

Cancer predisposition syndromes: lessons for truly precision medicine

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ABSTRACT

Cancer predisposition syndromes are typically uncommon, monogenic, high-penetrance disorders. Despite their rarity, they have proven to be highly clinically relevant in directing cancer prevention strategies. As such, they share notable similarities with an expanding class of low-frequency somatic mutations that are associated with a striking prognostic or predictive effect in the tumours in which they occur. In this review, we highlight these commonalities, with particular reference to mutations in the proofreading domain of replicative DNA polymerases. These molecular phenotypes may occur as either germline or somatic events, and in the latter case have been shown to confer a favourable prognosis and potential increased benefit from immune checkpoint inhibition. We note that incorporation of these variants into clinical management algorithms will help refine patient management, and that this will be further improved by the inclusion of other germline variants, such as those that determine the likelihood of benefit or toxicity from anti-neoplastic therapy. Finally, we propose that such integrated patient and tumour profiling will be essential if we are to deliver truly precision medicine for cancer patients, but in a similar way to rare germline mutations, we must ensure that we identify and utilise rare somatic mutations with strong predictive and prognostic effects.

KEYWORDS: Biomarkers, Precision Medicine, Oncology

INTRODUCTION

Cancer diagnosis and treatment have advanced hugely during the last few decades. However, for most common solid tumours, surgical and medical management still tend towards a one-size-fits-all approach within broad strata defined by clinicopathological, and in a few cases, molecular risk factors. The development and clinical application of technologies such as next generation sequencing (NGS) provide an opportunity to move beyond this paradigm towards a more personalised model of cancer care. In particular, NGS has vastly increased the capacity for both discovery and clinical detection of two classes of genetic variants central to the delivery of precision cancer medicine: the first are germline variants causative of cancer predisposition syndromes; the second are somatic driver mutations in tumours. Incorporating both types of variants into clinical management algorithms is no small task, but it will be essential to transforming the “one size fits all” approach into patient-tailored, genomics-driven, cancer therapeutics.

In this review, we note that, in many ways, cancer predisposition syndromes exemplify the concept of “precision” cancer medicine. Identifying patients who possess the underlying germline genetic variants, a form of “biomarker”, allows for the prescription of specific preventative interventions and counselling about long-term cancer risk. Furthermore, in the event that these patients do develop cancer, our understanding of the genetic mechanisms of oncogenesis permits tailoring of treatment regimens in several cases, such as the use of PARP inhibitors in carriers of *BRCA1/2* mutations [1]. Building on these observations, we highlight the similarities between the management of patients with such germline variants and those whose tumours harbour rare somatic

variants with large effects, with reference to our recent studies of mutations in the proofreading domain of replicative DNA polymerases. We note that treatment can be further refined by the incorporation of uncommon germline variants that substantially alter the chances of benefit or risk of toxicity from systemic anti-cancer therapy. Finally, we discuss the how these factors can be integrated to deliver truly precision medicine, with particular reference to colorectal cancer (CRC), but also drawing on examples from other malignancies where relevant.

MANAGEMENT OF PATIENTS WITH HIGH-PENETRANCE GERMLINE PREDISPOSITION VARIANTS FOR COLORECTAL CANCER

About 15 Mendelian syndromes exist in which inherited mutations confer a high lifetime risk of CRC, typically 50-90% without intervention (Table 1) [2]. The first to be discovered, familial adenomatous polyposis (FAP) accounts for less than 1% of all colorectal cancers, but yet is perhaps one of the best understood genetic diseases. It is an autosomal dominant disease caused by germline loss-of-function *APC* mutations, that are also found as somatic mutations in ~90% of sporadic CRC. *APC* is a prototypical tumour suppressor gene. To date there are more than 300 different *APC* mutations associated with FAP, and all are associated with multiple adenomatous polyps of the large bowel and a variety of extra-colonic features [3]. Of note for precision medicine, the position of the germline mutation within the *APC* gene is associated with different severities of disease [4, 5]. Those with mutations near codon 1309 tend to develop thousands of polyps, and to prevent CRC they may require pan-proctocolectomy and construction of an ileal pouch. Patients with mutations in most

