size was comparably low in this study, which may also negatively bias the kappa value as a result of statistical issues, both Mandard and Becker TRGs were prognostic for the outcome of the patients, but the Becker system was the most reproducible, with the highest kappa score between the two investigators (kappa value of 0.52). For the Mandard system, a kappa value of 0.44 was observed, and the Japanese Grading system had the lowest score of 0.28. In particular, the estimation of the percentage of residual tumor cells as a response parameter was more easily and reproducibly applicable compared to the determination of the degree of (tumor-induced) fibrosis. Similarly, in a comparative study, Karamitopoulou et al. [44] observed that both systems, Mandard and Becker, worked well in a series of 89 esophageal adenocarcinomas treated with neoadjuvant chemotherapy. The four-tiered Becker system, applied in cases with completely embedded tumor beds, performed better than the Mandard score (kappa values: Becker 0.83; Mandard 0.73) and was also better compared with the assessment of only one representative slide (kappa values: Becker 0.68; Mandard 0.71). Notably, in most cases, unambiguous categorization into one of the defined tumor regression grades is observed, and for example, the Mandard or the AJCC and the Becker system can readily be paralleled with only few exceptions (Table 1).

Another critical issue of the significance and comparability of TRG is the large variety of treatment protocols, particularly for rectal cancers [45]. This issue not only includes so-called "short-term treatments" but also different time frames between preoperative therapy and surgery [46–49]. Since tumor regression follows a time-dependent pattern that includes the occurrence of resorption and scarring after the first cytotoxic effects, characterized by necrosis and acute inflammation, the comparability between therapy regimes with different time settings may be biased or impossible. Notably, in this context, the determination of TRG at a fixed timepoint delivers a snapshot of response rather than a comprehensive status. Different alternative methods for the estimation of tumor regression have been proposed, such as clinical response evaluation by endoscopy and imaging [50] or response evaluation using fluorodeoxyglucose positron emission tomography (FDG-PET), which may facilitate early response evaluation during treatment, with the potential to adapt treatment protocols according to dynamic response behaviors [51, 52]. None of these systems, including histopathological TRG provide absolutely perfect prognostications, and every system has at least some limitations: histopathology cannot compare pre- with posttreatment sizes and cannot reliably assess the shrinkage of a polypoid and endoluminal tumor mass. Imaging, including endoscopy ultrasound, may miss small residual tumor areas or overestimate acellular mucinous pools as residual tumors. Analysis of the response dynamics based on FDG-PET is only feasible when the primary tumor shows uptake. A multidisciplinary approach may therefore be necessary in selected cases for the final determination of the tumor response.

Tumor regression in lymph node metastases

Tumor regression can also be observed in lymph nodes that are or have been metastatic [53]. The histopathological patterns are comparable to those observed in the primary sites of the tumors, such as fibrosis, resorptive changes, including histiocytic infiltrations, and foreign body reactions. Occasionally, lymph nodes show divergent regressive behaviors, even when located in close vicinity. However, regressive and fibrotic changes, particularly in the mediastinal lymph nodes, can occur independently from cytotoxic treatment. In contrast, small or micrometastases in lymph nodes may regress without significant scarring, and the reliable detection of small metastases before treatment is still difficult using imaging methods. These two factors may contribute to the fact that the determination of a TRG in lymph nodes has not yet been generally recommended. The presence of lymph node metastasis, however, is one of the most important prognostic factors in gastrointestinal carcinomas following neoadjuvant treatment and surgery [33, 36]. Therefore, residual tumors in

the lymph nodes should be carefully identified, and the use of step sectioning should be considered in case of the presence of fibrosis without a viable tumor in first sections. Although the grading of tumor regression in lymph nodes is typically not performed, the inclusion of the presence or absence of lymph node metastases, irrespective of regressive changes, into prognostic staging systems has been proposed [16, 54, 55]. In addition, many pathologists describe regressive changes in lymph nodes or lymph node metastases in their reports without further regression grading. Moreover, initial but few studies have shown that the presence of regressive changes in lymph node metastasis may be associated with patient outcome [23, 53, 56, 57]. These reports warrant future studies focusing on this particular aspect of tumor regression.

