Chapter 11

How does genome sequencing impact surgery?

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ABSTRACT

Cancer is a leading cause of death worldwide. Great efforts are dedicated to the development of prognostic and predictive biomarkers to improve diagnosis and achieve optimal treatment selection, thereby, introducing precision medicine in the multimodality treatment of cancer. Genomic aberrations are at the basis of tumor development, representing excellent candidates for the development of promising clinical biomarkers. Over the last decade, single-gene mutations and genomic profiling have been increasingly used in multidisciplinary consultations for risk-assessment and subsequent treatment planning for patients with cancer. We discuss the impact of such genetic-based information on surgical decision-making. Single-gene mutations have already influenced surgical decision-making in breast, colorectal and thyroid cancer. However, the direct impact of genomic profiling on surgical care has not yet been fully established. We discuss the direct and indirect influences of genomic profiling on surgery, and analyse the limitations and unresolved issues of a genotypic-approach to the surgical management of cancer.

INTRODUCTION

Despite early detection of cancer through screening programs and the development of new treatment modalities, the overall mortality as a consequence of this disease remains high [1]. The development of prognostic and predictive biomarkers for use in clinical practice has become a crucial part of cancer research. Single-gene mutations, which can be linked to cancer, have demonstrated promising prognostic and predictive value and have become increasingly used in multidisciplinary consultations for risk-assessment and subsequent individual treatment planning of patients with cancer [2-8]. Great examples are mutations within the *BRCA1* or *BRCA2* genes that are associated with a significantly increased risk of breast and ovarian cancer [9], and mutations in *KRAS,* which are extensively used for adjuvant treatment allocation in patients with colon cancer [2].

However, single-gene mutation analyses alone are unable to completely unravel the complexity of cancer. A more-global approach looking at changes in DNA, RNA or proteins that contribute to tumor growth and progression, is needed to capture the simultaneous interaction of many different mutated genes within malignant cells and their surrounding tissues. Genomic profiling, which enables gene expression profiles at a genome-wide level to be obtained, has already proven to have an impact on the diagnosis and prognostic classification of tumors, as well as on the prediction of response of individual patients to specific therapeutic regimens [10-12].

The promise of delivering precision medicine has been an incredibly strong driving force for the vast and rapid development of high-throughput genomic technologies. By definition, precision medicine is a multi-faceted approach to medicine that integrates molecular and clinical research with patient data and outcomes, with the aim of delivering a treatment targeted to the specific disease characteristics of an individual patient. Genomic, epigenomic, and environmental data are studied together with specific patient information to understand individual disease patterns and to design personalized preventive, diagnostic, and/or therapeutic solutions. Current regimens of cancer treatment are effective in a minority of patients, whereas adverse effects occur in many of the treated patients. Genome wide approaches may contribute to increase therapy benefit and decreasing adverse events by tailoring treatment decisions [13].

From a clinical perspective, the added value of genetic and genomic approaches is clear. However, their impact on surgery, which is still the cornerstone of cancer treatment, is less obvious. This Perspectives article discusses the effect and associated limitations of introducing single-gene mutations and genomic profiling in the surgical decision-making process in terms of timing, extent and subsequent treatment of the patient.

SINGLE-GENE MUTATIONS AND SURGERY

There are several examples of how single-gene mutations can guide surgical management, including mutations in *BRCA*1 and *BRCA2* in breast cancer, adenomatous polyposis coli (*APC*) in colorectal cancer (CRC), the mismatch repair genes (*MMR*) in hereditary colon cancer and other cancers, and *RET* in multiple encocrine-relared tumors [3;14-17].

BRCA mutations

Specifically, women carrying mutations in the tumor suppressor genes *BRCA1 or BRCA2* have a high (cumulative risk of 60–80%) lifetime risk of breast cancer [18]. The *BRCA* genes are normally expressed in breast cells and other tissues, where they have a crucial role in DNA damage repair. If a mutation occurs in one of these genes, DNA damage is not repaired properly, resulting in an increased risk of breast and ovarian cancer [19;20]. Nowadays, bilateral prophylactic mastectomy and oophorectomy are the most effective strategy available for risk reduction of breast and ovarian cancer in mutation carriers [15;20-22]. In a recent study, Neuburger *et al.* [23], showed that in the UK the number of women who had a bilateral mastectomy nearly doubled over the last decade, and more than tripled among women without breast cancer. Of note, bilateral prophylactic mastectomy has been shown to reduce breast cancer risk by 90% in *BRCA1* or *BRCA2* mutation carriers [24]. Despite this great risk reduction, nearly 64% of *BRCA1* or *BRCA2* carriers in the USA choose to avoid surgery as a result of the high sensitivity of MRI that allows early tumor detection [25]. Since ovarian cancer screening methods are largely ineffective, bilateral prophylactic salpingo-oophorectomy remains the standard of care in all *BRCA1* or *BRCA2* mutation carriers, leading to a risk reduction of 80-96% in women with *BRCA* associated gynaecologic cancers [26;27].