other parts of the *APC* gene usually develop hundreds of adenomas and often they are managed by an ileo-rectal anastomosis and surveillance of the remaining rectum. In a few cases, germline mutations at the extreme ends of the *APC* gene and in exon 9 cause an attenuated form of the disease, with tens of polyps that can often be managed using regular colonoscopy alone. A further variant of FAP has recently been recognised; known as gastric adenomatous and proximal polyposis of the stomach (GAPPS) syndrome, it is the result of point mutations in the *APC* promoter 1B. GAPPS sufferers develop polyposis of the gastric body and fundus but not of the colon and there is no apparent associated increased risk of CRC [6, 7]. Although these genotype-phenotype correlations are not perfect and unknown factors also affect the severity of colonic polyposis in FAP, they can be used as a means of choosing first-line cancer preventive treatment. The underlying cause for these correlations is probably that many truncated APC proteins are stable and retain some function, affecting the extent to which they activate Wnt pathway signalling and hence promote tumorigenesis.

Lynch syndrome (LS) is the commonest Mendelian predisposition to CRC, accounting for around 3% of all cases. LS is caused by inherited defects in DNA mismatch repair – a cellular process, responsible for correcting base-base mismatches and insertion-deletion loops following DNA replication [8]. LS is inherited in an autosomal dominant fashion, and sufferers are at greatly increased risk of CRC and endometrial cancer (EC), and at a lesser increased risk of several other cancer types [9]. Colorectal polyp numbers are not greatly increased in LS, and most patients are managed by regular colonoscopy, although both prophylactic surgery and chemoprevention are also offered. LS is caused by germline mutations in one of four genes encoding mismatch

repair proteins: *MSH2* [10], *MLH1* [11, 12], *PMS2* [13] and *MSH6* [14]. Additionally, Lynch syndrome can be caused by inherited mutations at the 3' end of the *EPCAM* gene, which leads to hypermethylation and epigenetic silencing of *MSH2* [15].

Genotype-phenotype correlations, including reduced penetrance of *MSH6* and *PMS2* mutations, have been reported, but few are strong enough to use in clinical practice. Loss of MMR function following a “second hit” causes a hypermutated state, with a 10–100-fold increased mutation rate in cancers, and a particular increase of small insertion and deletion mutations at DNA microsatellites, a phenomenon known as microsatellite instability (MSI) [16, 17]. MMR deficiency also occurs in sporadic tumours (~15% of CRC and ECs) most commonly because of somatic transcriptional silencing of *MLH1* (Figure 1) [18, 19]. Recent reports have also highlighted the existence of a small proportion (~4%) of MSI tumours that have been coined as “double somatic”, a term that refers to cancers that harbour biallelic somatic mutations in MMR genes in patients who lack germline MMR gene mutations [20]. The MMR status of CRC is important, because it is the most rigorously validated molecular prognostic biomarker currently available to clinicians. Both a meta-analysis and several large clinical trials have demonstrated that MMR deficiency is associated with a significantly better prognosis in stage II disease, with a risk of recurrence roughly half that of MMR-proficient cancers [21-23]. Furthermore, exciting recent data demonstrate that MMR-deficient (MMR-D) cancers respond particularly well to immune checkpoint inhibition therapy [24]. Thus, identification of Lynch Syndrome patients may allow for the implementation of a focused preventative strategy, and in the eventuality of malignancy developing, a specific treatment strategy.

Unlike *APC* and *MLH1*, the genes mutated in most of the Mendelian CRC syndromes play no clear role in sporadic CRC. One exception is *SMAD4*, which is mutated in the germline of patients with juvenile polyposis and in ~10% of sporadic CRCs [25] and another is *POLE*, which encodes the major subunit of the leading strand DNA polymerase Pol ϵ . The equivalent subunit of the lagging strand polymerase Pol δ is encoded by *POLD1*. Both of these proteins contain a polymerase domain, which extends the primer strand by addition of bases opposite the template strand, and an exonuclease domain which functions to proofread the newly synthesised DNA as replication proceeds [26, 27]. On the (uncommon) occasion that an incorrect base is incorporated by the polymerase, a conformational alteration results in pausing of the replication complex, and excision of the mismatch through a reaction catalysed by the highly conserved active site residues that lie within the exonuclease domain Exo motifs. Following this, the correct base is inserted, and DNA replication may continue. Polymerase proofreading is essential for accurate DNA replication, and amino acid substitutions within the exonuclease domain active site residues increase the mutation rate approximately 100-fold (Figure 1) [28]. Germline missense mutations in the exonuclease domains of *POLE* and *POLD1* disrupt polymerase proofreading and predispose to polyposis, early-onset CRC and EC [29]. The syndrome was named polymerase proofreading-associated polyposis, or PPAP [30].

