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## **Somatic POLE exonuclease domain mutations in endometrial cancer : Insights into the biology of POLE-mutant tumors**

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# Chapter 8

Discussion and future perspectives



## DISCUSSION AND FUTURE PERSPECTIVES

*POLE* exonuclease domain mutations (hereafter referred to as '*POLE* mutations') are found in a wide variety of human malignancies, including endometrial cancer. In all tumor types, cancers with *POLE* mutations are characterized by an exceptionally high mutational burden and a specific mutational signature.<sup>1,2</sup> Despite their so-called 'ultra-mutated' phenotype, patients with *POLE*-mutant cancers have significantly better outcomes compared to patients with *POLE*-wild-type tumors. This prognostic benefit has thus far been shown for early-stage endometrial and stage II/III colorectal cancers, and has been suggested for glioblastomas.<sup>1,3-10</sup> The studies described in this thesis aimed to gain insight into somatic *POLE* exonuclease domain mutations and especially into the underlying mechanism(s) by which these *POLE* mutations contribute to low risk of recurrence. This chapter puts the main findings of this thesis into perspective of current literature, and discusses the implications of these findings for the treatment of patients with *POLE*-mutant cancers, in particular endometrial cancer.

### Explaining the favorable clinical outcome of *POLE* exonuclease domain-mutant cancers

Three hypotheses that could explain or contribute to the favorable prognosis of *POLE*-mutant cancers were studied and will be discussed below.

#### 1. Induction of an antitumor immune response

To explain the good prognosis of *POLE*-mutant cancers, we hypothesized that the ultra-mutated phenotype would result in the presentation of neoantigens on the tumor cell surface. These neoantigens would elicit an antitumor immune response, which may contribute to the favorable outcome of these patients. To support this hypothesis, this thesis and work of others provide several lines of evidence that will now be discussed.

*POLE*-mutant cancers were predicted to display more neoantigens than *POLE*-wild-type cancers (chapters 4 and 5).<sup>11</sup> This prediction was based on *in silico* algorithms that assessed the binding affinity to MHC class I of all possible mutant peptides relative to that of the wild-type peptide. Similar prediction algorithms have been used in many reports to date to identify neoantigens.<sup>12-18</sup> Previous studies also showed, however, that only a small fraction of mutations in expressed genes resulted in the presentation of neoantigens that elicit T cell reactivity.<sup>13,15,19-21</sup> Therefore, the *in silico* algorithms could more accurately predict reflect the landscape of immunogenic neoantigens in cancers when filtering of the exome or RNA sequencing data would be improved. Still, the observed difference in predicted neoantigen numbers between *POLE*-mutant and *POLE*-wild-type is very large (~100-fold) with the currently employed methods. Therefore, regardless of

more stringent filtering, these differences suggest that *POLE* mutations indeed result in the presentation of immunogenic neoantigens.

An *in vitro* study supported the concept of increased immunogenicity of *POLE*-mutant cancers. In this study, autologous dendritic cells pulsed with lysate from *POLE*-mutant endometrial cancers induced stronger proliferation of cytotoxic T cells and T helper cells than lysate derived from *POLE*-wild-type cancers.<sup>22</sup> This suggests that *POLE*-mutant cancers are capable of eliciting an immune response and that this capacity is greater than in their wild-type counterparts. Whether the increased immunogenicity of *POLE*-mutant cancers is indeed caused by a higher neoantigen load remains to be elucidated.

*POLE*-mutant (endometrial) cancers are characterized by the presence of an enhanced cytotoxic T cell response (chapters 4 and 5).<sup>9,22,23</sup> In *POLE*-mutant cancers, tumor-infiltrating lymphocytes are present in larger numbers than in *POLE*-wild-type cancers. Moreover, these lymphocytes demonstrate upregulation of activation and effector markers, suggesting the T cells are cytotoxically active. These findings are supportive of increased immunogenicity of *POLE*-mutant cancers. Furthermore, the association between the presence of such an immune infiltrate and prognosis is well-established: a meta-analysis showed in a pooled cohort of over 12,000 patients with various tumor types that tumor-infiltrating lymphocytes have a positive effect on clinical outcomes.<sup>24</sup> A similar association has been previously described for endometrial cancers, albeit in a limited number of studies.<sup>25,26</sup>

For the T cells to contribute to the favorable prognosis, these lymphocytes would have to be tumor-reactive, recognizing the antigen presented by the tumor cell as being foreign. Evidence for this tumor reactivity is provided by the response of *POLE*-mutant cancers to immune checkpoint inhibition. It is important to note that this response is also clinically very relevant (details on therapeutic implications are given below). Through immune checkpoint inhibition, T cells are relieved of certain inhibitory signals (such as PD-1/PD-L1 signaling), leading to a release of their cytotoxic potential. Although the search for a robust biomarker to predict response to checkpoint inhibitors is still ongoing, response rates are higher in cancers with a high mutational load.<sup>27,28</sup> This is illustrated by the 40-53% objective response rates found in (mostly colorectal) cancers with mismatch repair deficiency, compared to 0% response in non-hypermutated cancers.<sup>29,30</sup> Following this rationale, the first few women with advanced-stage endometrial cancers carrying *POLE* mutations have been treated with anti-PD-1 antibodies and showed sustained responses.<sup>31-33</sup> These responses suggest that upon PD-1/PD-L1 blockade, T cells that are tumor-reactive are 'unleashed', resulting in effective antitumor activity contributing to prolonged survival.

Finally, a parallel can be drawn between *POLE*-mutant cancers and melanomas to provide evidence, albeit circumstantial, supportive of this 'antitumor immune response-hypothesis'. In melanomas, another highly mutated tumor type, lymphocytes have been identified in the tumor microenvironment that are reactive to neoantigens presented by the cancer cells.<sup>15,20</sup> Furthermore, the tumor-reactive T cells expanded in the peripheral blood after immune checkpoint inhibitor treatment, suggesting a role for these T cells in tumor regression.<sup>20</sup> Thus, the mutational load in melanomas is apparently sufficient to generate neoantigens that can be recognized by autologous T cells. Compared to melanomas, *POLE*-mutant cancers carry on average ten times as many mutations (10 mutations per Mb for melanomas vs more than 100 mutations per Mb for *POLE*-mutant cancers).<sup>34</sup> It can therefore be expected that the presence of neoantigens and their recognition by T cells are also common in *POLE*-mutant cancers.

