Summarizing Discussion & Future Perspectives
Chapter 6

SUMMARY

DNA in human cells suffers from approximately 70,000 lesions per day, either caused by endogenous or exogenous stressors. Fortunately, cells are equipped with a DNA damage response (DDR) pathway, which allow cells to arrest cell cycle progression in response to DNA lesions and activate DNA repair. Distinct from these DNA repair pathways, is the spindle assembly checkpoint (SAC) which acts as a checkpoint in mitosis and safeguards equal chromosome distribution among daughter cells. Malfunctioning or loss of expression of proteins involved in the DDR or SAC can lead to genome maintenance defects, which manifests as mutations, copy number alterations (CNAs), complex chromosomal rearrangements or whole-chromosomal gains or losses. Such genomic aberrations are central, among other diseases, to carcinogenesis. Some cancer subtypes are characterized by high levels of genomic instability. The high rate of acquisition of genomic aberrations in such genomically unstable cancer cells adversely affects cellular viability, and likely makes cancer cells increasingly dependent or addicted to certain pathways that allow their survival under these circumstances. Unravelling which genes are essential for the survival of genomically unstable cancer cells can provide insight into the genetic dependency of such tumors, and may be instrumental to the identification of drug targets for these tumors. Although genome instability facilitates the acquisition of genetic alterations that drive oncogenic properties (so-called oncogenic ‘driver’ mutations), many other genomic alterations are acquired as ‘collateral damage’. Such ‘passenger mutations’ do not necessarily affect the survival of cancer cells, but are a result of ongoing DNA maintenance defects. ‘Passenger mutations’ can include genes that are co-amplified or co-deleted because they reside in the same amplicon or deletion with an ‘oncogenic driver’. Additionally, passenger mutations encompass gene mutations which originate from ongoing mutagenesis.

Although passenger mutations do not drive tumor progression, they do have implications for cancer treatment. An important manifestation of passenger mutations is the appearance of neoantigens presented on the outside of the tumor cell. In addition, focal amplifications may result in elevated (tumor-restricted) expression patterns of specific genes. Tumor-specific antigens that are localized to the plasma membrane, and are not or hardly expressed on normal cells, could be of value. Specifically, antibody-drug conjugates (ADCs) can be used to target a cytotoxic load to tumor cells that express such tumor-specific antigens, and consequently lead to cancer cell killing. Finally, the patterns of gene mutations and genomic rearrangements reflect the presence of DNA repair defects in cancers. Such knowledge can for instance be used as a selection tool to identify HR-deficient cancers for PARP1 inhibitor treatment.

The development of new therapeutic treatment regimens is highly needed for patients with genomically unstable cancers, which are difficult to treat with the current standard of care. Therefore, the goal of this thesis was to find potential new therapeutic treatment regimens for patients with genomically unstable cancers. To this end, we aimed to uncover genes and proteins of which expression is upregulated in genomically unstable cancer cells in comparison to moderately genomically unstable cancer cells or healthy cells. Uncovering the genetic dependencies or differences in gene and protein expression levels of genomically unstable cancers can provide potential actionable targets for these cancers. Whereas genetic dependencies may point at direct targets for treatment, upregulated expression of tumor-specific antigens on tumor cells in contrast to normal cells, can provide targets for ADCs.

In Chapter 1, a general introduction is provided that elaborates on how genomic instability can arise, what the consequences are of genomic instability, how cancer cells cope with high levels of genomic instability and how genomic instability can be measured.