APC mutations

In CRC, familial adenomatous polyposis (FAP) is a syndrome in which the inherited defect in the gate-keeper tumor-suppressor *APC* gene leads to the development of multiple premalignant polyps throughout the colon as a result of uncontrolled growth, and subsequent malignant progression before the age of 40 years [28]. Therefore, a colectomy is advised after detection of a germ line mutation *APC*. Depending on the clinical features (such as patient age, the number, nature and location of polyps), a rectal or pouch-anal anastomosis is recommended [29]. Various aspects of surgical decision-making are influenced by both surgeons and patients, whose preferences should be taken into account with regard to optimal time for surgical intervention, extent of surgery and the type of anastomosis performed. Independent of mutation type, surgery will be recommended as soon as FAP syndrome is diagnosed because this is associated with an almost 100% risk of CRC [30]. However, since cancer is rare before the age of 20, surgery is often

deferred to the late teen years or in between major life changes, such as in academic transitions or between jobs [29]. The amount of polyps in the rectum are correlated with disease severity and are of crucial importance for deciding on the type of anastomosis [31]. When fewer than five rectal polyps are observed, an ileorectal anastomosis is advised as this correlated with mild disease. Conversely, if 20 or more rectal polyps are identified, indicating severe disease, an ileal pouch anal anastomosis will be recommended. Furthermore, morbidity quality of life and desired subsequent bowel function should be taken into account. Although pouch-anal anastomosis nearly eliminates CRC risk, it is associated with worse functional outcome, including an increased daily stool frequency, 24-hour incontinence, sexual dysfunction, decreased fecundity in females, impotence in men and decreased quality of life when compared to preservation of the rectum [32-35].

MMR mutations

Germline mutations in DNA *MMR* genes, *hMLH1*, *hMSH2*, *PMS2* or *hMSH6,* are responsible for another form of hereditary colon cancer, namely non-polyposis CRC (or Lynch Syndrome) [36]. MMR genes are involved in numerous cellular functions including DNA repair, apoptosis, anti-recombination and destabilization of DNA [37]. Lynch Syndrome is also associated with an increased risk of cancers of the stomach, small intestine, liver, bile ducts, upper urinary tract, brain, and skin [38;39]. Additionally, women with this disorder have a high risk of cancer of the ovaries and the endometrium [39]. Although the need for prophylactic surgery is less evident in Lynch syndrome patients than in FAP syndrome patients, those with Lynch syndrome who are diagnosed with CRC should consider total colectomy rather than a segmental colon resection due to the increased risk of metachronous neoplasia associated with this condition. A large observational study of 382 *MMR* gene mutation carriers (172 *MLH1*, 167 *MSH2*, 23 *MSH6* and 20 *PMS2*) followed for 9 years confirmed a high cumulative risk of metachronous CRC for 332 carriers treated by segmental resection for their primary CRC. In contrast, there were no diagnoses of metachronous CRC for the other 50 *MMR* gene mutation carriers treated by extensive colon resection [16].

RET mutations

Multiple endocrine neoplasia (MEN) are clinical inherited syndromes affecting different endocrine glands. The different patterns of MEN syndromes includes MEN1, MEN2A, MEN2B and medullary thyroid cancer (MTC) [17], which is commonly associated with pheochromocytoma (PHEO) and/or multiple adenomatosis of parathyroid glands with hyperparathyroidism (PHPT). These syndromes have very different clinical courses: MEN2B is very aggressive, MTC is almost indolent in most patients, and MEN2A is associated with variable degrees of aggressiveness [17]. Activating germline point mutations of the *RET* protooncogene—a 21-exon gene encoding for a tyrosine kinase transmem-

brane receptor involved in the transduction of signals for cell growth and differentiation—are present in 95% and 98% of families with MEN2A and MEN2B respectively, and in approximately 95% of families with MTC [17]. A presymptomatic gene diagnosis aimed at detecting the presence of *RET* mutations in patients with MEN2 syndrome has been established to improve morbidity and mortality for patients with this disease. The treatment of choice for primary MTC is total thyroidectomy with central neck lymph nodes dissection. However, even after radical surgery for MTC, there is a 30 percent chance of recurrence. Therefore, a prophylactic thyroidectomy is advised in patients with MEN2 carrying mutations in *RET* in order to guarantee a definitive cure and avoid morbidity of a central neck lymph node dissection [17].

The American Thyroid Association task force has suggested four different risk levels—from A (the lowest) to D (the highest)— for *RET* mutations , which are incorporated in their most recent management guidelines [40]. Specifically, children from families with MEN or MTC that carry *RET* mutations associated with a risk level D-(such as Met918Thr) should be surgically treated as soon as possible in the first year of life; whereas patients with level B and C risk levels (with *RET* mutations located in exons 10, 11, 13, 14, and 15) should be operated with a total thyroidectomy before 5 years of age; total thyroidectomy can be delayed till after the age of 5 or until the calcitonin positivity only for patients with a level A risk level (with *RET* mutations mapping to exon 5 and 8) [41]. Removing the thyroid in young children has a great impact on the child's life, as lifetime levothyroxine supplementation is required [42].