Prognostic significance of TRG

Numerous studies have investigated the prognostic relevance of TRGs. The strongest evidence for the association between TRG and patient outcome has been observed for upper gastrointestinal cancers. Patients with complete tumor regression generally have the best outcome [6, 33, 41, 55]. In addition, for esophageal squamous cell and adenocarcinomas, patients with subtotal and partial tumor regression (i.e., Mandard 2 and 3; Becker 1b and 2) showed a similar, intermediate outcome compared to complete and non-regression [6, 55, 58], whereas for gastric cancer, there is a clear discrimination between "responding" patients with complete and subtotal regression (i.e., Mandard 1 and 2; Becker 1a and 1b) and "non-responders" with partial and no regression (i.e., Mandard 3–5; Becker 2 and 3) [33]. It is therefore not appropriate to generally merge the different TRGs together into a simplified two-tiered classification scheme with "responders" and "non-responders," as this classification would incorrectly reflect the particular impact of subtotal and partial tumor regression as shown in esophageal adenocarcinomas [6, 55]. Depending on the case composition and the statistical models used, TRGs were also independent prognostic markers for survival for gastric and esophageal carcinomas, in addition to the presence of lymph node metastases [11, 33], while in other studies, the latter represented the only independent prognostic factor [18, 59, 60]. Interestingly, the depth of tumor infiltration, which determines the ypT category according to the AJCC/UICC classification, frequently performed worse compared to the TRG and yN categories [18, 55]. A recent, carefully conducted meta-analysis, however, confirmed the overall prognostic value of TRG in esophagogastric carcinomas [61]. Interestingly, an association between tumor localization and tumor type and TRG was demonstrated for gastroesophageal adenocarcinomas, as more distal cancers and carcinomas of diffuse type, according to Laurén, showed the lowest probability for significant tumor regression after neoadjuvant chemotherapy [62].

For rectal cancer, data concerning the clinical relevance of TRG are less conclusive. While complete tumor regression is constantly associated with improved disease-free and overall survival and a lower risk of local and distal recurrence prognosis [8, 29, 63], the impact of subtotal and partial tumor regression, which would be a major advantage of tumor regression grading over UICC/AJCC and TNM classification, is less clear. Some cohort studies have demonstrated a distinct prognostic impact of subtotal and/or partial regression [29, 40, 63–65], while other studies do not confirm these findings [66] or suggest the classification of complete and subtotal regression into one category [67]. In contrast, a recently published study, which also includes a comprehensive data assessment of the literature, showed that patients with a near-total regression show a worse prognosis compared to complete regression, which argues against merging total and subtotal regression into one prognostic group [65]. In summary, evidence for TRG as an important predictor of survival has been presented in several larger case-cohort studies and meta-analyses. In contrast to upper gastrointestinal cancers, however, the high variability of different treatment protocols may hamper the comparability of some rectal cancer studies, not only regarding TRGs but also the clinical end points. The most important prognostic factors for rectal cancers, with or without neoadjuvant therapy, are the status of the circumferential resection margin and the presence or absence of lymph node metastases [20]. Since tumor regression is strongly associated with these factors, this feature may lose its statistical independence in some calculation models, which explains why some studies failed to show an independent prognostic value for TRG compared to these parameters [68].

Conclusion and future outlook

The assessment and grading of tumor regression of gastrointestinal cancers, particularly esophageal, gastric, and rectal carcinomas, following neoadjuvant treatment are feasible through the histopathological examination of the post-therapeutic resected specimens. Therefore, it is highly recommended that TRG should be implemented in histopathological reports of treated gastrointestinal carcinomas treated by neoadjuvant therapy [69].

