Guideline

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S1-Guideline: Microscopically controlled surgery

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Summary

Microscopically controlled surgery (MCS) comprises various methods allowing histologically proven complete resection of malignant tumors while at the same time sparing the tumor-free tissue in the immediate vicinity as much as possible. All procedures subsumed under MCS have in common the marking of the excised tissue for topographical orientation, which provides an assignment of remaining tumor remnants. Indications for MCS are malignant skin tumors in problem localizations as well as aggressive subtypes of skin tumors. Established indications for MCS include basal cell carcinoma, cutaneous squamous cell carcinoma, Bowen's disease as well as Bowen's carcinoma, dermatofibrosarcoma protuberans, melanoma in chronically light-damaged skin as well as acral lentiginous melanoma and Merkel cell carcinoma. For other tumors such as extramammary Paget's disease and various cutaneous sarcomas, evidence exists that MCS has demonstrated benefits, such as local recurrence rates. In addition, MCS is indicated when it is foreseeable that a complex closure technique is required and complete resection of the tumor must be assured. Various methods of MCS have been described, including 3D histology, horizontal method and Mohs surgery. A close cooperation of qualified surgeons and (dermato)pathologists as well as laboratory staff is essential for the successful application of MCS.

Microscopically controlled surgery (MCS) aims at the complete removal of malignant tumors (R0 resection) with histological confirmation while sparing as much of the unaffected area surrounding the tumor, and thus of the healthy tissue, as possible.

Basic principles and indications of MCS

- MCS should be used in case of malignant skin tumors at problematic sites, for aggressive subtypes, or if the need for complex closure techniques is anticipated. Any region with foreseeable relevant esthetic and/or functional impairments due to increased safety margins, must be considered as a problematic site.
- Typical tumor indications for MCS include basal cell carcinoma (especially infiltrative types), recurrent basal cell carcinoma, or tumors with neural/perineural infiltration, as well as cutaneous squamous cell carcinoma (especially in case of subcutaneous infiltration, moderate to poor differentiation, or neural/perineural invasion), dermatofibrosarcoma protuberans at problematic sites, melanoma, Merkel cell carcinoma, as well as atypical fibroxanthoma/pleomorphic dermal sarcoma, extramammary Paget's disease, and adnexal carcinomas (for example, sebaceous carcinoma, microcystic adnexal carcinoma, and others).

Complete removal of the tumor is the prerequisite for local healing. For all solitary malignant and several benign skin tumors, the subclinical extent cannot be assessed based on macroscopic appearance prior to therapy. Accordingly, there is a risk that the required margin of safety for an excision is unnecessarily wide or too narrow. MCS is performed with various histological processing methods to ensure complete surgical removal of the tumor. In all procedures, the excised tissue is marked for precise topographical orientation. These procedures differ with respect to the surgical technique and the method of histological sectioning, which confirm R0 resection in various ways [1, 2].

MCS should be used, if *(1)* malignant skin tumors occur at problematic sites, *(2)* aggressive subtypes of skin tumors are present, or *(3)* it is anticipated that complex closure techniques will be required and, therefore, complete resection of the tumor has to be assured prior to surgical wound closure (Table 1).

Problematic sites include all regions for which narrower primary safety margins are beneficial for esthetic and functional reasons due to anatomical conditions (such as tumors on or near the eyelid, on the nose, the lips or areas near the lips, on the ear, in genital or acral regions) or if a wider primary safety margin would also include neighboring structures or esthetic entities (Table 2). More complex reconstruction techniques include, for example, local flaps for which defect size, configuration, and localization are of critical importance during planning of the flap. While re-excision is possible, in principle, it may yield unfavorable functional and/or esthetic results.

Table 1 Indications for microscopically controlled surgery (MCS).

(i)	malignant skin tumors at problematic sites
(ii)	aggressive subtypes of skin tumors
(iii)	confirmation of complete tumor removal prior to complex wound closure

Table 2 Special locations that indicate the use of microscopically controlled surgery (MCS).

- eyelids, area around the eyelid
- nose
- lips, area near the lips
- ear
- genital region
- acral areas

Common tumor indications in the literature, provided they involve problematic sites, include basal cell carcinoma, (especially infiltrative types), recurrent basal cell carcinoma [3–6], or tumors with neural/perineural infiltration, as well as cutaneous squamous cell carcinoma, especially if it infiltrates the subcutaneous tissue, is moderately to poorly differentiated, or shows neural/perineural invasion [7–10]. Additional indications include dermatofibrosarcoma protuberans at problematic sites [11–13], melanoma, [14–19], Merkel cell carcinoma [20, 21], and atypical fibroxanthoma/pleomorphic dermal sarcoma [22–25], as well as extramammary Paget's disease [26], or Bowen's disease [27, 28]. [22, 29, 30] (Table 3).

Table 3 Tumor entities that indicate marginal incision control using microscopically controlled surgery (MCS).