Recent data have shown that *RET* mutations carriers with undetectable levels of basal calcitonin have an almost no risk of developing MTC [43]. Moreover, serum levels of calcitonin <30–40 pg/ml are always associated with intrathyroidal micro-MTC without any evidence of lymph node metastases [43]. Elisei *et al.* [43] designed a study in which they operated on only *RET* mutation gene carriers depending on their basal and stimulated level of calcitonin. Total thyroidectomy was strongly indicated in patients when their basal or stimulated calcitonin levels were above 10 pg/mL. Importantly, this study showed that the time of surgical treatment could be personalized and safely planned once the positivity to calcitonin is detected at the annual assessment, independent of the type of *RET* mutation and its associated level of risk. This strategy obviously implies a high compliance of carriers of *RET* mutations to the scheduled follow-up if surgery is postponed as long as possible. The detection of mutations in the proto-oncogene *RET* has, therefore, become standard practice with surgical implications in MTC, that have crucially influenced the timing of surgery [41]. Furthermore, Xing *et al.* [44] have recently published an algorithm that incorporates cytology and molecular *(RET)* testing for the management of patients with thyroid nodules presenting with atypia of undetermined clinical significance, with the aim of limiting unnecessary and/or extensive surgery. This study suggests that in these patients, fine needle aspiration biopsy molecular analysis

should be performed for malignancy risk stratification. For example, a *BRAF* mutation in thyroid nodules from this specific patient group tends to be associated with increased risk of thyroid cancer and thus need for surgical intervention [44].

GENOMIC PROFILING

In the past decades, the technology for DNA and RNA analysis has evolved rapidly, shifting from single-gene mutation analysis to a genome wide, system-biology approach, well placed to assist in unravelling the complexity of cancer [5]. Since then, genomic profiling has been increasingly used in multidisciplinary consultations for risk-assessment and subsequent treatment planning for cancer patients. In the first part of this section the influence of these established RNA-based gene profiles on cancer management are discussed. The second part of this section focuses on the impact of genomic profiling on surgical decision-making in terms of timing and surgical extent.

Genome sequencing in cancer care

The first genome-wide approaches used to predict clinical outcome in patients with cancer were based on RNA microarray analyses [45]. In one study that used microarray analysis, a panel of 50 genes identified low-risk and high-risk lung cancer patients with significantly different survival outcomes. Since then, many RNA expression profiles have been published with varying clinical value (Table 1).

Specifically, the Onco*type* DX® profile (Genomic Health Inc., Redwood City, CA) showed a promising prognostic value and also proved beneficial for adjuvant treatment allocation for patients with breast cancer [46]. In this assay, the recurrence score is calculated using a 21-gene assay, which includes 16 cancer-related genes and five reference genes for standardization, and determined a recurrence risk estimate (low, intermediate, or high) for each patient [46]. In breast cancer, the recurrence score proved to be an independent predictor of distant recurrence in patients with node-negative, estrogen receptor (ER)-positive breast cancer treated with tamoxifen. The recurrence score was also shown to be a predictor of the magnitude of chemotherapy benefit, with patients with high recurrence score showing the greatest benefit from chemotherapy [46;47]. The recurrence score was also found to be prognostic and predictive for postmenopausal patients with hormone receptor-positive disease and with positive nodes who were treated with tamoxifen. However, these studies showed no benefit from chemotherapy in patients with low recurrence scores [10;47].

These results were validated in a separate study, in which the prognostic value of the recurrence score for postmenopausal hormone receptor-positive, node-negative and –positive patients with breast cancer treated with aromatase inhibitors was also

Table 1: Established RNA based prognostic and predictive profiles for breast and colorectal cancer

Breast Cancer Profiles								
Test	**Company**	**Technique**	**Proven value**	**Tissue requirements**	**Output**	**Results**	**Validation**	**References**
Oncotype DX	Genomic Health, Inc. (Redwood City CA, USA)	qRT-PCR (21 genes)	Prognostic	Fresh frozen or FFPE	**RS**: *Low: <18* *Intermediate: 18-31* *High: ≥31* 10-years distant recurrence risk for ER+ve, LN- BC patients	*Low risk:* 6.8% chance of distant recurrence (95% CI 4.0-9.6) *Intermediate risk:* 14.3% (95% CI 8.3-20.3) *High risk:* 30.5% (95% CI 23.6-37.4) *High risk* & LN-: significant benefit from CT (HR 0.26 (95%CI 0.13-0.53)). Same is seen for LN+ (HR 0.59). Not seen in *low risk* patients	Yes Current Prospective trials: TAILORx: LN- patients RxPONDER: LN+ patients	46, 47
Mamma-Print	Agendia BV (Amsterdam, Netherlands)	Micro-array based gene expression profiling (70 genes)	Prognostic	RNA of fresh tissue cores or frozen material or FFPE	**Mammaprint risk score:** *Low & high risk* to develop metastasis in five years follow-up in BC patients	*Low vs. High*: HR 4.6 (95% CI 2.3-9.2) Sensitivity>90%	Yes Current prospective trial: MINDACT: LN-/LN+ patients	12, 50, 51
Colorectal Cancer Profiles								
Oncotype DX	Genomic Health Inc. (Redwood City CA, USA)	qRT-PCR (12 genes)	Prognostic	Fresh frozen or FFPE	**RS**: *Low: <18* *Intermediate: 18-31* *High: ≥31* 10-years distant recurrence risk in stage II colon cancer patients	Chance of distant recurrence in 3 years: *Low risk:* 12% *Intermediate:* 18% *High risk:* 22% High vs. Low risk: HR 1.47 (95% CI 1.01-2.14)	Yes	11, 54
ColoPrint	Agendia BV (Amsterdam, the Netherlands)	Micro-array based gene expression profiling (18 genes)	Prognostic	Fresh frozen material	**Coloprint risk score:** *low & high risk* to develop metastasis in five years follow-up for stage II and III colon cancer patients	Five years distant metastasis free survival: *Low:* 94.9% *High:* 80.6% High vs. Low risk: HR 4.28 (95% CI 1.36-13.5)	Yes	55, 56