POLE proofreading domain mutations are also somatic events in sporadic cancers, occurring in around 3% of CRCs [18] and 10% ECs [19, 31]. The *POLE*-mutant cancers are termed 'ultramutated', as they harbour up to 1,000,000 detectable base substitutions, a burden which far exceeds that of MMR-D cancers [18, 19, 26, 32]. The most common somatic *POLE* mutation in sporadic tumours is a p.Pro286Arg

substitution flanking the Exo I motif, and other recurrent mutations include p.Val411Leu, p.Ser297Phe and p.Ser459Phe alterations [26]. These findings have now been confirmed in multiple different colorectal and endometrial cancer cohorts [18, 19, 31-36], and pathogenic *POLE* exonuclease domain mutations have also been reported in highly mutated tumours of the brain [37], stomach [38], breast and pancreas (CBioportal, accessed 3rd October 2016). Strikingly, very few clearly pathogenic somatic *POLD1* exonuclease domain mutations have been detected, despite the fact that several hundred cases of most common tumour types have now been sequenced [26]. Consequently, it appears that if somatic *POLD1* mutations do occur in sporadic cancers, they do so with a prevalence that is less than 1% of cases. Although very intriguing, the reason for this discordance with the germline is unclear.

In addition to their exceptional mutational load, tumours with pathogenic *POLE* exonuclease domain mutations also display a distinctive mutation spectrum, with a pattern of base substitutions and a striking relative increase in C>A transversions in the context of TCT trinucleotides [31, 32, 39]. In addition, the specific driver mutations found in *POLE*-mutant tumours differ somewhat from the majority of sporadic CRCs or ECs. The over-represented changes in CRCs include *APC* p.Arg1114*, *TP53* p.Arg213* and *KRAS* p.Ala146Thr. Similar phenomena occur in *POLE*-mutant ECs and these cancers also tend to acquire inactivating *APC* changes rather than oncogenic *CTNNB1* mutations as a means of activating the Wnt pathway. This combination of features means that these *POLE* mutant tumours are often identifiable using next generation sequencing panels in clinical use in academic centres [40]. This distinct tumour mutational signature may also help to identify patients who carry germline *POLE* mutations.

Endometrial cancers with somatic *POLE* mutations have a favourable prognosis, with a risk of relapse between one third and one tenth of that of other ECs, depending on risk stratum [35, 36, 41-43]. We have recently shown that CRCs with pathogenic *POLE* exonuclease domain mutations have a similarly good prognosis, which appears superior to that of MMR-D CRCs [44]. In both tumour types, this may relate to a strong immune response against these cancers, as evidenced by immunohistochemical analysis for the T cell markers CD8 and CD3, and increased expression of cytotoxic T cell genes in RNA from non-microdissected whole tumours [44-46]. Perhaps unsurprisingly, in view of their ultramutation, bioinformatic analysis has confirmed that *POLE*-mutant tumours are predicted to harbour a substantially greater number of neo-epitopes (that is mutations capable of eliciting an immune response) than other tumours [45-47], a feature that may account for their apparently enhanced immunogenicity. Both increased neo-epitope burden and a pre-treatment lymphocytic infiltrate appear to predict benefit from immune checkpoint inhibition [48, 49], and consistent with this very recent studies have reported complete response of *POLE*-mutant endometrial cancers to these agents [45, 50]. The efficacy of immune checkpoint inhibitors against *POLE*-mutant tumours along with hypermutated MMR-D cancers will be investigated by several prospective clinical trials currently recruiting or in set-up.

An unresolved issue is whether the management of cancers in Mendelian syndromes should follow that of sporadic cancers where the same gene is involved. Clearly, this is problematic, because the rarity of the Mendelian syndromes means that standard methods for answering this question such as randomised controlled trials are

infeasible. In the absence of such data, it is tempting to use similar therapeutic strategies for MMR-D cancer in the sporadic and LS settings, and also to treat sporadic and PPAP *POLE* ultramutated cancers in the same way. In both cases, however, there are clear differences between sporadic and inherited disease. For example, sporadic MMR-D CRCs tend to occur in older individuals and women, and are almost all in the proximal colon, whereas LS CRCs can present from the late teens onwards, are more prevalent in men and are often in the distal colon or rectum. Given that recent studies have suggested substantial variation in the impact of MMR deficiency depending on tumour location (proximal vs. distal colon) [51], it will be important to define the impact of these differences on tumour biology and management. Such efforts will require large-scale collaboration between multiple investigators.