- basal cell carcinoma (especially sclerodermiform type) and recurrent basal cell carcinomas
- cutaneous squamous cell carcinoma (deep infiltrating and/or G2-3), Paget's disease
- tumors with neural/perineural invasion
- dermatofibrosarcoma protuberans
- melanoma
- Merkel cell carcinoma
- atypical fibroxanthoma/pleomorphic dermal sarcoma
- extramammary Paget's disease
- adnexal carcinomas

The histological methods can be applied in single, dual, or multiple interventions. In principle, immediate wound closure is possible, especially if there are no disadvantages with respect to any potentially required re-excision, given that the precise topological assignment remains possible. Alternatively, a temporary defect coverage (by application of suitable protective dressings) may allow for secondary wound closure. In this approach, tumor resection is executed independently of the defect closure, which can be achieved by means of reconstructive plastic surgery (for example, skin graft, local flap, or microvascular tissue transfer), as necessary. If the tumor extends to the excision margins on the histological slides, exact topographical identification of the tumor-positive border zone or wound base in the defect is possible until completeness of the resection (R0) is assured.

With regard to histological analysis, a distinction is made between procedures that show the excision margin almost without any gaps and procedures that have predictable and possibly calculated diagnostic gaps (Table 4). Depending on tumor or tumor subtype, tumor size and localization, the importance of gapless procedures that demonstrate R0 resection with high sensitivity increases [31]. As gapless a visualization as possible of the lateral and basal excision margins is required, irrespective of the diameter of the excised tissue. Nevertheless, during method selection it should be considered that other processing methods are justified for smaller tumors (< 1 cm). Comparing the multitude of data in the literature with respect to the development of local recurrence shows that, overall, methods of gapless visualization perform better than conventional excision for a range of tumor entities [4, 9, 11, 16, 32–39].

Table 4 Procedures in MCS surgery that display excision margins almost without any gaps, as well as procedures that have predictable and possibly calculated diagnostic gaps.

Method	Advantages	Disadvantages
Methods of microscopically controlled surgery with complete visualization of excision margins		
Mohs surgery *Bowl-like excision, cryostat section*	– complete control of excision margins – prompt wound closure, usually on the same day	– prone to artifacts and errors – not reproducible – complicated and time-consuming – compromised quality due to cryostat sections possible
Munich method *Horizontal sections* *Cylinder-shaped excision* *Cryostat section*	– clear evaluation of complete excision with visualization of the entire tumor (3D) – prompt wound closure possible	– assessment of numerous sections – limited assessment of the epidermis – interpretation requires special experience
3D histology, techniques		
Marginal strip technique Muffin technique *Excision en bloc. Separation of margins and possibly base on unstained or fixed excised tissue*	– complete control of excision margins – excision *en bloc* within one procedure – suitable for large (marginal strip technique) and small (*muffin* technique) excisions – readily performed, with practice	– tumor center is difficult to assess in small excisions – geometrical idea is required
La Galette *Later removal of margins and base in situ*	– complete control of excision margins possible – good preparation of the tumor	– difficult incision – double hemostasis (tumor and margins)
Square procedure *Square excision for better separation of marginal sections*	– complete control of excision margins possible	– square wound defects – difficult incision
Quadrant technique *Removal of margins from fixed specimen*	– complete control of excision margins – no additional effort, apart from thread marking	– previous fixation makes making a flat incision from the periphery difficult
"*Wallgraben*" or perimeter technique *First, margin excision, tumor is initially "placeholder"*	– complete control of the lateral excision margin possible – tumor remains initially *in situ*	– the base can only be controlled in a later surgical step – only for superficial tumors

Table 4 Continued.

Method	Advantages	Disadvantages
Methods of microscopically controlled surgery with predictable and possibly calculated diagnostic gaps		
Vertical sections *(loaf of bread technique)*	– simple, rarely artifacts – assessment of complete excision for the respective incision level – simple in geometric perception – enables assessment of tumor architecture (particularly important for melanocytic tumors) – particularly advantageous for small excisions	– vertical random sections in case of larger excisions – diagnostic gaps of excision margin assessment (the larger the excised specimen, the larger the gaps) – possibly, assessment of many sections
Vertical sections and additionally samples from the margin *Biopsies are intended for "mapping"*	– see above – additional mapping biopsies, reproducible with extensive documentation	– see above – only random biopsies – biopsies with very large diagnostic gaps (< 1 % of tumor margin environment is examined)

Crucial for the quality of the technique is the expertise and experience of the surgeon and (dermato)pathologist, as well as their communication, though they may be one and the same person. Usually, however, the cooperation with the (dermato)pathologist is useful and necessary, given that two individuals with the respective expertise then work together [18, 40]. Ideal is a standardized approach with respect to macroscopic cutting of the tissue by the surgeon, identification of the specimen, and communication of the findings in concert with (dermato)pathologists and surgeons.

It may be assumed that in future non-invasive imaging techniques (such as optical coherence tomography) will be used as complementary methods for pretherapeutic assessment of the macroscopic tumor outgrowths in tumors with low invasiveness. While non-invasive imaging techniques may complement MCS in the future, the added diagnostic benefit will need to be proven with sufficient evidence [41].

Methods of MCS

- Several methods of MCS are available (Table 4). These include 3D histology, horizontal method, and Mohs surgery (in various described variants).
- In 3D histology, the tumor is excised with an individual safety margin with an incision perpendicular to the skin surface. Subsequently, margins and base of the excised specimen are separated and inserted separately into histology cassettes (or, as a variant, collected separately *in situ*) for fixation.