In summary, we and others showed that the ultramutated *POLE*-mutant (endometrial) cancers display an enhanced cytotoxic T cell response. This T cell response is accompanied by - and most likely caused by - a significant increase in the number of neoantigens presented at the tumor cell surface. Together, this provides a plausible mechanism by which *POLE* exonuclease domain mutations contribute to the good clinical outcome of the majority of patients.

A question worthy of further exploration is why *POLE*-mutant cancers, despite the antitumor immune response, are not eliminated by the immune system. Defects in antigen processing or presentation occurred at a similar frequency in *POLE*-mutant as in *POLE*-wild-type (chapter 4). Immunosuppressive molecules such as PD-1 and CTLA-4 were upregulated in *POLE*-mutant cancers compared to *POLE*-wild-type. However, considering the overall increase in expression of cytotoxic effector markers, the upregulation of immunosuppressive mediators appeared insufficient to fully suppress the T cell response in *POLE*-mutant endometrial cancers (chapter 4). Therefore, further research on immune escape mechanisms in *POLE*-mutant cancers, e.g. on the presence of immunosuppressive cells in the tumor microenvironment, is warranted.

The above-described immune-related mechanism does not explain the high survival rate of patients with *POLE*-mutant cancers completely. In chapter 3, *POLE*-mutant endometrial cancers were evaluated for the presence of tumor-infiltrating and peritumoral lymphocytes. Although these lymphocytes were prominently present in the majority of cases, ~15% of *POLE*-mutant endometrial cancers showed no evidence of a strong T cell response. In colorectal cancer, a lack of lymphocytic infiltrate seems associated with poorer clinical outcome in the 'hypermutated', mismatch repair-deficient cases.<sup>35,36</sup> The number of *POLE*-mutant endometrial cancers studied to date is too small to per-

form similar within-subgroup analyses. Considering the genomic similarities between *POLE*-mutant and mismatch repair-deficient cancers, the degree of immune response is likely to have prognostic impact in *POLE*-mutant cancers as well. Therefore, also other mechanisms that could (in part) account for the excellent prognosis of *POLE*-mutant cancers were investigated.

## **2. Increased sensitivity to adjuvant treatment**

The favorable prognosis of *POLE*-mutant cancers was, as described, found in several independent studies.<sup>1,3-10,37</sup> In these studies, the majority of patients (~66%) with *POLE*-mutant cancers received some type of adjuvant therapy (i.e. radiotherapy and/or chemotherapy). Therefore, increased sensitivity to adjuvant treatment might also contribute to the good outcome of *POLE*-mutant cancers. Outcomes were, however, consistently favorable for patients in the PORTEC-1 endometrial cancer trial and in the colorectal cancer cohort regardless of adjuvant therapy (chapter 6).<sup>4,9</sup> Moreover, in a mouse-derived embryonic stem cell model, *POLE* mutations did not result in greater sensitivity to ionizing radiation or to commonly used chemotherapeutics, including cisplatin, paclitaxel, and doxorubicin. This is in line with another preclinical study in which no significant difference in sensitivity to paclitaxel was found between *POLE*-mutant and *POLE*-wild-type endometrial cancer cell lines, while demonstrating increased resistance to carboplatin in their *POLE*-mutant cell line. The cell lines used in this study were, however, non-isogenic.<sup>38</sup> Moreover, the exonuclease activity of DNA polymerase epsilon is presumed not to have a role in the mechanisms of actions of the commonly used anti-cancer treatments: a *POLE* mutation is therefore unlikely to alter sensitivity to these therapies, providing further support for the clinical and *in vitro* findings. It should be noted that in the described model systems, an interaction between radiotherapy and the immune system could not be assessed.<sup>39</sup> Nevertheless, the clinical data and the *in vitro* data together suggest that the clinical outcome of *POLE*-mutant cancers is independent of the administered adjuvant treatment. Thus, increased sensitivity to adjuvant therapy does not seem to explain the favorable prognosis of *POLE*-mutant cancers.

## **3. Error catastrophe**

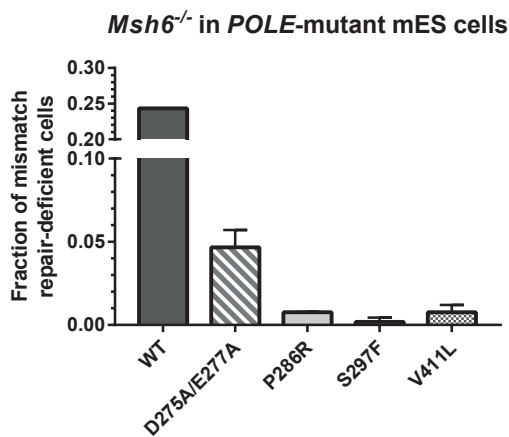
The third possible explanation for the excellent clinical outcome of *POLE*-mutant cancers may originate from one of their characteristic features: the ultramutator phenotype. This may be the Achilles' heel of *POLE*-mutant cancers. On one hand, the high mutation rate causes cells to rapidly accumulate mutations. This increases the genetic variation, which in turn facilitates adaptation of malignant cells to changing microenvironments, e.g. immune evasion.<sup>40-42</sup> On the other hand, rapid accumulation of mutations may compromise tumor viability. Many random mutations can arise that are neither directly beneficial, nor immediately deleterious. These mutations will not be subject to strong positive or

negative selection and can become fixed in the genome. Subsequently, these mutations may collectively exert a negative effect and can result in so-called error catastrophe.<sup>42,43</sup>