Abbreviations: FFPE: formalin fixed paraffin embedded; qRT-PCR: quantitative reverse-transcriptase polymerase chain reaction; RS: recurrence score; ET: endocrine therapy; LN-: lymph node negative; LN+: lymph node positive; CT: chemotherapy; ER+ve: estrogen receptor positive; HR: hazard ratio; 95% CI: 95% confidence interval

demonstrated [48]. Furthermore, recent findings have also suggested that the recurrence score is able to predict locoregional recurrence (LRR) in patients with node-negative ER-positive breast cancer treated with tamoxifen [49]. This same study further showed that patients who underwent a mastectomy had significantly less LRR compared with patients who received lumpectomy followed by breast radiotherapy. When subdivided by age categories (<50 or ≥50 years), patients aged <50 years with high recurrence score seemed to have better clinical benefit from mastectomy than from lumpectomy and radiotherapy. On the basis of these results, patients with breast cancer, aged <50 years, featuring a high recurrence score should be advised to undergo a mastectomy.

In addition to the Onco*type* DX® profile, the MammaPrint® (Agendia Inc. , Amsterdam, The Netherlands) RNA mini-array was developed for use in the high-throughput clinical setting for the diagnosis of breast cancer [12;50;51]. Using a supervised classification method, the correlation coefficient of the expression for approximately 5,000 genes was correlated with disease outcome in a retrospective cohort of 78 patients [12]. Classification was made on the basis of the correlations of the expression profile of the 'leave-one-out'sample with the mean expression levels of the remaining samples from the good and the poor prognosis patients, respectively. The accuracy improved until the optimal number of marker genes was reached (70 genes). In a validation study, this prognostic profile was tested in 295 consecutive patients. The estimated HR for distant metastases in the group with a poor-prognosis signature, was 5.1 (95% CI, 2.9-9.0; $p<0.001$) [51]. MammaPrint® is a 70-gene prognosis profile that was reported to be superior to standard clinical parameters, such as nodal status and grade, in predicting the occurrence of distant metastasis in patients with breast cancer [51]. Moreover, the MammaPrint® profile also showed predictive value in patients assigned to the 'high-risk' subgroup, who had a significant benefit of 12% for combined (chemotherapy and hormone therapy) treatment when compared with patients in the low risk subgroup [52]. Once available, the results of the randomized controlled trial MINDACT (Microarray in Node-negative Disease may Avoid ChemoTherapy) will contribute to the validation of the predictive role of MammaPrint® [53].

As in breast cancer, one of the clinically established RNA profiles for colon cancer is the Onco*type* DX® profile. This profile was established from four studies performed in over 1,800 patients with stage II or stage III colon cancer [54]. Genomic profiling in these studies allowed the identification of seven genes associated with tumor recurrence risk, six genes associated with chemotherapy benefit and five reference genes, that were predictive of recurrence in patients with resected colon cancer who were treated with surgery alone or surgery followed by 5-Fluorouracil and Leucovorin chemotherapy. This analysis led to the design of a 12-gene colon cancer recurrence score, which was validated in the QUASAR clinical trial [11]. According to this 12-gene score, predefined risk groups are categorized as low, intermediate or high risk for tumor recurrence, which

gives the possibility to allocate high-risk stage II colon cancer patients to adjuvant treatment, ultimately protecting patients from costly overtreatment. Of note, currently the Onco*type* DX® assay has prognostic value regarding outcome in colon cancer, however, no predictive value has been established for adjuvant treatment so far.

In addition, the ColoPrint® (Agendia,Amsterdam, The Netherlands), a prognostic 18-gene signature that was identified through unsupervised hierarchical clustering of a whole-genome oligonucleotide high-density microarray leading to unbiased gene selection, also showed promising results in patients with colon cancer [55]. The signature was validated in an independent set of patients with stage II colon cancer and identified a 5-year distant metastasis-free survival of 94.9 ± 2.2% for low-risk patients and 80.6 ± 6.6% for high-risk patients, (p=0.009) [56]. These results support the prognostic value of RNA profiling in patients with stage II colon cancer and herewith facilitate the identification of patients who may benefit from chemotherapy. Nevertheless, surgical treatment will not change at all, using this type of prognostication.

High-throughput genomic analysis have led to the identification of different genomic signatures (or profiles) that can be used for cancer management and can contribute to the multidisciplenary decision making process for cancer treatment. However, as described in the following section, the direct impact of genomic profiling on surgery, timing and/or extent of the procedure, is currently less clear.