Introduction

Apart from the skills of the surgeon, the effectiveness of MCS crucially depends on the qualification of the entire dermatopathological team. In this context, the professional qualification of the examining (dermato)pathologist and the assisting technical staff, the MCS method used (see below), different individual steps of the procedure (dye marking of tissue margins, levelling and sectioning of tissue), cryosectioning, thickness of tissue sections, use of routine stains and toluidine blue, manual versus automated staining, immunohistochemical staining, as well as infrastructural factors play a major role [42]. All these factors should be kept constant and at the highest possible level of quality. Histological pitfalls may significantly affect the quality of MCS findings and should be known: artifacts of cryosections, contamination of sections by so-called *tissue floaters*, are relevant and typical technical artifacts that pose a risk of misdiagnosis. *Tissue floaters* are fragmented and loose, unconnected pieces of tissue arising partly as a result of fragmented intraoperative tissue collection and partly during histological processing. Subsequently, these "dislocated" pieces of tissue are often difficult to assign on the stained slides and their topographical orientation is *de facto* impossible. While they are very rare, their presence will significantly impair the histological analysis [43, 44]. Additional pitfalls arise based on the tumor entities *sui generis*: the histological differential diagnoses of subtypes of basal cell carcinoma, such as mantleoma, fibroepithelioma of Pinkus, folliculocentric basaloid proliferation, and others, are a challenge for the histological diagnostic workup during

MCS, often performed under time pressure, and justify the necessary, high degree of qualification of the (dermato)pathologist. The same is true for the histological differential diagnoses of cutaneous squamous cell carcinoma, as well as of melanoma and its simulators [44]. In addition, 3D procedures involving the dissection of lateral and deep excision margins carry the risk that the actual tumor cannot be adequately assessed in its overall architecture if the central section of the tumor is not completely available. Therefore, care should be taken that the entire tissue from the clinical tumor center is available for diagnosis. The overall architecture (in particular, symmetry and definition of the borders) is particularly essential for the diagnostic workup of melanocytic tumors. When using 3D methods for surgical treatment of melanoma, in each case the macroscopically apparent tumor as a whole, as well as the lateral and deep excision margins, should be cut separately during histological processing in the laboratory, before the tumor piece is processed closely into vertical serial sections.

Mohs surgery

The procedure was introduced in 1941 in the USA by Frederic Mohs, initially as chemosurgery [45, 46]. Originally, he used zinc chloride paste for intravital tissue fixation of the tumor on patients prior to the actual excision in a procedure that was very painful. In 1974, Tromovitch published the cryostat technique [47]. Here, tumor excision is performed under local anesthesia and histological analysis is done by rapid cryostat sectioning. Mohs called the procedure "*microscopically controlled surgery*" [46]. It is used in specialized centers predominantly in the USA. In the literature, the terms Mohs micrographic surgery or Mohs surgery are used [48, 49]. Classic Mohs surgery is no longer used in German-speaking countries (Figure S1, Online Supplementary Information).

Horizontal method

The "Munich method" with an incision performed parallel to the skin surface is derived from Mohs technique (Figure S2, Online Supplementary Information) [1, 50]. In contrast to Mohs technique, cylindrical rather than cone-shaped excisions with perpendicularly cut margins are processed histologically in cryostats. This allows for assessment of the entire tumor. *The horizontal method is the only MCS method that allows for both the assessment of the tumor and the measurement of the excision margins [34].* Discarding a few intermediate steps, sequential sections in the micrometer range are prepared, resulting in numerous horizontal parallel sections. However, the evaluation of the sequential sections, from the base to the epidermis, will only allow for indirect evaluation of the three-dimensional growth behavior of the tumor while also requiring good imagination of the examining pathologists (3D histology). For large tumors that do not fit on the cryostat slide, the tissue is divided into several individual blocks with corresponding marking of the topography. Accordingly, histological processing necessarily requires greater effort. This method is suitable for histologically confirmed epithelial tumors, especially basal cell carcinomas and their recurrent diseases. The "Munich method" is inappropriate for histological assessment of melanomas and superficial tumors, such as extramammary Paget's disease and Bowen's disease, given that pathological cell structures are difficult or impossible to assess by horizontal processing and in cryostat sections. Moreover, this method does not enable the exact determination of the tumor thickness relevant for prognosis and therapy of melanoma and other tumors.

3D histology

Various similar alternatives to the mentioned techniques are described in the literature: after tumor resection, marginal and basal parts are resected systematically and their lateral sides are analyzed histologically [51], later referred to as "La Galette", histology of excision margins with no gaps – marginal strip technique or previously "Tuebingen cake" [52, 53], "*Flunder*" or *muffin* technique [54–56], *square procedure* [57], quadrant method [58], "*Wallgraben*" or perimeter technique (Figures S3–S7, Online Supplementary Information) [59]. While they are occasionally used synonymously, the terms refer to different techniques with individual advantages and disadvantages. In principle, all of these methods visualize the three-dimensional margin of the excised specimen without any gaps. The central section should be processed in numerous vertical serial sections and is used by the (dermato)pathologist to assess the tumor with all its characteristics while the marginal and basal parts are assessed to determine whether or not tumor residues are visible at the excision margins.

Techniques of 3D histology

These methods have in common that the incision is made perpendicularly to the skin surface. This improves the initial situation for the subsequent reconstructive defect closure. Usually, the excision of the tumor is performed with a safety margin *en bloc*. When using 3D histology, the size of the safety margin is between 1 and 10 mm. The safety margin depends on various factors:

1. *Tumor entity, clinical extent of the findings, and histological tumor type:* The initial surgical safety margin is defined based on the diagnosis (usually previously confirmed by biopsy) and the assessability of the clinical tumor border. The impact of the safety margin on the defect

size decreases with increasing area of the primary tumor. Additional revision surgeries are thus avoided [60, 61]. For locally aggressively or destructively growing tumors, deep invasive tumors, or if neural/perineural spreading has already been confirmed by biopsy, a primarily larger safety margin is also recommended [62].