In Chapter 2, we aimed to uncover which genes could play a role in the survival of genomically unstable cancer cells. We used functional genomic mRNA (FGmRNA) profiles of 16,172 cancer samples that were ranked based on their degree of genomic instability. We analyzed the top 250 genes, that showed a positive correlation between FGmRNA levels and the degree of genomic instability, in a co-functionality network. In our co-functionality analysis, a cluster of 11 cell cycle-related genes
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... appeared, including TPX2. TPX2 is involved in the assembly and maintenance of the mitotic spindle. Depletion of TPX2 or its associated kinase Aurora-A preferentially reduced cell viability in a panel of genomically unstable cancer cells, induced by BRCA2 inactivation. In line with these findings, BRCA2-depleted and BRCA2-mutant human cell lines, or tumor cell lines derived from Brca2−/−:p53−/− mice showed increased sensitivity to the Aurora-A kinase inhibitor alisertib, with delayed mitotic progression and frequent mitotic failure. Overall, our findings revealed that BRCA2-deficient cancer cells show enhanced sensitivity to inactivation of TPX2 or its partner Aurora-A, which points at an actionable dependency of genomically unstable cancers.

In Chapter 3, we investigated protein expression levels of TPX2 across breast cancer subtypes by immunohistochemical staining of resection specimens of 499 breast carcinomas. We found that triple-negative breast cancers (TNBCs), the subtype with the highest degree of genomic instability, had significantly higher levels of TPX2 in comparison to the other breast cancer subtypes. Furthermore, we associated protein expression of TPX2 to protein expression of the proto-oncogene Cyclin E, replication stress marker pRPA and DNA double strand break marker γH2AX. Elevated protein levels of pRPA and γH2AX are indicative of a high degree of genomic instability, whereas Cyclin E is considered a driver of genomic instability. We found that TPX2 expression was positively associated to expression of pRPA and Cyclin E (both cytoplasmic and nuclear Cyclin E). Although RPA, Cyclin E and TPX2 are cell-cycle related proteins and show elevated expression in rapidly proliferating cells, we speculate that TPX2 expression could be an indicator of replication stress and thereby also of genomic instability.

Head-and-neck squamous cell carcinoma (HNSCC) is characterized by a high degree of genomic instability, likely induced by increased levels of replication stress. The aim of the study in Chapter 4 was to gain insight into the degree of replication stress in HNSCCs, and to determine whether HNSCCs increasingly depend on genes that might suppress toxic levels of replication stress. Using FGmRNA profiles of 344 HNSCC tumor tissues, we associated the degree of genomic instability to the expression of individual genes. We found that expression of the ATR gene was positively associated to the degree of genomic instability. These data were confirmed by immunohistochemical analysis, which showed that ATR activity, assessed by phosphorylation of its substrate RPA, was elevated in HNSCCs. In addition, ex vivo analysis of DNA replication in individual DNA fibers of ten HNSCC tumors showed that replication speed increased, decreased or remained constant upon ATR inhibition. Although we were unable to identify why HNSCCs were differentially sensitive to ATR inhibition, we for the first time report DNA fiber analysis in fresh tumor tissue and observed replication speed differences upon ATR inhibition.

Tumor-specific antigens can be targeted by ADCs, which are a class of therapeutic agents that can bind to a tumor-specific antigen and deliver a cytotoxic agent. Advantages of ADCs are that their targets do not have to be drivers of tumor growth to selectively kill cancer cells. Instead, they can be efficiently targeted to cancer cells (when tumor-specific antigens are abundantly expressed on cancer cells in contrast to normal cells) to deliver a toxic payload, and induce cancer cell specific cytotoxicity while inducing minimal side effects. In Chapter 5, we used FGmRNA profiling on 344 HNSCC samples, to screen for the expression of genes, for which an ADC is currently available. Among those genes, we found that glycoprotein nmb (GPNMB) had the highest predicted FGmRNA expression in HNSCCs. Immunohistochemical analysis of 414 HNSCC tumors confirmed that 92% of the tumors expressed cytoplasmic and membrane-associated GPNMB, and that healthy tissues expressed GPNMB to a much lower level. Although GPNMB is likely not directly required for cells, its overexpression in tumor cells compared to healthy tissue might provide a starting point for an ADC-based therapy strategy in the treatment of patients with HNSCC.