Impact of genomic profiles on surgery

Breast cancer

Several studies have shown that gene expression profiling of biopsies is a succesful tool that can predict response to neo-adjuvant treatment [57;58]. Specifically, Ayers *et al.* [57] suggested that transcriptional profiling had the potential to identify a 74-gene expression pattern on biopsies of breast cancer that might lead to clinically useful predictors of pathological complete response (pCR) to the neo-adjuvant treatment regimen of sequential weekly paclitaxel in combination with 5-fluorouracil, doxorubicin and cyclophosphamide. However, this small sample study still needs further validation. Chang *et al.* [58] analysed core biopsy samples from 24 patients with breast cancer and found an association of a 92-gene signature with treatment response to neo-adjuvant monotherapy with docetaxel. These studies suggest that genomic-profiling on biopsies represents a clinically relevant progress in cancer management. It can be argued that current practice should focus on genomic profiling of the tumor biopsy, before assignment of a targeted neo-adjuvant treatment. Although this aspect does not have a direct impact on surgery, it could influence the extent and timing of surgery indirectly (Figure 1). Targeted neo-adjuvant treatment could potentially lead to downsizing of the tumor, with consequently less-extensive surgery or even a delay in surgery in case of a clinical

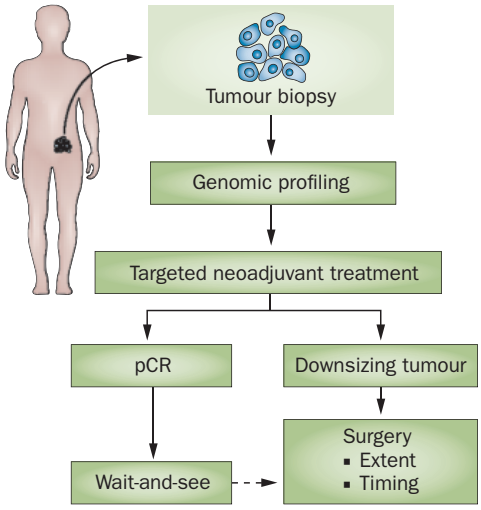

Figure 1: Impact of genomic profiling on surgery
This figure shows two ways by which genomic profiling might impact surgical intervention. Through genomic profiling of a tumor biopsy targeted neo-adjuvant treatment can be administered to a patient, possibly resulting in pathological complete response (pCR) or downsizing of the tumor. Downsizing of the tumor might influence surgery with regards to extent or timing of surgery. In case of pCR a wait-and-see approach can be followed, where surgery is no longer necessary and a strict follow-up is advised.

complete response (cCR). By using genomic profiling to tailor neo-adjuvant treatment, response rates may increase. This will result in lower mastectomy rates.

In breast cancer, there is already a shift from mastectomy to breast-conserving surgery after tumor shrinkage by neo-adjuvant chemotherapy, which proved to be oncologically safe in terms of survival outcomes [59;60]. This decrease of mastectomy rates is a result of response to chemotherapy. Although this response can be predicted by molecular profiling of the tumor, the surgical planning in itself is not directly influenced by any gene expression signature. For local control, the studies by Cho *et al.* [59] and Shin *et al.* [60], investigating the oncologic safety of conservative surgery versus mastectomy after neo-adjuvant chemotherapy also improved outcome in terms of local recurrence. However, the number of patients included and the number of local events were too small to draw a significant conclusion in terms of therapeutic safety. These studies imply that through targeted neo-adjuvant treatment, based on biopsy profiling, further downsizing of the tumor could occur and result in less invasive surgery. Today there are no known genomic profiles that guide surgical planning directly for breast cancer. Perhaps in the future, the risk of local regional recurrences can be predicted on the basis of genomic profiling in such a way that even after excellent response to neo-adjuvant therapy, a mastectomy is advised.

Pancreatic cancer

An other example of the potential impact of genomic profiling of biopsies is pancreatic cancer. Neo-adjuvant chemotherapy with gemcitabine and docetaxel in patients with borderline resectable cancer of the pancreatic head showed that operative exploration was associated with curative intent in 48% of the patients investigated [61]. Of the patients that underwent surgery, 87% had a R0 resection and 10% had a complete pathological

response. This treatment was associated with a low perioperative morbidity and favourable survival: 81% of patients with resected cancers were alive at a median follow-up of 21.6 months [61]. Although this result was not directly based on genomic profiling, it is expected that genomic analysis of these tumors (both mutation analysis and expression profiling) will better identify 'treatment sensitive'tumor characteristics, which may lead to optimization of allocation of directed neoadjuvant treatment per individual patient.

In the future, a more curative surgical intervention could be achieved for patient groups with limited resection options, as a result of genomic profiling of the tumor biopsy, when therapeutic regimens are further optimized by targeted neo-adjuvant treatments.

Rectal cancer

As described above, neo-adjuvant treatment sometimes leads to downstaging of the primary tumor or even a complete clinical or pathological response. Therefore, more R0 resections and less-extensive surgeries can be achieved. With the use of genomic profiling on biopsy samples, followed by targeted neo-adjuvant treatement, the impact on surgical intervention can be striking, possibly leading to the omission of surgery. One can argue that based on specific genomic profiles from tumor biopsies, a wait-and-see approach might be indicated following complete clinical response after tailored neo-adjuvant therapy [62]. With this wait-and-see approach surgery can be delayed or even omitted. In patients with rectal cancer, this wait-and-see approach, however, is under debate. Curative total mesorectal excision after preoperative chemoradiation is the current standard of care in rectal cancer, in which pCR is observed in nearly 14% of these patients [63]. This example highlighted the rationale of a wait-and-see policy, which was further suggested by the results from a series of retrospective studies from Brazil. The Brazilian studies reported similar survival rates in patients that after complete clinical response following neo-adjuvant treatment underwent radical resection or observation only [64-68]. Furthermore, Maas *et al.* [69] showed that a wait-and-see policy with strict selection criteria, up-to-date imaging techniques and follow-up is feasible with promising rates of 89% and 100% for cumulative probabilities of 2-year disease-free survival and overall survival, respectively, in patients with rectal cancer showing a complete clinical response. However, this study was small with a low local event rate, making clinical significance debatable. Recently, a study investigating criteria for determination of residual disease after neo-adjuvant chemotherapy showed that the majority of patients with a complete clinical response still had pathological residual disease [70]. For maximal benefit from a wait-and-see approach in rectal cancer, we should aim for better identification of patients with pathological complete response.