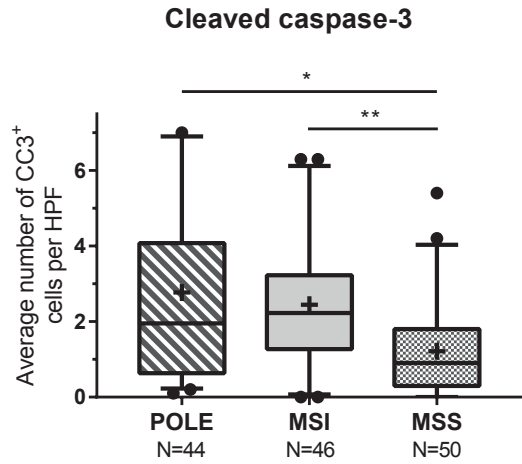
Error catastrophe occurs when the mutational burden exceeds a certain threshold above which the combined negative effect of the acquired mutations prevails, and cellular viability decreases. Considering the exceptionally high mutational load of *POLE*-mutant cancers, error catastrophe may take place and may thus contribute to the favorable outcome of these tumors. In the primary cancer, the mutation rate may have struck a balance between the potential to adapt to new states and maintenance of tumor viability, preventing tumor regression. This is supported by a study of biallelic mismatch repair-deficient brain cancers: after acquisition of a *POLE* mutation, the number of mutations rapidly increased to a certain threshold without surpassing it.<sup>44</sup> The high mutational burden may, however, render *POLE*-mutant cancer cells unfit to effectively metastasize: this could account for the rare incidence of distant metastases. Following Paget's 'seed and soil'-hypothesis, also in this scenario the immune system plays a pivotal role.<sup>45</sup>

The existence of an 'error threshold' is supported by the infrequency at which *POLE* exonuclease domain mutations co-occur with other defects that induce replication errors. Indeed, in yeast, combined polymerase  $\epsilon$  proofreading defects and complete mismatch repair deficiency proved synthetically lethal for the majority of cells.<sup>46</sup> We found similar results in preliminary experiments in the mouse embryonic stem cell model described in chapter 6. When attempting to knock-out *Msh6*, one of the mismatch repair genes, a mismatch repair-deficient clone was often obtained in the wild-type cell line (approximately 1 in 4 cells), while this proved rarely successful in the *POLE* exonuclease domain-mutant cell lines (~1 in 175 cells, Figure 1). These findings suggest that the absence of both DNA repair pathways results in a rapid and catastrophic accumulation of mutations incompatible with cellular survival. This synthetic lethality may also be the reason why *POLE*-mutant cancers generally are microsatellite stable.<sup>47</sup>

To gain further insight into error catastrophe, future studies could investigate cell death in *POLE*-mutant compared to *POLE*-wild-type cancers. We examined levels of apoptosis in the endometrial cancer series described in chapter 4 by performing immunohistochemistry of cleaved caspase-3. Cleaved caspase-3 is the effector marker of both intrinsic and extrinsic apoptotic pathways.<sup>48</sup> In *POLE*-mutant and *POLE*-wild-type microsatellite-unstable endometrial cancers, cleaved caspase-3<sup>+</sup> apoptotic cells were present in significantly higher numbers compared to *POLE*-wild-type microsatellite-stable cases (Figure 2). These results are promising and warrant further exploration.



**Figure 1.** Knockout of *Msh6* in *POLE* exonuclease domain-mutant mouse embryonic stem cells. A knock-out of *Msh6*, one of the mismatch repair genes, was attempted in *POLE*-wild-type (WT) and in *POLE* exonuclease domain-mutant mouse embryonic stem (mES) cells using CRISPR-Cas9. To this end, stem cells were transfected with a guide RNA targeting *Msh6*. Successfully transfected cells were positively selected using puromycin. The number of cells that survived a 6-thioguanine pulse, which selects for loss of mismatch repair, was counted and is shown (box and whisker signify mean and standard deviation, respectively). Results are shown for different homozygous *POLE*-mutant cell lines, namely double-mutant D275A/E277A, and the common cancer-associated *POLE* P286R-, S297F-, and V411L mutants.



**Figure 2.** Apoptosis based on immunohistochemistry of cleaved caspase-3 in endometrial cancer according to molecular subgroup. Apoptosis based on cleaved caspase-3 (CC3) counts was compared between *POLE* exonuclease domain-mutant (*POLE*), microsatellite-unstable (MSI), and *POLE*-wildtype, microsatellite-stable (MSS) endometrial cancers. For each endometrial cancer, the number of cleaved caspase-3-positive cells was counted in ten high power fields (HPFs; 325µm×225µm), consisting of at least 70% tumor tissue. The mean of these ten high power fields is used for each case. For each molecular subgroup, boxes represent the interquartile range, with the upper whisker indicating the 95<sup>th</sup> percentile and the lower whisker the 5<sup>th</sup> percentile. The median and mean values are indicated by a horizontal line and cross respectively. \**P*=0.0005, \*\**P*=0.0072.

Future studies could also focus on cases that apparently escaped error-induced extinction, e.g. *POLE*-mutant endometrial cancers with combined (partial) mismatch repair deficiency or *POLE*-mutant cancers that present at an advanced stage or that recur. A starting point for such future studies could stem from an interesting finding in the yeast study on polymerase  $\epsilon$  proofreading and mismatch repair: among the colonies that survived with combined polymerase  $\epsilon$  proofreading- and mismatch repair defects were colonies that had acquired secondary mutations in *POLE*. These secondary mutations resulted in a slower accumulation of mutations.<sup>46</sup> It would be of interest to explore whether such 'antimutator' variants also occur alongside *POLE* exonuclease domain mutations in human cancers and if present, what their effect is on the mutational burden. At the same time, as many *POLE* mutations outside the exonuclease domain remain 'variants of unknown significance', fundamental research is warranted to understand the functional consequences of such variants.

In conclusion, this thesis together with work of others proposes an enhanced antitumor immune response and a catastrophic accumulation of mutations as most likely mechanisms to explain the high survival rate of patients with *POLE*-mutant cancers. While these and other explanations require further research, they may provide a starting point for more tailored (adjuvant) treatment of *POLE*-mutant cancers, as will be discussed below.

### **Implications for endometrial cancer treatment: current practice and future directions**

Early-stage endometrial cancer is treated primarily with surgery; the indication for adjuvant treatment depends on risk factors for recurrence (chapter 1). Despite the use of risk-based indications, vaginal brachytherapy for patients with high-intermediate risk endometrial cancer still yields significant overtreatment: six out of seven patients would not have had a recurrence without vaginal brachytherapy.<sup>49-51</sup> To reduce overtreatment, screening for *POLE* mutations could be of use. *POLE* mutations predict an excellent prognosis in endometrial cancer.<sup>51</sup> Moreover, as discussed before, this prognostic benefit does not seem to depend on adjuvant treatment. Therefore, it could be argued that for patients with early-stage endometrial cancers carrying *POLE* mutations, surgery alone may suffice, thus reducing overtreatment. For *POLE*-mutant early-stage endometrial cancers, no additional treatment versus vaginal brachytherapy is currently under prospective evaluation in the PORTEC-4A trial.<sup>52</sup> In this randomized trial, vaginal brachytherapy based on standard clinicopathological indications (chapter 1, Table 2) is compared to molecular profile-based indication for either observation, vaginal brachytherapy or external beam radiotherapy. The PORTEC-4A trial is the first to bring molecular profile-based adjuvant treatment for endometrial cancer into the clinic, and results are eagerly awaited.