2. *Site:* Depending on functional and esthetic relevance of the site, the smaller the safety margin, the better to spare unaffected skin. For unproblematic sites, a larger safety margin may be selected to reduce revision surgeries. Especially at problematic sites, the use of MCS as a tissue-sparing method proves to be highly beneficial.
3. *Marking:* For topographical orientation, an incision or thread marking is performed intraoperatively, usually at 12:00 o'clock (orientation center of the head/vertex). The chosen marking has to be documented unequivocally in the medical report request (histological submission form).

Various techniques are available for macroscopic cutting of the unfixed excised specimen: in the *marginal strip method ("Tuebingen cake")* (Figure S3, Online Supplementary Information), the tumor margins on the excised specimen are dissected after surgery as strips with a width of approximately 1–3 mm, while the base is dissected as a disc. The prepared tissue portions are divided to such an extent that they fit into a histology cassette for routine processing. For this purpose, rules have been developed to facilitate the communication between surgeon and (dermato)pathologist [46, 63]. Margins and base are embedded unfixed in histology cassettes either by the surgeon or in the histology laboratory. For smaller excisions (up to 2 cm in diameter), margins and underside can be cut and leveled (*muffin technique*) (Figure S4, Online Supplementary Information). The diagnostic classification is aided by several cross-sections through the central portion of the clinically visible tumor. Since melanomas require numerous serial sections, either a correspondingly high number of sections must be made or the entire central part of the tumor must be fixed after appropriate labeling to allow serial section processing in the histology laboratory. Dye marking of the unfixed specimen facilitates topographic orientation of the histopathological sections.

In the *La Galette procedure* (Figure S5, Online Supplementary Information), re-excision from margins and base is performed *in situ* after the preliminary narrow tumor excision. Above all, this ensures the maximum integrity of the actual tumor part allowing for histopathological assessment of all its facets. In the *perimeter* or *"Wallgraben" technique*, initially only a marginal strip is excised around the tumor, which remains *in situ*. In the *square procedure* (or *quadrant technique*), the tumor is removed by a square excision using a double-bladed scalpel to facilitate the separation of the margins (Figure S6, Online Supplementary Information).

Usually, formalin fixation of the tissue with subsequent paraffin embedding is performed after cutting and embedding in histology cassettes. However, cryostat processing, with the advantages and disadvantages mentioned above, is also possible. Using the paraffin technique with rapid fixation (two hours in formalin solution heated to 60°C), the histological sections can be ready the next day. The preparation of margins and base (marginal strip technique, quadrant technique) can also be performed on fixed tissue by the histopathology laboratory, but is more time-consuming for the latter. After fixation, the dissected pieces of margins and base are first treated with paraffin. Then the wax-like tissue can be heated to 65°C with the outer side slightly bent for leveling. This procedure allows for the specimen to be sent to a laboratory for cutting and histological analysis.

In the peripheral method, substantially fewer excision margins have to be examined. Similar to Mohs technique, the distance between the tumor and the excision margin cannot be determined. A comprehensive presentation of the various methods has also been published in book form [63].

Method of vertical sections "loaf of bread" (syn. serial section histology): while this method is very often used, its classification as a method of MCS is controversial [2]. It is best used for small tumors (up to approximately 1 cm) where separation of the circular tumor margins is difficult or impossible and thus prone to error. In this procedure, the incision is made by close vertical sequential sections (serial sections) with a scalpel. Accordingly, this approach is figuratively referred to as loaf-of-bread technique. Sections are then prepared from the resulting tissue slices (Figure S7, Online Supplementary Information). While the many steps of sectioning allow for an optimal assessment of the tumor, there are diagnostic gaps with respect to the excision margins. For typical macroscopic tissue slices with a thickness of 1 mm and HE sections with a thickness of 10 µm, in mathematical terms only a minor fraction of the tumor border is examined by histopathology. Especially for tumors infiltrating the tissue in a diffuse manner and without sharply defined borders, these diagnostic gaps may mimic R0 resection. Although the method of close vertical sections (loaf-of-bread technique) meets the aim of histologically confirmed complete resection of malignant tumors (R0 resection) to a somewhat lesser degree, it has its place in the mentioned constellations. Apart from the technically simpler processing of small tumors and the good ability to assess tumor architecture, it is also beneficial for measuring the achieved tumor-free zone up to the excision border. For follow-up surgeries required for R1 resections and tumors at problematic sites, one of the peripheral techniques without gaps (3D histology) should be used.

Special aspects of MCS with respect to tumor entities

Basal cell carcinoma

- The MCS procedure is a suitable method for the therapy of basal cell carcinoma and shall be used especially in case of more aggressively growing tumor variants or at problematic sites.
- The local recurrence rate of basal cell carcinomas is significantly lower after MCS compared to serial section histology.