Oesophageal cancer

In oesophageal cancer, neo-adjuvant treatment can downstage tumors, thereby increasing R0 resections [71]. In one study, patients were randomly assigned to surgery alone or to chemoradiotherapy with carboplatin and paclitaxel followed by surgery [71]. Complete resection with no tumor within 1 mm of the resection margins (R0) was achieved in 92% of patients in the chemoradiotherapy-surgery group versus 69% in the surgery group ($p<0.001$). A pCR was achieved in 47 of 161 patients (29%) who underwent resection after chemoradiotherapy. In this scenario, targeted neo-adjuvant therapy based on the genomic profile of a biopsy was shown to influence surgery by improving the R0 resections and pCR rates.

In patients with locally advanced oesophageal cancer, the benefit from neo-adjuvant chemoradiotion is clear, but the benefit from surgery afterwards is less obvious [72]. Some patients with oesophageal cancer will have a pCR after neo-adjuvant chemoradiation and some of these patients would be able to forego surgery, but unfortunately evidence to guide treatment is scarce. For patients with squamous cell oesophageal cancer, those with a good clinical response after neo-adjuvant chemoradiation do not have a worse survival when undergoing observation only compared to surgery after chemoradiation [73]. The absolute benefit from surgery after neo-adjuvant chemoradiation seems to be relatively modest for patients with a good clinical response [72]. In selected patients with a complete clinical response following neo-adjuvant treatment, 3-year survival rates of 50% are seen irrespective of subsequent surgical intervention [74]. The accurate prediction of response to neo-adjuvant therapy can, therefore, have a direct influence on the surgical management of cancer. As treatment regimens improve and detection of earlier-stage disease increases (resulting in higher percentages of pCR), alternative approaches for patients at high risk of morbidity from surgery should be sought [75]. Even though evidence is not derived from randomized controlled trials, it might be reasonable to forego surgical intervention in patients with a complete clinical response, especially in elderly with comorbidities who are less fit to undergo surgery and more likely to experience adverse events. On the basis of these results, one can imagine that genomic-profiling could have an additional role in targeting the tumor with the most optimal neo-adjuvant treatment, possibly leading to an even better local control and survival outcome. However, in current clinical practice, this approach has not been routinely established yet.

FUTURE DEVELOPMENTS

Genomic profiling is gaining importance in the multidisciplinary treatment of cancer. A direct impact on surgical oncology, however, cannot yet be claimed. Genomic testing

on biopsies could potentially affect surgical management, but some important issues still remain unresolved and warrant further investigation before genomic profiling on biopsies can truly influence surgical decision-making.

First, several studies in different types of cancer have shown that in most cases sufficient tissue can be obtained from biopsies for performing genomic profiling [76;77]. However, in 20% of the cases limited tissue quantity is available from a biopsy, precluding further analysis [76]. Furthermore, low tumor content may need more in-depth sequencing or even a repeated biopsy to obtain more material for analysis, which is undesirable from the patient perspective. Therefore, improvement of profiling techniques is necessary to allow the identification of a valid profile in these more complicated circumstances.

Second, the risk of tumor seeding while performing the biopsy should not be underestimated. Case reports of malignant seeding following needle-biopsy have in fact been described in several tumors [78-80]. However, the clinical significance of this seeding is not known. In breast cancer, although data are limited, no increased morbidity has been observed as a consequence of tumor seeding [81].

Third, the heterogeneous nature of the tumor could contribute to unreliable prognostication and prediction. Genomic and epigenomic factors, among others, contribute to this heterogeneity and, consequently, newly developed targeted anti-cancer drugs will only be effective in a subset of patients, and perhaps only at a specific stage of their disease. A biopsy represents only a small fraction of the primary tumor, and owing to the heterogeneity of the tumor, important information could be missed, possibly resulting in a misleading phenotype. A solution for this issue is to obtain multiple biopsy samples from several locations throughout the tumor, although a higher risk of tumor seeding may be a consequence of this increased sampling.

Finally, the interactions of the tumor with the micro-environment influence tumor development and maintenance [82]. These patient-specific factors challenge adequate tumor sampling for biomarker discovery, warranting the use of techniques such as laser capture microdissection for separate analysis of tumor and normal tissue for biomarker profiling. Some profiles, such as MammaPrint®, were derived from the analysis of tissue sections containing both the tumor and its closely surrounding micro-environment, whereas others, such as Onco*type* DX® , analysed only cancer cells. The different gene signatures identified from these approaches reveal a great variety of differentially expressed genes, with minimal overlap between the signatures identified. For example, Varga *et al.* [83] showed that nearly 18% of breast cancer patients showed major-discrepancy between Endopredict and Onco*type* DX® assay. In current clinical practice, the use of these techniques would require highly trained personnel and are associated with high costs and, therefore, is not advisable. It is important to implement sample handling, processing and data analysis into a routine standardized practice, thereby increasing quality of the array and decreasing costs and inter-laboratory variability [84].