This discussion has thus far focused mostly on *POLE* mutations in early-stage endometrial cancer and the high survival rate observed in this patient group. However, as mentioned briefly, *POLE* mutations can also be found in advanced-stage endometrial cancer. The frequency of *POLE* mutations in advanced-stage disease is yet to be determined: a collaborative study by Bosse et al. show 1 (1.6%) *POLE*-mutant cancers among 61 grade 3, stage III-IV endometrial cancers, while this was more common in the TCGA endometrial cancer cohort (7.2%, 4/55 stage III-IV endometrial cancers).<sup>1,53</sup> Also the prognostic impact of *POLE* mutations in advanced-stage disease remains uncertain. For these advanced-stage endometrial cancers and for other cancer types with relatively poorer prognoses, the mechanisms underlying the good prognosis of *POLE* mutations can be exploited for therapeutic purposes. First of all, the antitumor immune response can be enhanced by immune checkpoint inhibition. Indeed, the first cases of advanced-stage *POLE*-mutant endometrial cancers treated with anti-PD-1 antibodies have been reported, as well as of pediatric, *POLE*-mutant, biallelic mismatch repair-deficient glioblastomas. These studies showed remarkably good responses of at least five months duration (maximum reported duration was 16 months).<sup>31-33,54</sup> Clinical trials testing the efficacy of immune checkpoint inhibitors in advanced-stage cancers are currently recruiting in which *POLE*-mutant cancers are included.<sup>55-58</sup> Whether *POLE* mutational status itself or other factors such as the mutational load, the number or type of predicted neoantigens or the expression of CD8, PD-1 and/or PD-L1 are the strongest markers to predict response to checkpoint inhibition remains to be elucidated. The outcomes of ongoing trials may further advance this field.

If immune checkpoint inhibitors also yield promising results in larger series of *POLE*-mutant cancers, these drugs may be quickly approved for this new indication. How fast this process of approval can be has been shown for the use of pembrolizumab in advanced-stage mismatch repair-deficient solid tumors: while the results of the first phase II study were only published in 2015, pembrolizumab was granted accelerated approval for this indication by the American Food and Drug Administration in May 2017.<sup>29</sup> In the Netherlands, PD-1 inhibitors pembrolizumab and nivolumab have been approved thus far only for the treatment of, among others, advanced-stage melanoma and advanced-stage non-small-cell lung carcinoma, although response rates in these tumors are similar to, or even lower than, those observed in mismatch repair-deficient cancers (e.g. for pembrolizumab: ~33% in melanoma, ~45% in PD-L1<sup>+</sup> non-small-cell lung cancer, 40-53% in mismatch repair-deficient colorectal cancers).<sup>29,30,59-63</sup> Nonetheless, the use of immune checkpoint inhibitors should be carefully considered because of the high treatment costs. While the exact costs of anti-PD-1 therapy are not known because of confidential price agreements between the Dutch government and the manufacturers, they are likely to approximate or even exceed the threshold value of €80,000 per quality-

adjusted life year, similarly to the anti-CTLA-4 antibody ipilimumab.<sup>64-66</sup> These costs raise important ethical and political discussions on how much society is willing - and able - to pay for such treatments. Ongoing trials investigating immune checkpoint blockade for *POLE*-mutant cancers will provide more insight into the response rates, and thereby into the cost-effectiveness of these therapies.

Along with the immune response observed in patients with *POLE*-mutant cancers, the ultramutated phenotype of these tumors may be exploited for treatment. As the mutational burden seems to plateau just below the threshold for cellular viability, drugs that drive the mutation rate past this threshold could be of interest.<sup>44</sup> The possibility to induce error catastrophe has been demonstrated in RNA viruses by using nucleoside analogs. RNA viruses have remarkably high mutation rates. Nucleoside analogs result in a critical increase of these mutation rates by mispairing after incorporation in the DNA or by preventing the DNA chain from being elongated during replication.<sup>42,67,68</sup> Taking advantage of their already ultramutated phenotype, a similar approach could be beneficial for cancers with *POLE* mutations. Moreover, the effects of nucleoside analogs may be increased in *POLE*-mutant cells: contrary to their wild-type counterparts, *POLE*-mutant cells may not be able to excise the analog once incorporated.

In mouse embryonic stem cells, we explored the sensitivity conferred by *POLE* exonuclease domain mutations to nucleoside analogs (chapter 6). *POLE*-mutant cells were indeed found to be significantly more sensitive to the nucleoside analogs cytarabine and fludarabine compared to *POLE*-wild-type cells: sensitivity to both compounds was increased approximately three-fold. Increased sensitivity to cytarabine is supported by a previous study in *POLE*-mutant chicken lymphoma and human lymphoblast cell lines, which showed an estimated six-fold increase in sensitivity.<sup>69</sup>

Sensitivity to cytarabine (and fludarabine) was thus far demonstrated in cell lines homozygous for the specific *POLE* mutations. For the S297F hot spot mutation, sensitivity to these two nucleoside analogs was also evaluated in the heterozygous state: sensitivity was lower than in the homozygous *POLE*-mutant lines, but higher than wild-type, reaching significance for cytarabine. Tsuda et al. also showed moderate sensitivity in heterozygous lines.<sup>69</sup> As the majority of cancers – if not all – are heterozygous for the somatic *POLE* mutations, the effects of cytarabine and fludarabine in *POLE*-mutant cancers remain to be determined. Moreover, while cytarabine and fludarabine are used in the treatment of leukemias and lymphomas, their efficacy as (mono-)treatment for solid tumors appears to be limited, and toxicity can be considerable.<sup>70-75</sup> Despite these considerations, the sensitivity conferred by *POLE* mutations to certain nucleoside analogs is substantial and may be very specific for *POLE*-mutant cells. Another advantage of

nucleoside analog treatment may be that by the induction of additional mutations, the probability of neoantigen formation increases. This may further enhance the antitumor immune response. Therefore, studies on the use of nucleoside analogs and other similar treatments in the context of *POLE* mutations are warranted.