Although challenging, the management of rare sub-groups, such as patients with Mendelian cancer syndromes or rare (but well-defined) sub-groups of sporadic cancers (e.g. *POLE*-mutant CRCs) will be central to the promised era of precision medicine.

THE UNCERTAIN FUTURE OF PRECISION CANCER MEDICINE

Somatic *POLE* mutations occur in a modest fraction of endometrial cancers, a smaller proportion of colorectal cancers, and at a very low frequency in all other tumours in which they have been found; pathogenic germline *POLE* and *POLD1* mutations are probably present in <1:1000 people. Yet, as discussed in the preceding section the apparent effect of *POLE* on tumour prognosis and, potentially, on therapeutic response is striking. We contend that, in the light of these characteristics, *POLE*-mutant tumours

highlight the challenges posed in delivering personalised or precision medicine in the genomic era, because true precision requires the identification of clinically important patient sub-sets that comprise at most a few per cent of the total.

For most of the common cancers, the number of tumours sequenced to date is such that essentially all mutations that occur in more than 20% of cases have already been discovered. In fact, many of these mutations, such as *TP53*, *APC* and *KRAS* were already known well before the use of NGS became widespread in the latter part of the last decade. As the number of cancers analysed continues to grow, so the mutation prevalence of any novel driver genes discovered will tend to decrease in parallel, reflecting the greater power to detect these variants provided by the larger number of cases. Indeed, an elegant study published in 2014 demonstrated that the number of modest frequency (5-10% cases) drivers was increasing linearly and that of low frequency (2-5% cases) drivers was increasing in a log-linear fashion [52]. This profusion of mutations with possible prognostic and predictive utility raises challenging questions for the research community. For example, consider a candidate biomarker present in 1% of cases, which confers risk of recurrence one third that of biomarker negative cases, for which the risk of recurrence is 20%. In the absence of confounding from other clinical or pathological factors, confirming this effect with conventional levels of type I and II error (two-sided α of 0.05 and $1-\beta$ of 0.8) requires a total of 3,226 cases. Clearly this is a major undertaking that requires analysis of a greater number of cases than that available in all but the largest of clinical trials. In our recent study of *POLE* mutations in colorectal cancer, we analysed three large clinical trial sample sets and several additional large sample biobanks, comprising more than 4,500 CRC cases in all, to confirm the prognostic effect [44].

Determining that low-frequency biomarkers have a predictive effect poses additional, particular challenges. Screening large numbers of people to detect a small fraction of biomarker-positive cases is costly and inefficient, and means that many patients face disappointment when they are unable to participate in the study. Though logistically demanding, perhaps the best solution to this problem will be to embed treatment of low-frequency biomarker groups within the context of a larger precision medicine trial, where patients are allocated to a particular therapy tailored to their tumour molecular profile [53]. This, of course, could reasonably be allocation to no additional treatment after surgery in the case of markers of favourable prognosis.

While NGS promises the potential for significant advances in cancer medicine, at least in part through the identification of novel biomarkers, we suggest a note of caution. There is a danger that the careless application of these technologies will not only fail to benefit patients with sporadic cancers, but may even cause harm as a consequence of abandoning the principles of evidence-based practice. There are no simple solutions to this problem. As we note above, adherence to a frequentist statistical approach means that, for rare somatic biomarkers, large sample sizes are required to detect all but substantial effect sizes. Bayesian approaches offer some advantages in this regard, but are often criticised for the subjectivity of assigning prior probabilities, based on assumptions that may be quite mistaken. For example, the impressive responses of melanomas to specific inhibitors of the mutant BRAF kinase might have suggested that these agents would demonstrate similar efficacy in other tumour types with this mutation. In fact, the effect of these drugs against *BRAF*-mutant CRCs was essentially negligible, due to compensatory upregulation of EGFR signalling. We contend that the

use of such priors should not be completely abandoned, but rather used with caution when translating results between different cancer types.