Lack of clarity regarding how to assess a pCR, the ideal timing for a clinical, radiological and pathological assessment of response, the uncertainty of the long-term efficacy of this strategy and new follow-up protocols are all factors that currently influence the surgical implication of genomic profiling [85]. Of note, the decision of when to have surgery after chemoradiation is still an important issue. Patients should be given adequate time to recover from chemoradiation-associated toxic effects and sufficient time should be allowed for the tumor to respond to treatment. The optimal time-frame between neo-adjuvant treatment and surgery remains unclear and is most probably dependent on the specific tumor as well as on the individual patient. However, retrospective data in patients with rectal cancer and oesophageal cancer indicate that, in general, delaying surgery after neo-adjuvant therapy improves neo-adjuvant treatment response and decreases surgical complications [86;87]. These studies reported an increased pCR rate among patients who had a greater time frame between neo-adjuvant treatment and surgery [86;87], and an improved 5-year survival and a lower recurrence rate [88].

Finally, an important issue is that if genomic profiling is performed on tumor biopsies prior to the targeted neo-adjuvant treatment, the genomic signature identified might not be factual as the treatment could alter the genomic profile of the remaining tumor, possibly resulting in unreliable prognostication and prediction of adjuvant treatment benefit owing to this prespecified genomic profile [62]. Hannemann *et al.* [62] analyzed changes in gene expression patterns of breast tumors induced by chemotherapy, and compared the profiles of the pretreatment tumor-biopsy with the profiles of the remaining tumors after treatment. The researchers found that major changes in gene expression in locally advanced breast cancer were observed in responders to neo-adjuvant treatment, defined as patients with a tumor shrinkage >50%, but not in patients with resistant tumors [62]. Furthermore, Buchholz *et al.* [89] showed that genomic profiles of biopsies obtained from one patient before treatment or 24h and 48h after initiation of treatment clustered together more than samples obtained from different patients with comparable tumor stage [89]. The fact that no differences were observed before and after treatment in the study from Buchholz *et al.* [89] might be due to the time-points chosen for the biopsies. In fact, changes in gene expression might only occur at later time points (after 48 h). From a surgeon's perspective, neo-adjuvant-induced tumor shrinkage is desirable as it leads to less extensive surgery with a higher chance of free surgical margins. However, not knowing the blueprint of the tumor left behind when radical surgery is avoided still leaves us in the dark. Overall, the value of this prespecified genomic tumor biopsy profile before neo-adjuvant treatment is largely unknown, owing to the fact that redetermination of the genomic profile of the remaining tumor after neo-adjuvant treatment cannot be ruled out.

CONCLUSION

The multimodality treatment of cancer has witnessed an increasing influence of genomic profiling in clinical decision-making. The complex interplay of genetic and epigenetic alterations in our genomes leads to disrupted biochemical interactions in multiple pathways, which are responsible for tumor development (Box 1). Ultimately, identifying these genomic abnormalities will lead to accurate prediction of tumor recurrence or to cancer-related death, non-responsiveness to therapy, and might even provide potential new targets for cancer therapy.

BOX 1: IMPACT OF EPIGENETIC CHANGES ON SURGERY

Epigenetics, including DNA methylation and histone modifications, is defined as the study of inherited changes in gene expression or cellular phenotype, caused by mechanisms other than changes in the underlying DNA sequence. Epigenetic changes have shown to be critical for the development and progression of all cancer types [93-95]. Of note, these changes are intrinsically reversible and are therefore attractive targets for therapeutic intervention [93;96-98]. Drugs for both DNA methyl transferases (DNMTs) and histone deacetylases (HDACs), involved in addition of methyl-groups to DNA and removal of acetyl groups on histone tails, are available [99;100]. DNMT inhibitors have shown promising results in cancer therapy, but unfortunately their activity is genome-wide rather than targeting specific genes [101]. A number of HDAC inhibitors have been designed to drive re-expression of aberrantly silenced genes, leading to inhibition of cell proliferation, hormone receptor reactivation and/or apoptosis [102]. In the future, these directed epigenetic treatments could potentially have the same impact on surgery as seen with targeted neo-adjuvant chemotherapy after biopsy profiling. Furthermore, epigenetic changes can be detected in tumor-derived DNA in stool, tissues or blood [103-105], allowing the use of epigenetic markers in a clinical setting. This advance could lead to earlier tumor detection with an indirect impact on surgical care, influencing extent and timing of surgery with less delay in surgical intervention [106].

In prostate cancer, DNA hypermethylation of glutathione *S*-transferase pi 1 (*GSTP1*) [107] can be detected in urine, serum and ejaculate [108], which was able to increase sensitivity of prostate cancer diagnosis [109] and distinguish between primary cancer tissue and benign tissue [110].

In CRC, identification of hypermethylation of *P16* [111] , *DAPK* (death associated protein kinase)[112], *RUNX3* [113] and *ALX4* (aristaless like homeobox-4) [114] in blood or stool also served as a screening tool. Recently, a panel of highly sensitive and specific biomarkers for methylated DNA in plasma was identified, which resulted in three genes (*TMEFF2*, *NGR2* and *SEPT9*) specific in discriminating healthy subjects from patients with colorectal neoplasia [115].