DISCUSSION and FUTURE PERSPECTIVES

Functional genomic mRNA profiling as an explorative tool for studying genomic instability and its consequence on gene expression. Throughout this thesis, we made use of functional genomic mRNA (FGmRNA) profiling, which
is a tool that allows to unravel the effects of somatic copy number alterations (SCNAs) on gene expression levels. FGmRNA profiling uses data from mRNA microarray analyses and employs principal component analysis (PCA), a commonly used technique in gene expression studies to correct for batch effects and environmental (non-genetic) factors\textsuperscript{13,14}. By applying PCA on a large data set containing gene expression data of normal tissue samples and of samples annotated as cancer samples, the principal components responsible for variation of gene expression caused by non-genetic factors are revealed. Removal of these non-genetic factors results in ‘corrected’ gene expression profiles or functional genomic mRNA profiles (FGM profiles), which strongly correlate to copy number variations (CNVs)\textsuperscript{14}. These FGM profiles can be used to predict with high accuracy where in the genome (up to the individual gene level) amplifications or deletions occur. This allows sample stratification based on similar genomic alterations or association to *e.g.* the degree of overall genomic instability. By associating all individual amplifications and deletions of a set of FGmRNA profiles to the overall degree of genomic instability, we were able to determine which gene amplifications or deletions occur often in cancers that have a higher degree of genomic instability.

In the context of analyzing genome instability, the past decade observed a shift from detection techniques by which a small set of gene amplifications/deletions are detected towards genome-wide techniques that capture more types of genomic aberrations in one analysis. Three widely-used techniques that allow genome-wide detection of genomic aberrations are:

1) **Array comparative genomic hybridization (aCGH).** aCGH is based on hybridization of fluorescently labelled DNA fragments from an experimental sample to an array with thousands of oligonucleotides, representing multiple genomic loci\textsuperscript{15}.

2) **Single nucleotide polymorphism (SNP) arrays.** SNP arrays were originally designed to identify polymorphisms between individuals with high-throughput analysis. SNP arrays typically contain many more probes when compared to aCGH, while they also detect local amplifications/deletions similar to aCGH. For this reason, SNP arrays are more attractive than aCGH in measuring genomic instability\textsuperscript{16,17}. Advances in SNP array technologies improved the resolution of these arrays in recent years, allowing the detection of microdeletions and duplications at a resolution of 40-80 base pairs (bp).

3) **Next-generation sequencing (NGS).** NGS relies on fragmentation, amplification and subsequent labeling of DNA fragments with fluorescently labelled bases, resulting in the precise sequence of nucleotides\textsuperscript{16}. When NGS is used to assess copy-number alteration, low-coverage NGS can be used to relate the numbers of DNA reads to the abundance of genomic loci. Although typically only a small percentage of the genome is sequenced, NGS has an even higher resolution when compared to aCGH and SNP arrays. In addition to microdeletions and duplications, NGS also detects translocations and insertions.

A main difference between FGmRNA profiling and aCGH, SNP array or NGS is that FGmRNA profiling is an mRNA-based technique whereas aCGH, SNP array or NGS are DNA-based techniques\textsuperscript{18}. Additionally, FGmRNA profiling predicts rather than measures copy number alterations and provides insight into the downstream consequences of copy number alterations on mRNA levels. Since FGmRNA profiling allows for a fast prioritization of potentially important pathways, this technique appeared particularly appealing to us and was applied in chapter 2, 4 and 5. Analysis of the prioritized gene targets *in vitro* confirmed the relevance of the gene targets that were previously prioritized using FGmRNA profiling. Apart from that, gene expression data is often obscured by many non-genetic factors. FGmRNA profiling filters out gene expression alterations caused by non-genetic factors, resulting in a more accurate picture of the effect of genomic alterations on gene expression levels.