The prognostic value of TRG may even exceed that of currently used staging systems (e.g., TNM staging), which primarily originate from data for untreated or unspecified tumors. The AJCC [26], but not the UICC [70], considers TRG as an additional prognostic factor for rectal carcinomas after preoperative treatment but does not integrate this information into a defined staging system. In their 8th edition, the AJCC has also proposed a specific staging system for esophageal

Table 2 Proposal for standardized work-up and reporting of TRG (described in [71])

Photographic documentation
Photocopy or photograph of resection specimen (for orientation and documentation of blocks and of histologically proven residual tumor)
Macroscopic description, according to standard macroscopy tumor size (three-dimensional), distance to resection margins
Work-up
Inking of relevant circumferential margins (esophagus, rectum)
Complete embedding of the macroscopically identifiable tumor bed in 0.5-cm levels (e.g., orientated from proximal to distal) for tumors/tumor beds ≤ 5 cm
If tumor/tumor bed > 5 cm take blocks following the longitudinal and vertical largest dimension at first step (significant regression is unlikely). If no or less residual tumor by histology, embed remaining tumorbed in a second step.
All slides stained by hematoxylin/eosin (HE), selected blocks by periodic acid-Schiff (PAS), Elastica van Gieson staining (EvG);
Immunohistochemistry may be helpful for discrimination between histiocytes and alterated tumor cells. Conventional light microscopy is usually sufficient for the identification of residual tumor.
If no residual tumor in first section: order three step sections to confirm complete response
Lymph node stations. Step sections if signs of regression in lymph nodes without residual tumor in first section.
Resection margins oral, aboral, others
Additional macroscopic findings

cancers following preoperative treatment; however, the application of a TRG is not recommended in the present TNM classification. If a tumor with significant regression shows a residual tumor in deeper layers of the walls of the organ, then this tumor is assigned a high ypT category, despite the beneficial prognostic value of tumor regression. A sub-analysis of the data from 353 gastric cancers and 169 esophageal adenocarcinomas with > ypT2 categories showed that even in this subgroup, TRG retains its prognostic impact ($p < 0.001$ in the gastric cancer cohort; $p = 0.005$ in the esophageal adenocarcinoma cohort; unpublished data), with better outcomes for tumors showing regression, with a significance similar to those of case cohorts of 480 and 360 cases, respectively, comprising all ypT categories [54, 55]. Such findings would strongly argue in favor of the inclusion of TRG into clinical staging systems.

However, in view of the high number of various TRG systems, it should be a major task for international and interdisciplinary commissions to identify a consensus on TRG reporting. Critical issues, such as interobserver variability, can also be addressed through individual and institutional training. Both pathologists and clinicians should work on the standardization of specimen processing and the reporting of tumor regression. Although TRG may serve as a morphological "biomarker," evidence obtained from controlled clinical trials will not be generated from even larger cohort studies. Clinical trials will never have the comparison of different TRG systems as a major aim, but rather a careful work up of histology, and standardized reporting of TRG within trials may help to further strengthen the evidence of the value of TRG in locally advanced gastrointestinal tumors treated with neoadjuvant therapy.

A proposal for a standardized macroscopic and histopathological work-up of resected specimens of gastrointestinal tumors following neoadjuvant therapy has previously been described by the authors of this review [71] and is reproduced with slight modifications in Table 2. We prefer 4-tiered grading, using the Becker system with the determination of the percentage of residual tumor in our daily routine, which closely resembles the 4-tiered AJCC/CAP TRG system that also provides simple, reproducible, and prognostically relevant categories for TRG, considered as a "good choice" [72]. Based on this notion, further evidence-based data regarding the prognostic impact of TRG may render TRG as a powerful prognostic morphologic biomarker for clinical decision-making, improvements of surgical strategies, adjusting postoperative adjuvant treatment and surveillance intensity, and a potential end point and surrogate marker for patient outcomes in clinical trials and research projects, which currently has not been proposed [73].

Moreover, novel therapeutic approaches for tumor treatments beyond conventional radiation, chemotherapy and surgery have emerged in recent years, such as targeting Her2 in upper gastrointestinal adenocarcinomas or, most recently, the introduction of immune checkpoint inhibition [74]. However, little is known about the tissue changes resulting from these emerging treatment strategies. An initial description of the changes observed after treatment with trastuzumab in gastric cancers has recently been published [75]. Careful histopathological examination of the post-therapeutic tissue may provide important aspects of the effects and resistance mechanisms of these novel drugs. However, this examination should not only include a description of a TRG at this stage but also provide a more

detailed description of histologic findings comparable to studies that initially introduced TRGs into pathology.

Compliance with ethical standards The authors adhere to institutional ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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