The question remains as to whether we will ever be able to provide currently accepted levels of proof for some well-defined patient sub-groups. For example, even for LS, let alone PPAP, a phase 3 clinical trial of immune checkpoint inhibitors is currently unthinkable even though LS is claimed to account for ~3% of CRCs. Whether patient registries, international collaborations and alternative statistical methods, for example propensity score matching, can deal with these issues requires careful evaluation. If not, there is a very real risk that what is termed “precision medicine” will in fact be a return to the bad old days of patient care based on small case reports – a model subject to multiple confounders, biases and well recognised as being fundamentally unsound. The Exceptional Responders Initiative coordinated by the National Cancer Institute is approaching this issue in a reverse manner by attempting to define the molecular characteristics of small patient subsets that exhibit remarkable responses to cancer therapies. It remains to be seen whether the results of this study, which is focusing on groups of “outliers”, can be generalised to a wider patient population [54, 55].

INTERSECTION BETWEEN GERMLINE AND SOMATIC VARIANTS IN PRECISION MEDICINE

The previous sections have highlighted the clinical relevance of low frequency genetic variants in both the germline and soma in clinical practice. We propose that, to work towards truly precision medicine, clinical management algorithms must incorporate

both classes of alteration. In the case of germline variants, these are not limited to those that predispose to cancer, but also may include those that influence response to treatment or therapeutic toxicity. Examples of such germline variants that may predict toxicity are described in Table 3 [56-59].

For example, a woman presents with a stage III colorectal cancer that displays a prominent lymphocytic infiltrate, but is found to retain expression of mismatch repair protein expression on IHC. Molecular testing confirms that her tumour harbours a somatic *POLE* p.Pro286Arg mutation. Although the prognosis for these tumours appears somewhat better than that of MMR-P cancers of similar stage that lack *POLE* mutations, current evidence is insufficient to recommend omission of adjuvant chemotherapy. The patient is considered for a clinical trial of chemotherapy with an immune checkpoint inhibitor, but declines this and instead opts for standard post-operative treatment with oxaliplatin and the oral fluoropyrimidine capecitabine. However, genetic testing reveals that she carries a rare loss-of-function *DPYD**2A allele, meaning that standard dose capecitabine treatment would probably cause substantial toxicity [60]. In keeping with recent reports and emerging consensus [61], she is therefore treated with capecitabine at 50% dose, at which it is well tolerated.

While this illustrative case is taken from the field of colorectal cancer, there are similar examples in many other tumour types, where both germline and somatic mutation testing have the potential to improve patient care from the point of diagnosis through to individualised treatment (Figure 2). Frustratingly however, although in many cases the evidence supporting such testing is clear and unequivocal, clinical practice has lagged

behind, as illustrated by the patchy implementation of MMR testing for colorectal cancers in UK practice.

The 100,000 Genomes Project, launched in the UK in 2012, is a government funded project that aims to sequence 100,000 genomes from patients with cancer (or rare genetic diseases), recruited from the within the National Health Service (NHS). In addition to providing a wealth of data, this project also represents an important step in fully integrating genomic research with routine clinical practice [62, 63].

CONCLUSIONS

In the last 5 years, NGS has hugely increased our understanding of cancer genomes. As we have highlighted in this review, the application of NGS has also helped to identify an increasing number of rare somatic variants of potential value as biomarkers. Translating these discoveries into clinical practice poses a considerable challenge, but will be central to the implementation of precision cancer medicine. We contend that consideration of the clinical management of germline cancer predisposition variants provides some clues as to how this may be achieved. The same principles of detailed consideration of the pathogenicity of mutations, careful documentation of any prognostic or predictive effect of the variant (through a randomised controlled clinical trial where appropriate), and the rapid dissemination of this through evidence-based consensus guidelines all apply to the study of rare somatic variants. Other parallels can be drawn from phenotypes associated with germline variants that display lower penetrance, such as prostate cancer *BRCA2* mutation carriers. The challenge faced in confirming this association is similar to that we face when considering the many biomarkers with a more modest effect. Understanding the effect of these both alone and in combination will require large-scale collaborative working, meticulous data collection and rigorous statistical analysis.

During the last two decades, advances in the diagnosis and clinical management of patients with rare, high-penetrance, germline tumour-predisposition variants has substantially reduced morbidity and mortality from cancer [64, 65]. Applying similar principles to the rapidly expanding number of rare somatic mutations in common

cancers is essential if we are to realise the benefits that NGS offers, and move towards the goal of truly precision medicine for cancer patients.