To be able to optimally exploit the high mutational burden of *POLE*-mutant cancers, it is important to understand the mechanisms leading to this mutator phenotype. The somatic mutations found in cancers often lie close to, or within, the exonuclease domain motifs, and frequently concern residues that are highly conserved across species (chapter 2). An *in vitro* study showed that *POLE* mutations such as P286R, S459F, and V411L, despite not being located at the exonuclease domain catalytic sites, severely diminished proofreading activity. The degree to which each mutation reduced this activity, however, varied.<sup>76</sup> Interestingly, when somatic *POLE* hot spot mutations such as P286R were introduced into yeast and mouse embryonic stem cells, they resulted in a higher mutation rate than could be explained by proofreading deficiency alone (chapter 6).<sup>77,78</sup> dNTP pool imbalances have been put forth by a study in yeast to explain the ultramutator phenotype.<sup>79</sup> However, this explanation remains to be tested for cancer-associated variants and for mammalian cells, as their dNTP pool regulation differs greatly from yeast. Another explanation for the high mutational load is provided by structural analyses of somatic *POLE* mutations; the *POLE* substitutions found in cancers were predicted to perturb DNA binding.<sup>11</sup> Based on these analyses, Barbari et al. suggested that the altered DNA binding may reduce the efficiency of extrinsic proofreading, for example by polymerase  $\delta$ .<sup>78</sup> These possible mechanisms, together with their potential utility in treatment of *POLE*-mutant advanced-stage cancers, should be addressed in future studies.

### **Implementation of *POLE* exonuclease domain mutation screening in clinical practice**

In consideration of the prognostic and promising therapeutic implications of *POLE* mutations, implementation of screening for these mutations in clinical practice would be valuable. For which tumor types *POLE* mutation screening should be introduced could be based on the incidence and on prognostic and/or predictive implications. Endometrial cancer would be a logical cancer type to implement screening for: the frequency of *POLE* mutations is relatively high, and the prognostic and predictive implications of these mutations in this cancer type have been described. In early-stage disease, *POLE* as a biomarker is unique in the sense that it may decrease rather than increase costs by reducing indications for adjuvant treatment; this will probably make *POLE* mutational screening cost-effective in this patient group. Moreover, *POLE* mutational screening in patients with advanced-stage disease could help in identifying candidates for targeted therapies.

To determine *POLE* exonuclease domain mutational status, the gold standard is to sequence exons 9 through 14 of the *POLE* gene. Because sequencing is not available in every clinical pathology laboratory, we aimed to identify histopathological and immunohistochemical characteristics to prescreen for *POLE* mutations in endometrial cancer (chapter 3). A combination of tumor type (endometrioid), tumor grade (high), peritumoral lymphocytes, and two routinely performed immunohistochemical stainings (MLH1 and p53) was found to increase the positive predictive value approximately five-fold, from 7% to 33%. Although this prediction model requires validation, the majority of these histopathological features had been ascribed to *POLE*-mutant endometrial cancers in previous studies.<sup>80,81</sup> Alongside these histopathological features, the age at diagnosis can be taken into account: the likelihood of a *POLE* mutation increases when a patient is diagnosed at a lower than average age, especially below 60 years (average age of an endometrial cancer patient is ~65-67 years).<sup>3,4</sup> Moreover, although their origin remains to be clarified, the finding of tumor giant cells in an endometrioid-type endometrial cancer may also increase the likelihood of a *POLE* mutation being present. Together, these clinicopathological and immunohistochemical characteristics can aid in selecting cases for pathology consultation at medical centers with a molecular diagnostics laboratory equipped to determine *POLE* mutational status.

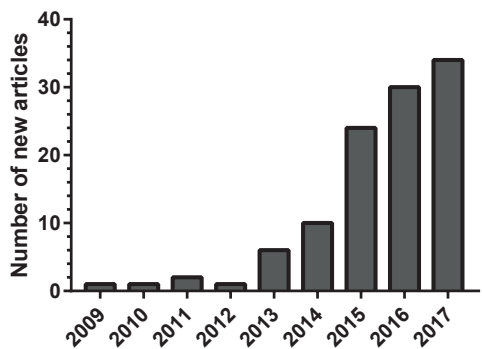
Different molecular diagnostic techniques can be used to identify *POLE* exonuclease domain mutations. While Sanger sequencing is a straightforward method, sequencing of all six exonuclease domain exons separately is laborious, especially when taking into account that in only ~1% of malignancies a pathogenic *POLE* mutation will be identified. To make Sanger sequencing more feasible, studies have screened only exons 9, 13 and at times exon 14, since these exons contain the majority of cancer-associated variants thus far identified (chapter 2). However, as massively parallel sequencing of targeted gene panels becomes increasingly available for clinical use, the *POLE* exonuclease domain could also be incorporated in such cancer gene panels. Ideally, these gene panels would be large enough to also establish mutational burden and spectrum. With this approach, *POLE* mutational status would be determined as part of the molecular work-up and would not require an additional laboratory test; this would greatly facilitate its implementation. Moreover, variant identification and pathogenicity testing would be performed in a single test. Through this method, cancers would also be identified with a high mutational burden and/or with the same mutational signature due to other molecular alterations, e.g. *POLD1* exonuclease domain mutations or *POLE/POLD1* polymerase domain variants. As the mutational load of a cancer likely influences tumor immunogenicity and sensitivity to immune checkpoint inhibition, a screening method including the number of mutations may be advantageous.

An important aspect to keep in mind is that after implementation of *POLE* exonuclease domain mutation screening, more *POLE* mutations will be identified. A fraction of these *POLE*-mutant cancers will also harbor other molecular alterations, such as *TP53* mutations. The implications of multiple prognostically relevant alterations for clinical outcome of patients are unknown. Large-scale collaborations are required to assemble a sufficient number of these relatively uncommon cases, and consequently, to determine which molecular alterations have the strongest impact on prognosis.