The detailed treatment of basal cell carcinoma was illustrated in a recent AWMF guideline [64, 65]. Micrographically controlled surgery represents a suitable method for the therapy of basal cell carcinoma [3, 5, 37, 66–68]. This applies especially to recurrent tumors [58]. The ability to completely visualize tumor growth in basal cell carcinoma is based on the continuous growth of this tumor [69]. However, this limits the ability to assess superficial basal cell carcinomas by MCS, given that this tumor type exhibits a discontinuous growth pattern [69, 70]. MCS is beneficial for the assessment of marginal and basal sections. Concerning histopathological processing, however, the importance of a correct tumor diagnosis must be emphasized, as well. In this context, it has to be ensured for three-dimensional processing techniques that the clinically recognizable tumor portion is available separately for serial processing, if possible.

The procedure of MCS is optimally suited for basal cell carcinoma, especially for more aggressively growing tumor variants (histological evidence of sclerodermiform/morpheaform, basosquamous, or micronodular tumor portions), recurrences of basal cell carcinomas, additional risk factors, such as infiltration of subcutaneous tissue or musculature, ulceration, or neural/perineural invasion, as well as tumors at functionally/esthetically critical sites [71–75]. One option available for risk stratification in basal cell carcinoma is the *National Comprehensive Cancer Network Stratification*, which includes clinical and histological parameters [76].

In a prospective study with more than 5,200 basal cell carcinomas (3,320 patients), Häfner et al. showed a local recurrence rate of 0.7 % for all tumors processed by 3D histology after a follow-up period of five years. This result was confirmed by a local recurrence rate of 0.8 % observed by Wetzig et al. in 671 basal cell carcinomas processed by complete control of excision margins [37, 68]. In 2004, Smeets et al. presented the first prospective, randomized, controlled trial on MCS for basal cell carcinomas [58]. Here, patients with primary and recurrent basal cell carcinomas of the face were randomized into two groups and treated with either Mohs surgery or serial section histology. At first, no significant benefit in favor of Mohs surgery was shown in the initial analysis. During follow-up, however, the same group reported both 5-year data [77] and 10-year data and analyzed both methods with respect to local recurrence among other outcomes [6]. After five years, a significant benefit was shown for recurrent tumors, and after ten years a significant benefit for both primary basal cell carcinomas (4.4 % versus 12.2 %) and recurrent basal cell carcinomas (3.9 % versus 13.5 %) was observed [6]. In a recent prospective study, 347 patients with nodular basal cell carcinoma and a tumor diameter of up to 10 mm were treated by either curettage or excision with subsequent serial sectioning or 3D histology [3]. The patients from the curettage and excision groups were compared with patients whose excised specimen was processed by 3D histology. After a median follow-up period of 3.9 years, significantly fewer local recurrences were observed in the 3D group [3]. In a study by Boehringer et al., it was shown that tumor outgrowths of basal cell carcinomas were detected significantly more often by 3D histology compared to serial section histology [66]. These results were demonstrated for both basal cell carcinomas without differentiation of the subtype and individually for nodular or sclerodermiform basal cell carcinomas [66]. Another recent prospective, randomized, blinded study compared 3D histology and loaf-of-bread histology for basal cell carcinomas with a diameter of up to 30 mm [78]. A significantly lower local recurrence rate for processing by 3D histology was shown (median follow-up of 4.5 years).

Moreover, Muller et al. could show in a smaller prospective, randomized study that MCS (Mohs technique) in basal cell carcinomas results in a smaller defect size in case of R0 resection [79].

Cutaneous squamous cell carcinoma

- The MCS procedure is a suitable method for the therapy of cutaneous squamous cell carcinoma and shall be used especially in case of more aggressively growing tumor variants or at problematic sites.
- For various techniques of MCS, a lower local recurrence rate of cutaneous squamous cell carcinomas compared to serial section histology has been demonstrated.
- Additional immunohistochemical staining may contribute to reducing the recurrence risk of desmoplastic or dedifferentiated squamous cell carcinomas.

The detailed treatment of squamous cell carcinoma was illustrated in a recent AWMF guideline [80, 81]. The excision margins of squamous cell carcinomas can be processed with high accuracy by MCS [82–84]. In 2019, the group of Marrazzo et al. analyzed the course of high-risk squamous cell carcinomas after surgical therapy and processing by Mohs

technique [82]. After analyzing the collective of 647 tumors, the authors concluded that Mohs technique allows for an excellent margin control with low local recurrence and metastasis rates [82]. In a recent retrospective cohort study, Lee et al. compared local recurrence rates of cutaneous squamous cell carcinomas after processing by Mohs technique and standard excision on 672 tumors [84]. After a median follow-up of 4.9 years, fewer recurrences (3 % versus 8 %; $P = 0.013$) and a three times lower recurrence risk adjusted to tumor size and invasion depth (adjusted HR 0.31) were observed in the Mohs group [84]. Furthermore, Montuno et al. could show that high-risk criteria (corresponding to T3 stage of the 8th edition of the AJCC classification) were detected with greater certainty in specimens processed by Mohs technique compared to biopsies [83]. In the analyzed cohort, 10.5 % of squamous cell carcinomas were classified as T3 tumors after processing by Mohs technique. Compared to the previous biopsy, 70 % of these tumors were assigned to a higher stage by Mohs technique (*upstaging*) [83]. In addition to the data on Mohs technique, a lower local recurrence rate of cutaneous squamous cell carcinomas was also demonstrated for 3D histology [8, 37]. Häfner et al. reported a local recurrence rate of 3 % across all subtypes of squamous cell carcinoma after 3D histology, with desmoplastic squamous cell carcinomas presenting with a considerably higher recurrence rate [37]. For cutaneous squamous cell carcinomas with non-desmoplastic subtype, a local recurrence rate of 1 % was observed after a median follow-up of five years [37]. Given that desmoplastic or dedifferentiated squamous cell carcinomas exhibit a higher local recurrence rate, additional immunohistochemical processing of the specimens plays an important role [7, 85]. Schweinzer et al. used 3D histology to re-examine marginal sections diagnosed as tumor-free in HE sections by means of restaining for cytokeratin AE1/AE3 [86]. This revealed that in 27.8 % of the re-examined sections tumor outgrowths were still visible in immunohistochemistry that had not been detected by HE staining [86]. A major advantage of MCS procedures using paraffin sections (for example, 3D histology) is on the one hand the possibility to perform immunohistochemical staining and on the other hand the improved tissue fixation with the resulting improved ability to assess the various growth patterns [31]. This includes, for example, sarcomatoid or spindle cell-like growth patterns, as well as single-cell infiltrations of tissue by squamous cell carcinomas.