It is hoped that these screening methods will lead to earlier tumor detection, however, this will not necessarily translate to increased survival and reduced mortality. Future studies, especially randomized controlled trials are warranted to tackle these issues and increase sensitivity of this exciting diagnostic field.

In current clinical practice, surgery still is the cornerstone of cancer treatment and the most valuable outcome predictor. Whereas some single-gene mutations described

here have successfully impacted on cancer surgery, genomic tumor profiling has no direct impact on surgical decision-making, thus far. Today's research, however, is showing promising results, in particular genomic profiling of tumor biopsies, before and/or after targeted neo-adjuvant treatment, may result in less-extensive surgical techniques owing to optimal tumor shrinkage, or even lead to a wait-and-see approach.

The data disscussed in this Perspectives article are mainly derived from retrospective analyses in prospectively designed studies. These studies were not conducted in a randomized setting; therefore, confounding may be present. Furthermore, patient numbers were often limited, thereby decreasing statitiscal power and clinical significance. Currently, two large randomized controlled trials in the adjuvant setting are ongoing, where according to risk stratification using Onco*type* DX® or MammaPrint®, patients are randomly assigned for adjuvant chemotherapy in the TailorX or Mindact Trial, respectively [53;90]. The results of these trials will help define the true surgical implication of genomic profiling.

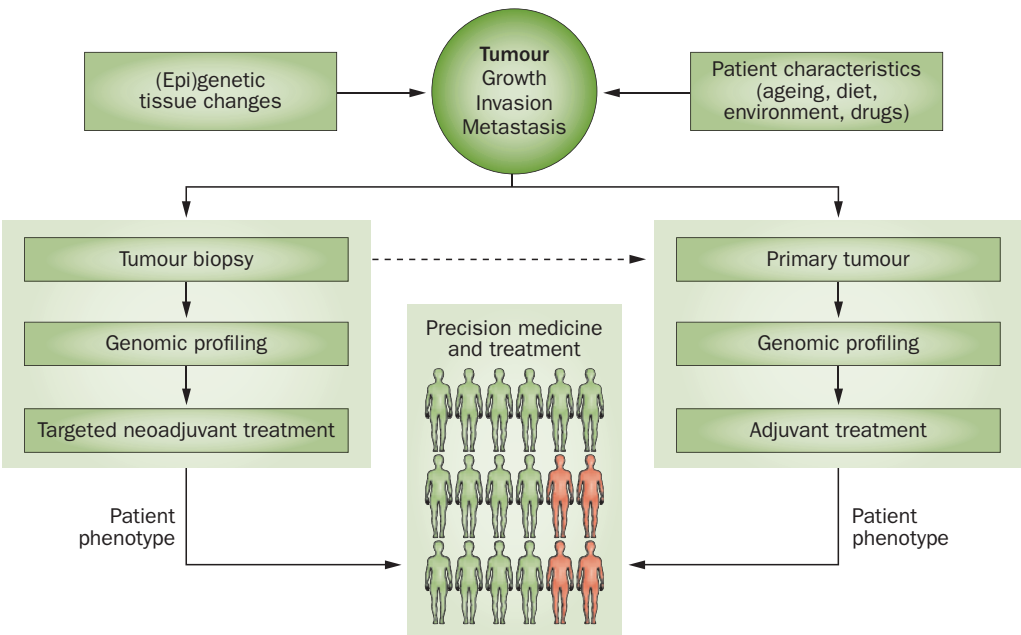

Figure 2: Global overview of the effect of genomic profiling on precision medicine
This figure shows the effect of genomic profiling on precision medicine. (Epi)genetic tissue changes and patient characteristics influence tumor growth, invasion and metastasis. Genomic profiling can result in targeted neo-adjuvant treatment and adjuvant treatment through profiling of tumor biopsies or primary tumors consecutively, with as main goal targeted treatment of the individual patient, better known as precision medicine. However, a patient's phenotype, e.g. comorbidities, frailty and poly-pharmacy, must be taken into account for optimal targeted treatment and to reduce therapeutic morbidity, as written in the discussion session.

More comparable trials, for example, in the neo-adjuvant setting, are needed with the aim of limiting the extent of surgery.

Molecular targeted therapy might radically alter cancer treatment in the future and have the potential to greatly improve cancer survival by delivering the most effective drugs to the right patients [91]. Nevertheless, the treatment of cancer, especially in older patients or in patients with multiple comorbidities, should also take into account these comorbid conditions, quality of life, patient resilience, and preferences. Despite the great contribution of genetics and genome profile to cancer therapy, considering only the sum of genetic aberrations in cancer is insufficient for developing and deciding adequate cancer treatment, especially in elderly patients. In the USA, the estimated number of cancer patients older then 65 years of age will rise from 850,000 cases in 2012 to 1.3 million in 2025 [92]. This population is characterized by a great heterogeneity in terms of comorbidities, quality of life and patient preferences. These factors are as crucial as the molecular signature of the tumor in the multidisciplinary approach to cancer. Thus, phenotypic profiling must be part of the vanguard of cancer research (Figure 2).

In conclusion, genomic profile-directed cancer therapy is still in its infancy. Much more is expected from this field of research, which might contribute to precision medicine in the future of cancer treatment. Currently, it is not clear if genomic profiling will ever gain full ground in direct surgical decision-making. It might contribute to improved informed decision and better outcome, however, surgery still is, and will remain the most important cornerstone in cancer management.

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