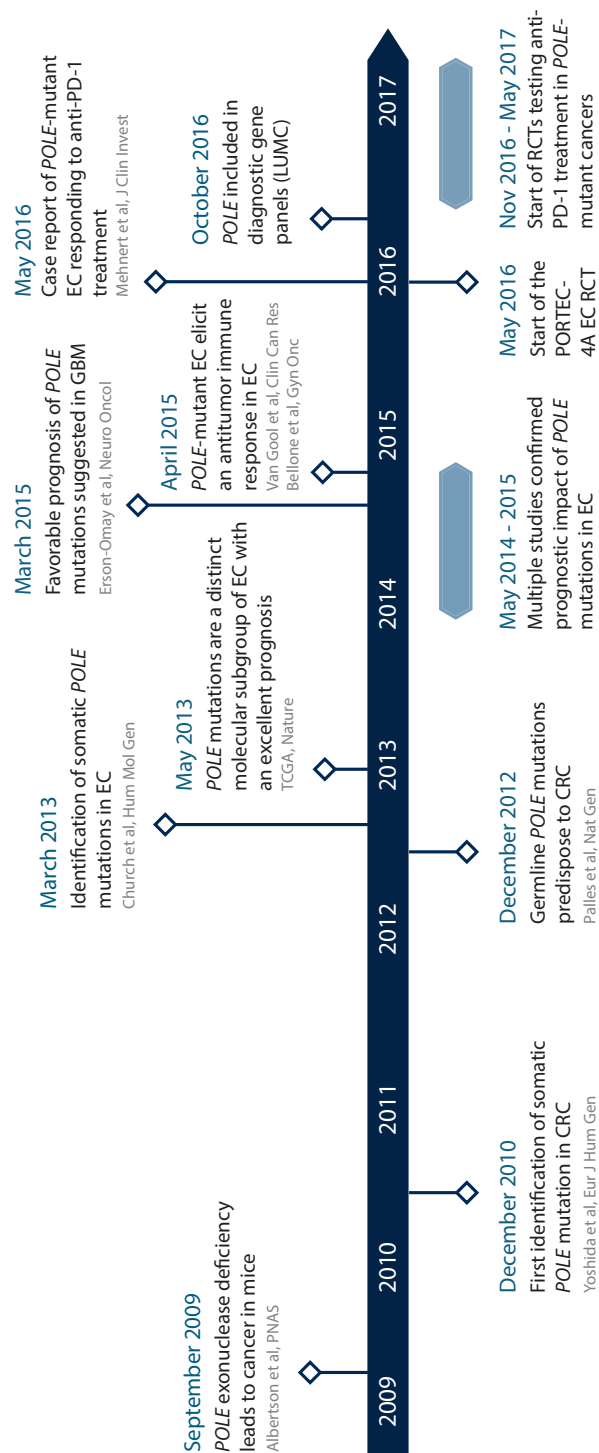
### ***POLE* exonuclease domain mutations in personalized medicine**

Somatic *POLE* exonuclease domain mutations have the potential to become one of the success stories of (genomics-based) personalized medicine. After the discovery of these mutations during extensive genomic analyses, not only their prognostic significance was established in subsequent studies, but also their implications for immunotherapies. Moreover, thanks to close collaboration between clinicians, translational and basic scientists, the research that has led to these findings was performed over a relatively short period of approximately six years (Figures 3 and 4). Finally, as the prognostic and predictive value of somatic *POLE* exonuclease domain mutations seems generalizable to other cancer types, these mutations may provide an important step towards implementation of personalized medicine not only in endometrial cancer, but in a wide variety of malignancies.

**Journal articles on *POLE* EDMs and cancer**



**Figure 3.** Journal articles on *POLE* exonuclease domain mutations and cancer by year. The graph represents the number of new publications per year on *POLE* exonuclease domain mutations (EDMs) and cancer as referenced by PubMed (through October 2017). Source: PubMed, a division of the US National Library of Medicine and the National Institute of Health (available from: <http://www.ncbi.nlm.nih.gov/pubmed/>).



**Figure 4.** Timeline of important findings and events regarding POLE exonuclease domain mutations in cancer.

POLE exonuclease domain mutations are referred to as 'POLE mutations' in the timeline. Abbreviations: EC, endometrial cancer; CRC, colorectal cancer; GBM, glioblastoma multiforme; TCGA, The Cancer Genome Atlas; LUMC, Leiden University Medical Center; RCT, randomized controlled trial.

## REFERENCES

1. The Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature* 2013;497(7447):67-73.
2. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012;487(7407):330-337.
3. Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, et al. Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. *Mod Pathol* 2015;28(6):836-844.
4. Church DN, Stelloo E, Nout RA, Valtcheva N, Depreeuw J, ter Haar N, et al. Prognostic significance of POLE proofreading mutations in endometrial cancer. *J Natl Cancer Inst* 2015;107(1):402.
5. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer* 2015;113(2):299-310.
6. McConechy MK, Talhouk A, Leung S, Chiu D, Yang W, Senz J, et al. Endometrial Carcinomas with POLE Exonuclease Domain Mutations Have a Favorable Prognosis. *Clin Cancer Res* 2016;22(12):2865-2873.
7. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. *Cancer* 2017;123(5):802-813.
8. Meng B, Hoang LN, McIntyre JB, Duggan MA, Nelson GS, Lee CH, et al. POLE exonuclease domain mutation predicts long progression-free survival in grade 3 endometrioid carcinoma of the endometrium. *Gynecol Oncol* 2014;134(1):15-19.
9. Domingo E, Freeman-Mills L, Rayner E, Glaire M, Briggs S, Vermeulen L, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. *Lancet Gastroenterol Hepatol* 2016;1(3):207-216.
10. Erson-Omay EZ, Caglayan AO, Schultz N, Weinhold N, Omay SB, Ozduman K, et al. Somatic POLE mutations cause an ultramutated giant cell high-grade glioma subtype with better prognosis. *Neuro Oncol* 2015;17(10):1356-1364.
11. Church DN, Briggs SE, Palles C, Domingo E, Kearsey SJ, Grimes JM, et al. DNA polymerase epsilon and delta exonuclease domain mutations in endometrial cancer. *Hum Mol Genet* 2013;22(14):2820-2828.
12. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res* 2014;24(5):743-750.
13. Castle JC, Kreiter S, Diekmann J, Lower M, van de Roemer N, de Graaf J, et al. Exploiting the mutanome for tumor vaccination. *Cancer Res* 2012;72(5):1081-1091.
14. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature* 2012;482(7385):400-404.
15. Robbins PF, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med* 2013;19(6):747-752.
16. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 2014;515(7528):577-581.
17. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature* 2014;515(7528):572-576.