In addition, data are available for Bowen's disease supporting the importance of margin-controlled excision by MCS [27, 87, 88].

In a retrospective analysis, Hansen et al. reported a 4-year recurrence rate of 6.3 % after Mohs surgery in 406 cases of Bowen's disease [87]. In a prospective multicenter analysis, Leibovitch et al. could include 270 cases of Bowen's disease, with 95 patients completing a five-year follow-up [27]. Again, a recurrence rate of 6.3 % was observed [27]. Apparently, the recurrence rates after MCS are higher for Bowen's disease compared to other tumor entities. While Bowen's disease occurs most frequently in the particularly UV-exposed head-and-neck region, analogous changes (Bowenoid papulosis) may occur in the genital region [89] and Bowen's disease may also occur in acral areas [27, 88, 90, 91], with MCS playing again an important role. Moreover, the complexity of the nail apparatus needs to be considered, given that standard excision does not allow for its accurate visualization and anatomical correlation [88].

A major benefit of margin- and base-controlled processing in Bowen's disease is the definite exclusion of invasive tumor portions. Eimpunth et al. reported that 16.3 % of all tumors of the type of Bowen's disease diagnosed as carcinoma in situ by biopsy, were diagnosed as invasive Bowen carcinomas after margin-controlled excision (Mohs technique) (*upstaging*) [92]. The most likely explanation is the non-representative character of a biopsy. Chuang et al. could show an even higher percentage of invasive tumors, although the cohort was considerably smaller [93]. Accordingly, complete excision with the use of MCS is recommended also in histologically confirmed Bowen's disease and should be used at least in Bowen's disease with high-risk factors (localization on lips, ears, nose, eyelids, diameter > 10 mm, or basal portions not represented in the biopsy) [92].

Dermatofibrosarcoma protuberans (DFSP)

- In DFSP, use of MCS allows for a smaller clinical safety margin. In this tumor, MCS is associated with a very low local recurrence rate.
- In DFSP with fibrosarcomatous transformation (DFSP-FS), the criteria for surgical therapy of *high-grade* soft-tissue carcinomas generally apply.

In the current AWMF guidelines, complete surgical excision is recommended for dermatofibrosarcoma protuberans (DFSP). In conventional surgery, re-excision with a safety margin of at least 2 cm is recommended; when using 3-dimensional MCS methods, *"a safety margin of 1 cm may be considered as sufficient"* [94, 95]. Accordingly, a method with margin-controlled excision is preferred [11, 79, 94, 96–100]. For both, MCS methods with cryostat fixation and MCS methods based on paraffin sections, very low local recurrence rates have been reported in case of R0 resection [11, 96, 98–101]. Patients undergoing surgery involving MCS showed considerably lower recurrence rates than those patients undergoing surgery with fixed safety margins [79, 94, 99]. Based on available data, the question whether processing by paraffin sections is superior to

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cryostat-based processing cannot be answered conclusively at present. Very similar recurrence rates for both techniques are reported in the available literature. Lee et al. compared cryostat technique and paraffin sections with respect to the recurrence rate in 71 cases [101]. While the authors found slightly more recurrences in the group analyzed by paraffin sections in this retrospective analysis, this difference did not reach significance [101]. In a prospective study on 70 patients with processing by 3D histology (paraffin technique), Häfner et al. found local recurrences in 1.4 % during a median follow-up period of five years [11]. Two additional studies from Irarrazaval et al. and Martìn-Fuentes et al. found no local recurrences after follow-up periods of 5.6 and 6.5 years, respectively [96, 98]. In a retrospective study on tissue processing by cryostat technique, Lee et al. observed also no recurrences [100]. Paradisi et al. reported a total of 1.3 % local recurrences after processing by cryostat technique [99]. In DFSP with fibrosarcomatous transformation (DFSP-FS), the criteria for surgical therapy of *high-grade* soft-tissue carcinomas generally apply [102]: if soft tissue sarcoma/DFSP-FS is suspected based on clinical features and/or imaging results, this shall be confirmed primarily by histology. If soft tissue sarcoma/DFSP-FS with a diameter of less than 3 cm or superficial localization is suspected based on clinical features and/or imaging results, primary R0 resection may be performed. The methodology of MCS itself is not addressed in the current S3 guideline on adult soft tissue sarcomas [102].