A *role for TPX2/Aurora-A in promoting survival of genomically unstable cancers.*

In chapter 3 of this thesis, we found that TNBCs, the most genomically unstable breast cancer subtype, had elevated protein levels of TPX2 compared to the other breast cancer subtypes. This suggests that TNBCs might have become dependent on TPX2 for their survival. Our hypothesis was that genomically unstable cancer cells are somehow genetically ‘rewired’ to survive high levels of genomic instability.
and thereby become increasingly dependent on pathways that secure their survival. Furthermore, in chapter 2, we found that depletion of TPX2 or its associated kinase Aurora-A preferentially reduced cell viability in BRCA2-deficient cells compared to BRCA2-proficient cells. Video microscopy analysis showed that depletion of TPX2 or Aurora-A resulted in an increased amount of mitotic aberrations, especially in BRCA2-deficient cells. These increased amounts of mitotic aberrations could lead to DNA lesions in subsequent rounds of cell division and install a dependency on DNA repair mechanisms such as homologous recombination (HR). As a consequence, BRCA2-deficient cells, which are unable to faithfully repair DNA breaks by HR, become increasingly dependent on faithful mitotic progression to limit further DNA lesions, securing their survival. The inability to resolve such lesions due to BRCA2 loss could eventually lead to mitotic catastrophe and cell death.

Specifically, we observed that BRCA2-deficient cells, which are depleted of TPX2 or Aurora-A had a significantly longer duration of mitosis compared to BRCA2-deficient cells. TPX2 and Aurora-A are required for the mitotic spindle apparatus and in this capacity are important factors in chromosome alignment and progression past the spindle assembly checkpoint (SAC). Interestingly, Aurora-A was recently shown to have the ability to promote mitotic exit even when the SAC is activated. Specifically, Retinoblastoma (RB1) mutant cells appeared to be particularly sensitive to Aurora-A inhibition, since RB1 mutant cells were unable to escape mitosis in the event of a hyperactivated SAC. These results suggested that cancer cells that instigate a “wait anaphase” signal are particularly depend on the TPX2-Aurora-A pathway to override the SAC and to prevent mitotic death. We observed in this thesis that inactivation of BRCA2 leads to an increase in structural aberrations, and inactivation of BRCA2 has previously been shown to lead to increased levels of aneuploidy, which might impede chromosome segregation. It is therefore conceivable that BRCA2-deficient cells are particularly sensitive to TPX2 or Aurora-A depletion due their increased levels of DNA damage and their inability to override the SAC. Consequently, this could lead to an unacceptably long ‘wait anaphase’ signal that finally results in mitotic cell death.

Besides the role of TPX2 and Aurora-A as mitotic regulators, a recent study showed that TPX2 and Aurora-A are also involved in regulating DNA double-stranded break repair as well as DNA fork protection in the event of replication stress. These previously unknown roles of the TPX2/Aurora-A complex ensure genomic integrity by counteracting 53BP1, a DNA repair factor that promotes error-prone DNA repair non-homologous end joining (NHEJ). The choice to repair DNA double-stranded by NHEJ or HR is balanced by 53BP1 and BRCA1. Whereas 53BP1 blocks the processing of DNA ends and thereby promotes DNA breaks to be repaired by NHEJ, BRCA1 promotes DNA end resection to stimulate repair through HR. The recent study showed that the TPX2/Aurora-A complex binds and thereby sequesters 53BP1, and consequently promotes BRCA1 localization to double-stranded breaks. Conversely, depletion of TPX2 or Aurora-A resulted in a decrease of BRCA1 and RAD51 at irradiation-induced foci and reduced HR efficiency. No effect, however, was observed in the recruitment of 53BP1 foci upon TPX2 depletion, which is also in line with our findings, and suggests that 53BP1 recruitment to sites of DNA damage is performed independent of TPX2/Aurora-A. Apart from repair of DNA breaks, the TPX2/Aurora-A complex was also shown to impact fork protection. Specifically, removal of either TPX2, Aurora-A or BRCA1 resulted in degradation of nascent DNA at stalled replication forks. It was found that binding of the TPX2/Aurora-A complex with 53BP1, decreases the amount of 53BP1 binding to newly replicated DNA which thereby protects newly replicated DNA from MRE11-dependent degradation. This pathway works fundamentally different than replication fork protection by FANCD2 or BRCA1/2 proteins whereby newly replicated DNA is protected from MRE11-dependent degradation by stabilization of RAD51 at stalled replication forks. We observed in this thesis that co-depletion of either TPX2 or Aurora-A and BRCA2 led to many genomic aberrations, ultimately leading to frequent cancer cell death. One could imagine that simultaneous inactivation of multiple pathways to protect newly replicated replication forks may eventually lead to cancer cell death due to aberrant replication intermediates that are propagated into mitosis. The authors therefore also suggest that Aurora kinase A inhibitors would be particularly useful for treatment of cancer cells that are HR-deficient or cancer cells that harbor defects in the replication fork machinery. This suggestion is completely in line with our findings in chapter 2, in which we
showed that HR-deficient cancer cells showed enhanced sensitivity towards depletion of Aurora-A or TPX2. It will therefore be of interest to determine whether cancer cells, in which replication fork protection is defective due to other mutations (e.g. Fanconi mutations), are also more sensitive to Aurora kinase A inhibitors.