18. Karasaki T, Nagayama K, Kuwano H, Nitadori JI, Sato M, Anraku M, et al. Prediction and prioritization of neoantigens: integration of RNA sequencing data with whole-exome sequencing. *Cancer Sci* 2017;108(2):170-177.
19. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015;348(6230):69-74.
20. van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J Clin Oncol* 2013;31(32):e439-442.
21. Rajasagi M, Shukla SA, Fritsch EF, Keskin DB, DeLuca D, Carmona E, et al. Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. *Blood* 2014;124(3):453-462.
22. Bellone S, Centritto F, Black J, Schwab C, English D, Cocco E, et al. Polymerase epsilon (POLE) ultra-mutated tumors induce robust tumor-specific CD4+ T cell responses in endometrial cancer patients. *Gynecol Oncol* 2015;138(1):11-17.
23. Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, et al. Association of Polymerase e-Mutated and Microsatellite-Unstable Endometrial Cancers With Neoantigen Load, Number of Tumor-Infiltrating Lymphocytes, and Expression of PD-1 and PD-L1. *JAMA Oncol* 2015;1(9):1319-1323.
24. Gooden MJ, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer* 2011;105(1):93-103.
25. Kondratiev S, Sabo E, Yakirevich E, Lavie O, Resnick MB. Intratumoral CD8+ T lymphocytes as a prognostic factor of survival in endometrial carcinoma. *Clin Cancer Res* 2004;10(13):4450-4456.
26. de Jong RA, Leffers N, Boezen HM, ten Hoor KA, van der Zee AG, Hollema H, et al. Presence of tumor-infiltrating lymphocytes is an independent prognostic factor in type I and II endometrial cancer. *Gynecol Oncol* 2009;114(1):105-110.
27. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348(6230):124-128.
28. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-Line Nivolumab in Stage IV or Recurrent Non-Small-Cell Lung Cancer. *N Engl J Med* 2017;376(25):2415-2426.
29. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015;372(26):2509-2520.
30. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017;357(6349):409-413.
31. Santin AD, Bellone S, Buza N, Choi J, Schwartz PE, Schlessinger J, et al. Regression of Chemotherapy-Resistant Polymerase epsilon (POLE) Ultra-Mutated and MSH6 Hyper-Mutated Endometrial Tumors with Nivolumab. *Clin Cancer Res* 2016;22(23):5682-5687.
32. Mehnert JM, Panda A, Zhong H, Hirshfield K, Damare S, Lane K, et al. Immune activation and response to pembrolizumab in POLE-mutant endometrial cancer. *J Clin Invest* 2016;126(6):2334-2340.
33. Ott PA, Bang YJ, Berton-Rigaud D, Elez E, Pishvaian MJ, Rugo HS, et al. Safety and Antitumor Activity of Pembrolizumab in Advanced Programmed Death Ligand 1-Positive Endometrial Cancer: Results From the KEYNOTE-028 Study. *J Clin Oncol* 2017;35(22):2535-2541.
34. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature* 2013;500(7463):415-421.

35. Zlobec I, Minoo P, Terracciano L, Baker K, Lugli A. Characterization of the immunological micro-environment of tumour buds and its impact on prognosis in mismatch repair-proficient and -deficient colorectal cancers. *Histopathology* 2011;59(3):482-495.
36. Guidoboni M, Gafa R, Viel A, Doglioni C, Russo A, Santini A, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. *Am J Pathol* 2001;159(1):297-304.
37. Billingsley CC, Cohn DE, Mutch DG, Hade EM, Goodfellow PJ. Prognostic Significance of POLE Exonuclease Domain Mutations in High-Grade Endometrioid Endometrial Cancer on Survival and Recurrence: A Subanalysis. *Int J Gynecol Cancer* 2016;26(5):933-938.
38. Bellone S, Bignotti E, Lonardi S, Ferrari F, Centritto F, Masserdotti A, et al. Polymerase epsilon (POLE) ultra-mutation in uterine tumors correlates with T lymphocyte infiltration and increased resistance to platinum-based chemotherapy in vitro. *Gynecol Oncol* 2017;144(1):146-152.
39. Brix N, Tiefenthaller A, Anders H, Belka C, Lauber K. Abscopal, immunological effects of radiotherapy: Narrowing the gap between clinical and preclinical experiences. *Immunol Rev* 2017;280(1):249-279.
40. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(5):646-674.
41. Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res* 1991;51(12):3075-3079.
42. Fox EJ, Loeb LA. Lethal mutagenesis: targeting the mutator phenotype in cancer. *Semin Cancer Biol* 2010;20(5):353-359.
43. McFarland CD, Mirny LA, Korolev KS. Tug-of-war between driver and passenger mutations in cancer and other adaptive processes. *Proc Natl Acad Sci USA* 2014;111(42):15138-15143.
44. Shlien A, Campbell BB, de Borja R, Alexandrov LB, Merico D, Wedge D, et al. Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. *Nat Genet* 2015;47(3):257-262.
45. Paget S. The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* 1989;8(2):98-101.
46. Williams LN, Herr AJ, Preston BD. Emergence of DNA polymerase epsilon antimutators that escape error-induced extinction in yeast. *Genetics* 2013;193(3):751-770.
47. Campbell BB, Light N, Fabrizio D, Zatzman M, Fuligni F, de Borja R, et al. Comprehensive Analysis of Hypermutation in Human Cancer. *Cell* 2017;171(5):1042-1056.
48. Tait SW, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat Rev Mol Cell Biol* 2010;11(9):621-632.
49. Creutzberg CL, Nout RA, Lybeert ML, Warlam-Rodenhuis CC, Jobsen JJ, Mens JW, et al. Fifteen-year radiotherapy outcomes of the randomized PORTEC-1 trial for endometrial carcinoma. *Int J Radiat Oncol Biol Phys* 2011;81(4):e631-638.
50. Nout RA, Smit VT, Putter H, Jurgenliemk-Schulz IM, Jobsen JJ, Lutgens LC, et al. Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial. *Lancet* 2010;375(9717):816-823.
51. Stelloo E, Nout RA, Osse EM, Jurgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts. *Clin Cancer Res* 2016;22(16):4215-4224.
52. Nederlands Trial Register [Internet]. Dutch Cochrane Center: Dutch prospective trial register. PORTEC-4A, een onderzoek naar de waarde van een individueel moleculaire risicoprofiel om de nabehandeling na operatie voor vroeg stadium baarmoederkanker te bepalen (NTR5841).