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Table 1. Germline cancer predisposition variants

Syndrome	Gene responsible (allelic status)	Molecular mechanisms	Clinical features	Ref
Familial adenomatous polyposis	<i>APC</i> mutation (monoallelic)	Activation of the Wnt signalling pathway through the accumulation of B-catenin; development of chromosomal instability and consequential aneuploidy.	Numerous adenomatous colorectal polyps (>100), with an almost 100% lifetime risk of developing CRC. Also associated with upper GI polyps, desmoid tumours and other extra colonic malignancies.	[3]
Lynch Syndrome	Mutations in MMR genes: <i>MSH2</i> (most commonly), <i>MLH1</i> , <i>MSH6</i> , <i>PMS2</i> and <i>EPCAM</i> (monoallelic).	Loss of mismatch repair machinery, which is responsible for post replicative DNA surveillance, results in a hypermutated state known as microsatellite instability	CRC development at earlier age than population; predominance of right sided CRC tumours (>70% proximal to splenic flexure). Increased risk of extra colonic malignancies: endometrial, small intestine and urothelial malignancies.	[66]
Constitutional mismatch repair deficiency (CMMRD) syndrome	Mutations in <i>MSH2</i> , <i>MLH1</i> , <i>MSH6</i> and <i>PMS2</i> (biallelic)	Loss of mismatch repair machinery.	Early onset colorectal cancer development, in addition to haematological and CNS malignancies.	[67]
MUTYH associated polyposis (MAP)	<i>MUTYH</i> mutation (biallelic)	Loss of base excision repair	"Attenuated FAP phenotype": fewer polyps and later age of onset than FAP. Patients also develop polyps of stomach and duodenum; gastric and thyroid cancers.	[68]
Juvenile polyposis syndrome (JPS)	<i>BMPR1A</i> or <i>SMAD4</i> mutation (monoallelic)	Loss of <i>BMPR1A</i> or <i>SMAD4</i> regulation of TGF- β signalling, and consequent neoplastic transformation.	Associated with hamartomatous polyps of the GI tract, in particular stomach, small intestine, colon and rectum. Incidence of CRC is 17-22% by age 35 and 68% by 60.	[69]
Petz-Jeghers syndrome (PJS)	<i>STK11</i> (<i>LKB1</i>) mutation (monoallelic)	<i>STK11</i> controls cellular proliferation through G1 cell cycle arrest and p53 mediated apoptosis	Mucocutaneous pigmented lesions in 95% of patients and hamartomatous GI tract polyps (60-90% small bowel and 50-64% colon). Associated with a CRC risk ratio of 84, and a mean age of development of 46.	[70]
Polymerase Proofreading Associated Polyposis (PPAP)	<i>POLE</i> or <i>POLD1</i> mutations (monoallelic)	Loss of DNA polymerase proofreading function leads to genomic instability and an ultramutated state	<i>POLE</i> and <i>POLD1</i> variants predispose to colorectal adenoma and carcinoma development; <i>POLD1</i> also increases risk of endometrial cancer	[29]
Hereditary Mixed Polyposis Syndrome	<i>GREM1</i> (monoallelic)	Increased <i>GREM1</i> causes loss of BMP signalling	Sufferers develop polyps with mixed and varied morphology; apparent absence of extra colonic features.	[71]

NTHL1 syndrome	<i>NTHL1 (biallelic)</i>	<i>NTHL1</i> encodes for a glycosylase responsible for targeting a wide variety of DNA lesions as part of the BER pathway; tumours exhibit a mutational signature of increased C:G > T:A transitions.	Sufferers exhibit adenomatous polyposis, and develop colorectal cancer and endometrial cancer.	[72, 73]
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Table 2. Selected uncommon somatic variants of prognostic or predictive significance

Somatic variant	Clinical significance	Cancer types (frequency)	Ref
<i>POLE</i>	Associated with a HR of 0.34 for disease recurrence in CRC vs MMR-proficient tumours; HR of 0.43 in endometrial	Endometrial (6-15%) Colorectal (1-3%)	[35, 44]
<i>CDX2</i>	Loss of CDX2 expression in CRC associated with increased rate of disease recurrence (HR 3.44)	Colorectal cancer (7%)	[74]
<i>HER2</i>	Targeted therapy with HER2 blockade (trastuzumab and lapatinib)	Colorectal cancer (5%)	[75]
<i>ALK</i>	<i>ALK</i> rearrangements identify patients with advanced non-small cell lung cancer that may benefit from Crizotinib therapy	Non-small cell lung cancer (3-5%)	[76]