Melanoma in chronically photodamaged skin and acral lentiginous melanoma (ALM)

- For lentiginous melanomas in chronically photodamaged skin (lentigo maligna [LM] or lentigo maligna melanoma [LMM]) and acral melanomas (ALM), it has been shown that MCS does not result in higher local recurrence rates compared to excision with fixed safety margins.
- The use of additional immunohistochemical staining for the control of excision margins in melanoma in situ should be viewed critically, given that melanocytes in chronically sun-exposed skin may be overinterpreted after staining with MART 1 (melan A).

Micrographically controlled surgery can also be used for melanomas where it allows for reduced safety margins in acral areas or on the face [103]. Especially for lentiginous melanomas in chronically photodamaged skin (lentigo maligna [LM] and lentigo maligna melanoma [LMM]) and acral melanomas (ALM), it has been shown that MCS does not result in higher local recurrence rates compared to excision with fixed safety margins [14–19, 104, 105]. This is of major importance, given that lentiginous melanomas occur typically on the scalp and the face while ALM occurs in acral areas. In a meta-analysis, local recurrence rates of 1.17 % and 2.4 % were observed for LM and LMM, respectively, after Mohs surgery [19]. Hansen et al. performed a retrospective analysis on melanomas in the head-and-neck region obtained from a US-based cancer registry. After multivariate analysis, the authors demonstrated a significant survival benefit of Mohs surgery only for melanomas of up to 0.74 mm [106]. Given that the study has numerous methodological shortcomings, however, the informative value was considerably limited. In the US-based cancer registry, survival is not documented in a tumor-specific manner. Moreover, MCS was also performed with safety margins of more than 1 cm. In contrast to MCS, the majority of excisions with large safety margins were not performed in academic institutes. In a retrospective analysis of more than 188,000 invasive melanomas and melanomas in situ localized on trunk and extremities, Demer et al. could show no differences concerning total survival after MCS compared to excision with fixed safety margin [107]. Schulz et al. could show for ALM, as well, that excision with reduced safety margins and processing by 3D histology is not inferior to excision with fixed safety margin [18]. No differences between both groups were observed with respect to local recurrence behavior or melanoma-specific 10-year survival [18]. For ALM, Lichte et al. reported better melanoma-specific 5-year survival rates after 3D-histological margin control compared to conventional excision with fixed safety margin [15]. Here, significantly smaller resections (7 mm versus 20 mm) were required with 3D histology to achieve complete resection [15].

The literature on the use of additional immunohistochemical staining for the assessment of excision margins in melanomas in situ, especially those on chronically sun-exposed skin, should be viewed critically [108]. MART1 (melan A), in particular, may result in overinterpretation in chronically sun-exposed skin and thus in unnecessary re-excisions [104, 108, 109].

Merkel cell carcinoma (MCC)

- It has been shown that MCS presents no disadvantage for overall survival of patients with Merkel cell carcinoma compared to excision with safety margin.

In 1997, O'Connor et al. could show on a retrospective cohort a superiority of MCS over standard excision with fixed safety margin for Merkel cell carcinoma (MCC) [21]. Since then, low local recurrence rates after MCS have been confirmed for Merkel cell carcinomas [20, 110]. A recent meta-analysis of Singh et al. with altogether 868 included patients showed, similar to a retrospective

multicenter analysis of Tarantola et al., that MCS presents no disadvantage for overall survival of MCC patients compared to excision with safety margin [111, 112]. With respect to surgical therapy, it is recommended in the AWMF S2k guideline for MCC *that primary tumors without evidence for the presence of organ metastases shall be surgically excised completely with adequate safety margin. In case of clinically suspected MCC, complete excision is preferred over biopsy*. For special sites, the possibility of a narrower safety margin is discussed taking functional outcomes into consideration. In the current guideline, MCS is not clearly recommended for MCC, *given that the authors point out that it is not sufficiently established whether microscopically controlled surgery can provide better outcomes than excisions with fixed safety margin* [113]. In this context, the currently pending update of the guideline on Merkel cell carcinoma is awaited.

Extramammary Paget's disease

For extramammary Paget's disease, lower local recurrence rates have been reported after MCS compared to serial section histology.

MCS enables low recurrence rates for extramammary Paget's disease, too [114–117]. In a retrospective study, O'Connor et al. reported 22 % local recurrences after standard excision and 8 % after MCS [115]. This is also reflected by a pooled meta-analysis of Bae et al. showing a significantly lower local recurrence rate after MCS [26].

Cutaneous sarcomas

– For cutaneous sarcomas, data on MCS are limited and depend on the specific tumor entity.

For cutaneous sarcomas, only limited data on MCS are available, depending on the tumor entity [22, 29, 30, 118]. It has been shown that angiosarcomas, in particular, may have extensive tumor outgrowths. Accordingly, MCS again plays a crucial role, with discontinuous growth in the form of *skip lesions* requiring attention [119]. For both atypical fibroxanthoma (AFX) and pleomorphic dermal sarcoma (PDS), radical excision is required, with the current AWMF guideline recommending MCS [120].

For AFX, MCS minimally into healthy tissue or a safety margin of at least 0.5 cm is recommended. For PDS, a larger safety margin of 2 cm, if possible, is recommended when using MCS, although an adjustment of the safety margin to the anatomical condition is possibly required. The final decision on the used safety margin should be made by the surgeon in agreement with the informed patient [120].

Complications of MCS

The majority of data on complications of MCS originate from the field of Mohs surgery. It is anticipated that the other methods of MCS have a similar spectrum of complications.