- Accessed May 2017. Available from: <http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=5841>.
53. Bosse T, Nout RA, McAlpine JN, McConechy MK, Britton H, Gonzalez C, et al. Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups. *Am J Surg Pathol* 2018.
  54. Bouffet E, Larouche V, Campbell BB, Merico D, de Borja R, Aronson M, et al. Immune Checkpoint Inhibition for Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic Mismatch Repair Deficiency. *J Clin Oncol* 2016;34(19):2206-2211.
  55. ClinicalTrials.gov [Internet]. U.S. National Library of Medicine: clinical studies registry and results database. Avelumab with hypofractionated radiation therapy in adults with isocitrate dehydrogenase mutant glioblastoma (NCT02968940). Accessed August 2017. Available from: <https://clinicaltrials.gov/ct2/show/NCT02968940>.
  56. ClinicalTrials.gov [Internet]. U.S. National Library of Medicine: clinical studies registry and results database. Avelumab for MSI-H or POLE Mutated Metastatic Colorectal Cancer (NCT03150706). Accessed August 2017. Available from: <https://clinicaltrials.gov/ct2/show/NCT03150706>.
  57. ClinicalTrials.gov [Internet]. U.S. National Library of Medicine: clinical studies registry and results database. Study of Avelumab in Patients With MSS, MSI-H and POLE-mutated Recurrent or Persistent Endometrial Cancer (NCT02912572). Accessed August 2017. Available from: <https://clinicaltrials.gov/ct2/show/record/NCT02912572>.
  58. ClinicalTrials.gov [Internet]. U.S. National Library of Medicine: clinical studies registry and results database. TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer (TAPUR; NCT02693535). Accessed August 2017. Available from: <https://clinicaltrials.gov/ct2/show/NCT02693535>.
  59. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. *Lancet Oncol* 2015;16(8):908-918.
  60. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* 2015;372(26):2521-2532.
  61. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. *JAMA* 2016;315(15):1600-1609.
  62. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Czoszi T, Fulop A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* 2016;375(19):1823-1833.
  63. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015;372(21):2018-2028.
  64. Nederlandse Zorgautoriteit. Monitor Uitgaven geneesmiddelen in de medisch-specialistische zorg. Eerste kwartaal 2017.
  65. Schippers EI. Vergoeding geneesmiddelen pertuzumab en pembrolizumab. Kenmerk: 1142602-164656-GMT [Kamerbrief], 09-06-2017.
  66. Schippers EI. Beeindiging 'sluis' nivolumab per 1 maart 2016. Kenmerk: 905753-146268-GMT [Kamerbrief], 28-01-2016.
  67. Anderson JP, Daifuku R, Loeb LA. Viral error catastrophe by mutagenic nucleosides. *Annu Rev Microbiol* 2004;58:183-205.
  68. Jordheim LP, Durantel D, Zoulim F, Dumontet C. Advances in the development of nucleoside and

- nucleotide analogues for cancer and viral diseases. *Nat Rev Drug Discov* 2013;12(6):447-464.
69. Tsuda M, Terada K, Ooka M, Kobayashi K, Sasanuma H, Fujisawa R, et al. The dominant role of proofreading exonuclease activity of replicative polymerase epsilon in cellular tolerance to cytarabine (Ara-C). *Oncotarget* 2017;8(20):33457-33474.
  70. Rodriguez G. Fludarabine phosphate. A new anticancer drug with significant activity in patients with chronic lymphocytic leukemia and in patients with lymphoma. *Invest New Drugs* 1994;12(2):75-92.
  71. Ajani JA, Abbruzzese JL, Faintuch JS, Blackburn R, Levin B, Boman BM. Phase II study of fludarabine phosphate in patients with advanced colorectal carcinoma. *Invest New Drugs* 1988;6(1):47-50.
  72. Mittelman A, Ashikari R, Ahmed T, Savona S, Arnold P, Arlin Z. Phase II trial of fludarabine phosphate (F-Ara-AMP) in patients with advanced breast cancer. *Cancer Chemother Pharmacol* 1988;22(1):63-64.
  73. Weiss GB, Metc B, Von Hoff DD, Taylor SA, Sainers JH. Phase II trial of fludarabine phosphate in patients with head and neck cancer: a Southwest Oncology Group Study. *Cancer Treat Rep* 1987;71(12):1313-1314.
  74. Rainey JM, Hill JB, Crowley J. Evaluation of fludarabine phosphate in small cell carcinoma. A Southwest Oncology Group Study. *Invest New Drugs* 1988;6(1):45-46.
  75. Dhani NC, Emmenegger U, Adams L, Jongstra J, Tannock IF, Sridhar SS, et al. Phase II study of cytarabine in men with docetaxel-refractory, castration-resistant prostate cancer with evaluation of TMPRSS2-ERG and SPINK1 as serum biomarkers. *BJU Int* 2012;110(6):840-845.
  76. Shinbrot E, Henninger EE, Weinhold N, Covington KR, Goksenin AY, Schultz N, et al. Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. *Genome Res* 2014;24(11):1740-1750.
  77. Kane DP, Shcherbakova PV. A common cancer-associated DNA polymerase epsilon mutation causes an exceptionally strong mutator phenotype, indicating fidelity defects distinct from loss of proofreading. *Cancer Res* 2014;74(7):1895-1901.
  78. Barbari SR, Shcherbakova PV. Replicative DNA polymerase defects in human cancers: Consequences, mechanisms, and implications for therapy. *DNA Repair (Amst)* 2017;56:16-25.
  79. Williams LN, Marjavaara L, Knowels GM, Schultz EM, Fox EJ, Chabes A, et al. dNTP pool levels modulate mutator phenotypes of error-prone DNA polymerase epsilon variants. *Proc Natl Acad Sci USA* 2015;112(19):E2457-2466.
  80. Hussein YR, Weigelt B, Levine DA, Schoolmeester JK, Dao LN, Balzer BL, et al. Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. *Mod Pathol* 2015;28(4):505-514.
  81. Bakhsh S, Kinloch M, Hoang LN, Soslow RA, Kobel M, Lee CH, et al. Histopathological features of endometrial carcinomas associated with POLE mutations: implications for decisions about adjuvant therapy. *Histopathology* 2016;68(6):916-924.