Table 3. Non-predisposition germline variants of clinical utility

Germline variant	Population Frequency	Clinical Utility	Ref
Dihydropyrimidine dehydrogenase (DPYD) polymorphisms	1-2%	Deficiency in dihydropyrimidine dehydrogenase associated with polymorphism DPYD*2A can result in toxicity with fluorouracil treatment. Testing for the polymorphism can help with appropriate dosage adjustment.	[58, 77]
Cytochrome P450 polymorphisms	<i>CYP2C8</i> *3 > 5% frequency in Caucasians	Testing for <i>CYP2C8</i> *3 may identify patients are at an increased risk of paclitaxel-induced neuropathy.	[78]
<i>FGD4</i> polymorphisms	Unknown	<i>FGD4</i> , rs10771973 is a marker of early onset paclitaxel induced sensory neuropathy in breast cancer patients. Additional variants, <i>EPHA5</i> and <i>FZD3</i> , may also act as markers for the onset and severity of peripheral neuropathy	[79]
<i>TPMT</i> polymorphisms	~0.3% with undetectable <i>TPMT</i> activity, and 11% with low activity.	Testing for <i>TPMT</i> polymorphisms prior to treatment with 6-mecaptopurine or 6-thioguanine to allow for appropriate dose adjustment	[80, 81]