Apart from general risks of surgery, such as bleeding, nerve or vessel injury, and wound infections, an increased prevalence of infections or bleeding complications in case of open defects is often discussed for MCS. Since the defect is usually only covered temporarily (for example, by wound dressings) until confirmed tumor clearance, bleeding and wound infections in the moist environment of the wound are critically discussed. The currently available data offer little information with respect to an increased complication rate. Kimyai-Asadi et al. could show on almost 4,000 patients treated by Mohs surgery that MCS can be performed safely both in outpatient and inpatient settings [121]. In 1,683 procedures with Mohs technique on 949 patients aged 85 or older, Nemer et al. identified a total of 30 complications (1.78 %). The most common complications were wound infections, followed by wound dehiscence, hematoma, and bleeding [122]. In a retrospective study with 633 defects (591 patients), Miller et al. examined the risk of complications after Mohs surgery as a function of the time of wound closure [123]. The authors could show that the time period between excision and definite closure did not increase the risk of complications [123]. Furthermore, Rzepecki et al. could show that both Mohs surgery and slow Mohs techniques with immunohistochemical processing can reduce the risk of complications for melanomas at special sites compared to conventional excision with subsequent margin assessment [122]. In this study, special sites included melanomas on head and neck, acral areas, genital region, and pretibial leg [122].

Practical aspects of MCS

The most important recommendations of this guideline are summarized in Table 5. The use of all mentioned MCS procedures should be based on the expertise of the surgeon and the (dermato)pathologist and should be performed in mutual cooperation. In some countries, the combined role of surgeon and (dermato)pathologist for Mohs surgery is a prerequisite relevant for cost settlement. In the ideal case, the surgeon can also assess the incisions, thus ensuring close clinical (dermato)pathological correlation. In peripheral margin techniques, the surgeon can process the marginal sections immediately after surgery to facilitate the cooperation with the histology laboratory [18]. Normally, the assessment is performed by the (dermato)pathologist or, in special cases, by surgeons specifically trained in histological analysis. All procedures of microscopically controlled surgery must be documented on the basis of records (for example, surgeon's protocol, request

Table 5 Summary of the most important recommendations of this guideline.

- MCS should be used in case of malignant skin tumors at problematic sites, for aggressive subtypes, or if the need of complex closure techniques is anticipated. Any region with foreseeable relevant esthetic and/or functional impairments due to increased safety margins, must be considered as a problematic site
- Several methods of MCS are available. These include 3D histology (in various described variants), horizontal method, and Mohs surgery.
- Typical tumor indications for MCS include basal cell carcinoma (especially infiltrative variants), recurrent basal cell carcinoma, or tumors with neural/perineural infiltration, as well as cutaneous squamous cell carcinoma (especially in case of subcutaneous infiltration, moderate to poor differentiation, or neural/perineural invasion), dermatofibrosarcoma protuberans at problematic sites, melanoma, Merkel cell carcinoma, as well as atypical fibroxanthoma/pleomorphic dermal sarcoma, extramammary Paget's disease, adnexal carcinomas, or Bowen's disease.
- The MCS procedure is a suitable method for the therapy of basal cell carcinoma and shall be used especially in case of aggressive tumor entities or at problematic sites. The local recurrence rate of basal cell carcinomas is significantly lower after MCS compared to serial section histology.
- The MCS procedure is a suitable method for the therapy of cutaneous squamous cell carcinoma and shall be used especially in case of aggressive tumor entities or at problematic sites. For various techniques of MCS, a lower local recurrence rate of cutaneous squamous cell carcinomas compared to serial section histology has been demonstrated.
- Processing of Bowen's disease by MCS can exclude invasive portions of the tumor with higher certainty compared to biopsy.
- In DFSP, use of MCS allows for a smaller safety margin and is associated with a very low local recurrence rate.
- For lentiginous melanomas in chronically photodamaged skin (so-called lentigo maligna [LM] and lentigo maligna melanoma [LMM]) and acral melanomas (ALM), it has been shown that MCS does not result in higher local recurrence rates compared to excision with fixed safety margins. In case of melanoma in situ, the use of additional immunohistochemical staining for the control of excision margins may result in overinterpretation and should be used critically.
- MCS presents no disadvantage for overall survival of patients with Merkel cell carcinoma compared to excision with safety margin.
- For extramammary Paget's disease, lower local recurrence rates after MCS have been reported.
- For cutaneous sarcomas, only limited data on MCS are available.
- Close cooperation of qualified surgeons and (dermato)pathologists as well as laboratory staff is essential for the successful implementation of MCS.

for histology, and histological analysis) and histological slides so that the individual steps can be easily reconstructed. None of the MCS methods can ensure complete cutting of all sections. During cutting of paraffin blocks, for example, the first sections are discarded until a flat surface is achieved. In principle, this can result in false-positive excision margins, which in turn may cause avoidable secondary resections. Even with all due care, it is not always possible to ensure that the entire marginal section is completely in one plane. *However, MCS can ensure an almost complete assessment of excision margins.*

The implementation of microscopically controlled surgery for resection of malignant skin tumors requires qualified surgeons working in close cooperation with (dermato)pathologists or the respective combination in one person, as well as laboratory staff trained and experienced in the methods of microscopically controlled surgery. The selection of the appropriate procedure depends on the experience of the users.

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The reference list can be retrieved from the online version.