For this study, we induced genomic instability by inactivation of DNA repair protein BRCA2. Of course, there are many genetic causes that underlie genomic instability, including oncogene activation, inactivation of other DNA repair genes, or defective cell cycle checkpoint mechanisms. Since the samples in our FGmRNA analysis were ranked according to their degree of genomic instability, regardless of underlying gene mutations, we do not expect that depletion of TPX or Aurora-A signaling only leads to increased cell death in BRCA2-deficient cells. Also, since the increased cell death upon TPX2 or Aurora-A depletion can be caused by mitotic catastrophe, the expectation is that cells with other DNA repair defects will face the same fate. This implies that genomically unstable cancer cells with similar DNA repair defects may also be particularly reliant on faithful mitotic progression to prevent mitotic catastrophe. So far, no studies have compared the sensitivity of TPX2 or Aurora-A depletion in cancer cells with defects in other DNA repair proteins. It will therefore be interesting to study the effects of TPX2 or Aurora-A inactivation in isogenic cell line panels with cancer-relevant mutations in DNA repair genes. In this context, particularly interesting, are genes that are involved in HR (e.g. BRCA1, RAD51C, RBBP8, MRE11A, PALB2), Fanconi anemia repair or other DNA damage repair pathways such as non-homologous or alternative end joining (e.g. ATM, H2AFX, DCLRE1C, RAD52), mismatch repair, nucleotide excision repair and translesion synthesis (TLS).

The principle of further enhancing genomic instability until cancer cells have reached intolerable levels of DNA lesions and undergo mitotic catastrophe opens a therapeutic window for the treatment of patients with genomically unstable cancers with anti-mitotic drugs. Anti-mitotic drugs including taxanes have been used for decades clinically in the treatment of multiple cancer types. While treatment with anti-mitotic drugs provides benefit for patients, it also leads to adverse effects as normal cells are also affected. It will be particularly interesting to see whether combination therapy of anti-mitotic drugs with an agent that induces unresolved DNA lesions could be used to further promote mitotic catastrophe in genomically unstable cells due to the accumulation of intolerable levels of genomic aberrations. Possibly, this may lead to using lower doses of the anti-mitotic drugs, which could limit the amount of adverse effects.