FIGURE LEGENDS

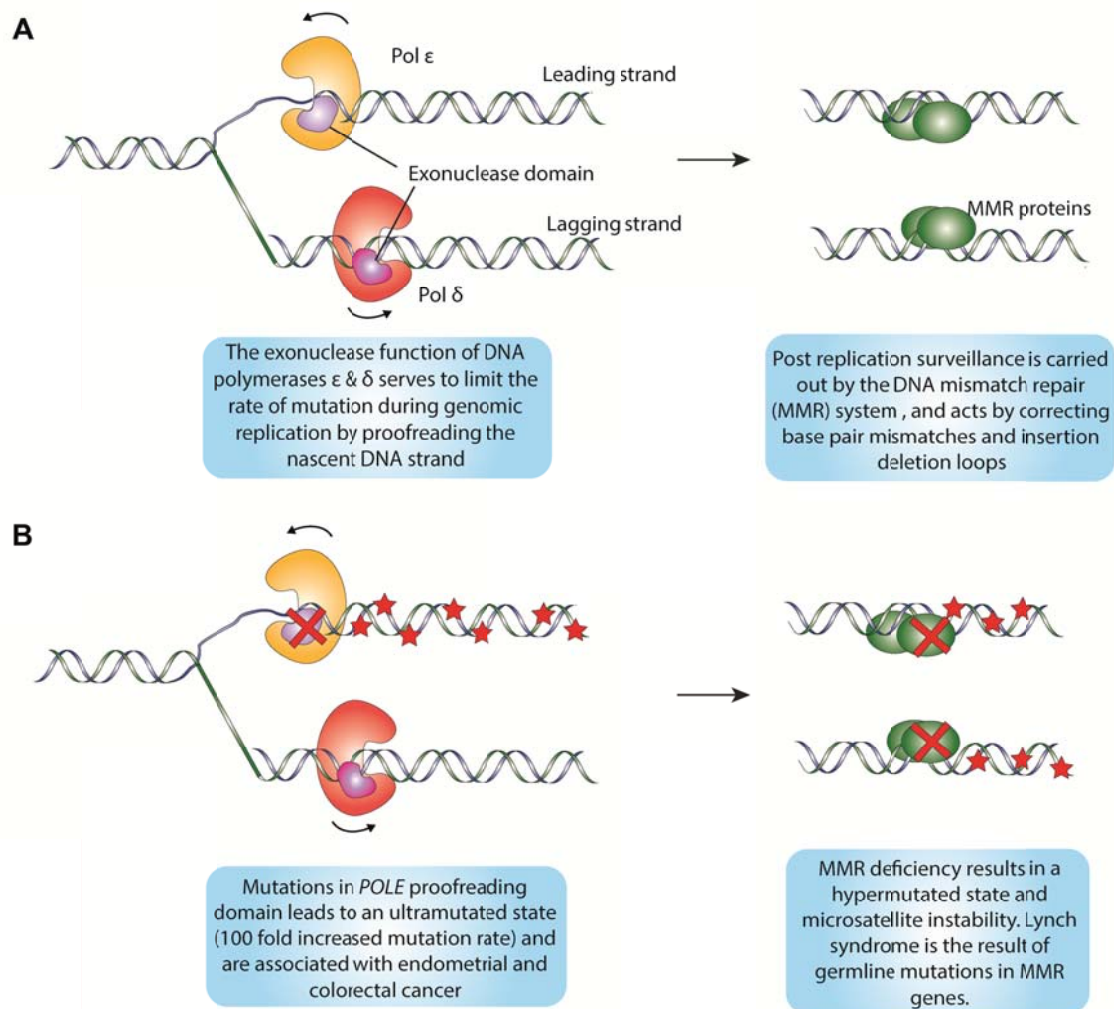


Figure 1: (A) DNA replication is one potential source of genomic instability. Under normal conditions, this is limited in human cells by two principle mechanisms. The first is the exonuclease domain of the two DNA replicative polymerases that serve to proofread the nascent DNA strand. The second is the DNA mismatch repair (MMR) system, which conducts post-replication surveillance and serves to correct errors introduced during DNA replication. (B) Loss of function in either of these systems, resulting from either germline or somatic mutations, greatly increases the rate of DNA mutation and can contribute to cancer development

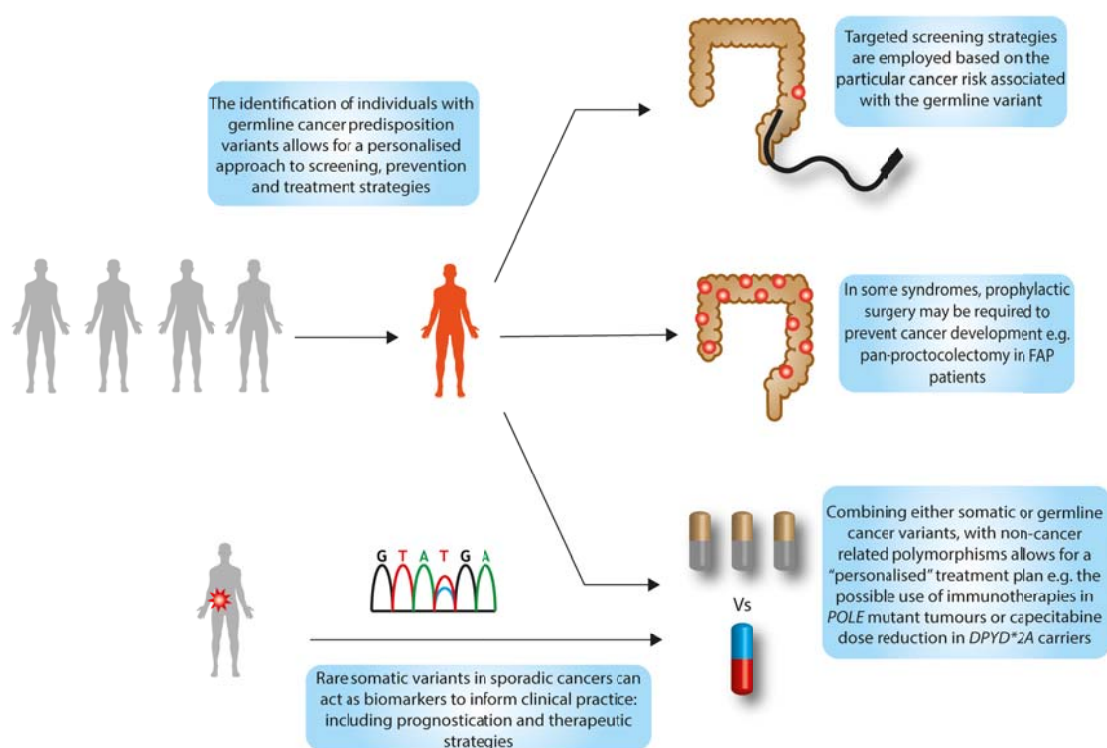


Figure 2: This diagram illustrates how the identification of somatic and germline variants can be used to inform clinical and assist in the delivery of “personalised” cancer medicine.