Currently, a variety of anti-mitotic drugs is used clinically, which all are classified as microtubule poisons (e.g. Paclitaxel and Docetaxel). These agents have been approved almost two decades ago and achieve good clinical response rates in the treatment of breast, ovarian and lung cancer. Other classes of anti-mitotic drugs, including inhibitors of Polo-like kinase-1 and kinases, appeared to have promising preclinical results, however, showed minimal activity in clinical studies. Aurora inhibitors, comprising the Aurora-A inhibitor alisertib, were proven to be particularly effective in the treatment of multiple different tumor types with long-term progression-free survival or complete response in some instances. In summary, investigation of the clinical activity of alisertib with an agent that induces DNA lesions that remain unresolved can provide further insight into the therapeutic effectiveness and feasibility of anti-mitotic drugs in the treatment of genomically unstable cancers. Thus, future clinical studies using anti-mitotic drugs should further elucidate whether genomically unstable tumors are particularly sensitive to drugs that induce mitotic aberrations and with which DNA damaging agent this can best be combined.

A role for ATR in preventing lethal replication stress

In chapter 4 of this thesis, we aimed to determine the levels of replication stress in HNSCCs and to determine whether ATR is required to suppress toxic levels of replication stress in HNSCCs. The exact mechanism of how ATR protects replication forks from breakage or ‘collapse’ during replication stress is not completely clear, although a few mechanisms have been proposed. The first mechanism is based on the observation that ATR can prevent replication fork collapse by remodeling the structure of stalled replication forks and allowing access of HR repair proteins. In parallel, it has been shown that ATR can replenish the supply of deoxynucleotides (dNTPs) thereby facilitating fork progression.
Finally, ATR has been shown to prevent progression into mitosis, and through this effect prevent mitotic entry in the presence of stalled replication forks38. Our data showed that HNSCCs indeed harbor high levels of replication stress, as measured by increased phosphorylation levels of RPA2, a downstream target of ATR. Also, we were able to identify variation in replication fork speed among the tumors compared to control-treated tumors using DNA fiber analysis. Due to the small sample size of ex vivo tumors treated with the ATR inhibitor VE-821, we were unable to draw conclusions on the dependency of HNSCCs on ATR. Likely, other factors or mutations play a role in the degree to which cells are dependent on ATR for their survival. However, our study paved the way for performing DNA fiber analysis in fresh tumor tissues. In the future, DNA fiber analysis could be a valuable tool to assess replication stress in tumor material, and to possibly predict whether a patient with HNSCC could benefit from ATR inhibitor treatment.

The observation that cancer cells harbor high levels of replication stress compared to normal cells makes replication checkpoint inhibitors such as ATR inhibitors promising therapeutic targets for those cancers. Several ATR inhibitors are currently being evaluated in phase I/II clinical trials in solid tumors and hematological cancers which should elucidate whether replication checkpoint inhibitors could be effective in the treatment of genomically unstable cancers38.

Better detection methods allow patient stratification and more personalized therapy

The increased availability of anti-cancer drugs, either approved or in development, has resulted in an increased demand for biomarkers to select patients and to predict or measure treatment response. Biomarkers can be categorized into several classes:

1) protein-based biomarkers. Examples of protein-based biomarkers include proteins of which expression can predict the severity of a disease or expression of proteins that can predict therapy response40. The estrogen receptor, progesterone receptor or HER2/neu receptor are classic examples of protein-based biomarkers. These protein-based biomarkers are valuable in predicting whether a patient will respond to hormonal therapy or not. Analysis of a biomarker that can identify genomically unstable cancers was also one of our aims in chapter 3. Herein, we performed association analyses to assess the relation between known biomarkers indicative of high levels of genomic instability or proto-oncogenes that cause genomic instability and a biomarker that so far has not been identified as an indicator of the degree of genomic instability. We found that protein levels of pRPA and Cyclin E were positively associated to protein levels of TPX2, a potential new biomarker indicative of the degree of genomic instability. Although more research is needed, this could suggest that these three proteins can be used for selection of patients that could benefit from agents that preferentially affect genomically unstable cancers, including inhibitors of Aurora-A. Moreover, literature research showed that Cyclin E-amplified and -high cancer cell lines appeared to have upregulated levels of TPX2 protein expression and that TPX2 appeared essential in the survival of Cyclin E amplified or overexpressing ovarian cancer cell lines41. Also, TNBC cell lines with high levels of MYC were shown to be more sensitive to TPX2 depletion than TNBC cell lines with low MYC levels42. This is in accordance with studies showing increased sensitivity towards anti-mitotic drugs in cells with MYC activation or Cyclin E overexpression22. Collectively, these observations demonstrate that protein expression of MYC, Cyclin E and perhaps pRPA can act as biomarkers predicting response to an Aurora-A inhibitor.

2) genetic biomarkers. Genetic biomarkers reflect the expression, function or regulation of a gene and are indicators of normal/abnormal biological processes43. Examples of genetic biomarkers are mutations in the BRCA1 and BRCA2 genes, which predispose individuals to the development of breast and ovarian cancer. Moreover, mutation analysis has allowed risk assessment of breast cancer occurrence based on the location and type of the mutation in BRCA1 and BRCA244,45. Thus, the availability of genetic biomarkers can aid in medical decision-making and provides a rationale for regular screening which could detect disease in an early stage.

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3) functional biomarkers. Functional biomarkers provide information on the function of a particular process or pathway. An example of a functional biomarker that is directly related to the ability of cancer cells to repair DNA damage using HR, is the formation of irradiation-induced RAD51 foci. This assay is already used in a number of studies in ex vivo tumors and appears to accurately predict response to PARP inhibitors\textsuperscript{46,47}. Another example of a functional biomarker are elevated levels of phosphorylated RPA which are indicative of ATR activation, as a consequence of stretches of single-stranded DNA and a consequence of prolonged replication stress\textsuperscript{48}. Additionally, DNA fiber analysis could be used to detect the degree of replication stress\textsuperscript{49} as performed in this thesis in ex vivo HNSCC tumors. DNA fiber analysis allows to examine DNA replication dynamics by visualization of individual replication forks in living cells. Cells suffering from replication stress show a perturbed replication fork speed\textsuperscript{50}. Advantages of DNA fiber analysis is that it is a direct measurement of replication stress and also allows to monitor response to a certain treatment\textsuperscript{50,51}. Despite some challenges of this technique, such as logistic transfer of the tumor to the lab and in vitro culture challenges of the tumor, it is conceivable that the DNA fiber analysis may become a diagnostic essay in the future and will provide a good estimate of the levels of replication stress and a prediction of a patient’s response to DNA replication-targeted agents.

The last two decades have seen a new approach in the treatment of cancer where tumor-specific molecular aberrations are systematically linked to targeted therapies. This ‘precision medicine’ or ‘precision oncology’ integrates clinical, molecular and therapy-related data to tailor the best suitable therapy to individual patients. A classic example of precision oncology, which is already part of standard of care, includes the treatment of patients with BRCA1, BRCA2 mutant cancers with PARP inhibitors\textsuperscript{52}. Advances in immunotherapy have further expanded the field of precision oncology\textsuperscript{53}. Multiple clinical studies have confirmed the efficacy and minimal toxicity of immunotherapy and a number of immunotherapy treatment regimens are standard of care in different cancer types. Despite promising responses to immunotherapy, only a small group of patients benefit from immunotherapy in the long-run, and similar to targeted therapies, acquired resistance can occur\textsuperscript{54}. Combining targeted therapies and immunotherapy have pointed to improved efficacy, manageable toxicity and longer-lasting responses\textsuperscript{55}. Currently, a vast number of clinical trials are investigating the combination of both therapies, which will shed light on potential incorporation of targeted therapies and immunotherapy in the clinic. Thus, precision medicine to date integrates clinical, molecular, therapy-response and also immune response data which will better guide treatment options for cancer patients\textsuperscript{55}. It is expected that the integration of different precision medicine platforms will lead to treatment options that are tailored to individual patients and will result in better response rates and fewer side effects.

REFERENCES

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