Therapeutic implications of germline genetic findings in cancer

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Abstract

Cancer is a genetic disease. To date, translational cancer genomics has focused largely on somatic alterations, driven by the desire to identify targets for personalized therapy. However, therapeutically relevant information is also latent within the germline genome. In addition to cancer susceptibility, alterations present in the germline can determine responses to both targeted and more traditional anticancer therapies, as well as their toxicities. Despite this, many algorithms designed to analyze somatic mutation conversely continue to 'subtract' information on germline genetics during analysis. In the light of low actionable yields from somatic tumour testing, a need exists for diversification of the sources of potential therapeutic biomarkers. In this Review, we summarize the literature on the therapeutic potential of alterations in the germline genome. The therapeutic value of germline information will not only be manifest as improvements in treatment, but will also drive greater levels of engagement and co-operation between traditional oncology services and familial risk management clinics.

[H1] Introduction

Several decades have passed since the first gene mutations conferring an increased risk of cancer were identified (1). *RB1* was the first cancer susceptibility gene to be cloned in 1986 (2). Traditionally, the clinical identification of hereditary syndromes led to the identification of the implicated gene, often with distinct molecular and histological tumour characteristics (1). The pattern of clinical recognition of a syndrome followed by genetic verification has been the norm in familial cancer clinics, until recently. From twin studies, the hereditary component of cancer varies according to type, and ranges from minimal to more than 40% in the case of prostate cancer (3). In general, less than 10% of the heritable fraction of cancer can attributed to currently recognized, monogenic and dominantly inherited syndromes (4).

As the use of next-generation sequencing in patients with cancer increases, an appreciation of the true burden of germline variations across cancers continues to grow (5-8). In a 2017 study involving 1,040 adults, of whom the majority had advanced-stage cancer, sequencing of tumour and nontumour DNA demonstrated that 18% of patients had germline variants conferring cancer susceptibility. More than half of these variants were not indicated in any clinical testing guidelines, and more than a quarter led to discussions regarding, or the initiation of a change in therapy (9). In another large cohort, one in six patients with advanced-stage cancer were found to carry pathogenic germline variants (6). Similarly, in a cohort of paediatric patients (mean age of 7.4 years, one in ten children had a pathogenic germline variant, most, at least one germline variant of uncertain significance (98%) and a variant with known pharmacogenetic consequences (89%) (7). Another pan-cancer analysis of a cohort of paediatric patients (95% were <18 years of age at diagnosis) found that 6% of all patients with childhood cancers carry a causative germline variant, with 3% of all 'potentially druggable genomic events' – herein referred to as therapeutically actionable, having a germline origin (10). The recognition of the germline mutation burden is increasing and becoming clinically relevant, with a surprisingly high level of clinical and phenotypic diversity. The presence of a germline variant was not concordant with family history, age at diagnosis, or tumour type in more than half of all germline mutation carriers in several of the studies described previously (6, 8, 9). This increase in our awareness of the germline mutation burden is the result of increased sequencing of tumour and nontumour samples in patient populations that were previously largely unexplored, and owing to expanded opportunities for the use of targeted therapy on the basis of these germline variants. Thus, the more widespread application of genomic technologies is enabling the identification of a previously unappreciated and frequently subclinical burden of pathogenic germline variations with potential clinical utility. Furthermore, emerging evidence suggests that, regardless of cancer type, patients respond similarly to therapies selected based on the presence of these targetable germline alterations (11). However, caution is warranted in extrapolating, biomarkers that are predictive in one context to another, although emerging data suggest that many patients with advanced-stage cancers that are not typically associated with an expected germline mutation might nonetheless benefit from therapies selected based on the presence of that mutation (12).

The emerging interest in the role of germline alterations in cancer creates both challenges and opportunities. An increased understanding of pathogenic germline variants not only has implications for risk management; the presence of such mutations is increasingly likely to inform therapeutic strategies. These considerations are important because the actionable yield from the somatic component of tumour sequencing panels has, thus far, been disappointing (13, 14). For example, in an interim analysis of data from the NCI–MATCH study, investigators reported a 9% actionability by molecular tumour profiling – 56 of the 645 tumours tested harboured a genomic alteration that made patients suitable for an affiliated study treatment (14). In the SHIVA study, 40% of tumours were found to have an actionable target alteration (including alterations in hormone, mTOR, or MAP kinase signalling pathways) with 25% of patients actually randomized to receive either the relevant targeted therapy or chemotherapy of the physician's choice (13). Additionally, the clinical actionability of somatic, but not germline, mutations is affected by both intratumour and intertumour heterogeneity. Germline genetic variants are by definition clonal (shared by all tumour cells in a host), whereas somatic mutations can be clonal or subclonal (only present in a subpopulation of tumour cells) (15). An agent targeting a clonal alteration has the potential to affect a larger proportion of tumour cells than an agent targeting a subclonal alteration. However, the emergence of secondary mutations, or revertant mutations (those causing reversion back to the wild-type phenotype) under the selective pressures of a targeted therapy, suggests that tumours are able to lose their addiction to germline, as they are to somatic, mutations.

Various therapeutic considerations are likely to affect the thresholds of clinical actionability of germline variants. Such thresholds have hitherto been defined primarily on the basis of implications for modifiable risk through early detection or prevention (16). The threshold for actionability of any biomarker is fundamentally driven by the perceived potential benefit to patients and is offset by the potential risks. The risk:benefit ratio of any intervention is, therefore, dependent on the clinical context. For example, the benefit of reporting the presence of a germline susceptibility variant to individuals who have no detectable cancer might be offset by the potential adverse effects or harms in an otherwise healthy state. These harms include the psychological burden of an increased lifetime risk of cancer, which must be offset by the potential benefits of early detection. By contrast, the frequently urgent need for treatment of patients with advanced-stage cancer shifts the risk:benefit ratio substantially. In patients with progressing cancer who lack, or have already failed standard therapies with proven efficacy, receiving a biomarker-based experimental option, whether based on a germline or somatic alteration, might offer a greater level of benefit than receiving a non-biomarker-based therapy in a phase 1 study (17). This therapeutic consideration improves the benefit side of the risk:benefit equation, thus lowering the threshold for clinical actionability in this setting. Notably, clinical awareness of germline therapeutic alterations as biomarkers is often substantially lower than is the case for somatic alterations, especially in settings in which genetics are less well integrated into multidisciplinary care. In this Review, we summarize the established and potential therapeutic implications of information on germline genetics and provide an overview of a range of pathways for which targeted therapies exist, or are emerging.

Quantifying therapeutic actionability

The findings of a study published in 2018 revealed the incidence of germline variations in The Cancer Genome Atlas (TCGA; FIGURE 1) (18). Investigators reported the presence of pathogenic or likely pathogenic germline variants in 8% of patients (853 of 10, 389) and possibly pathogenic variants in an additional 5.2% (540 of 10, 389). Briefly, the method of pathogenic variant discovery involved an automated variant classification pipeline called CharGen (Characterisation of Germline Variants) (19), according to guidelines provided by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) for the specific classification of rare variants in cancer (20). Each variant was evaluated using available data from 12 pathogenic evidence levels and 4 benign evidence tags, culminating in a composite score. Established pathogenic variants, according to ClinVar and other curated databases, were classified as pathogenic, those scoring >8 as being likely pathogenic and those scoring >4 as possibly pathogenic (18).

On the basis of a comprehensive review of the literature, we identified germline genes with therapeutic potential, and classified their actionability using the available evidence. An issue is that the standard of the available evidence is variable, and evolving rapidly. In the genomic era, many cancers can be classified as rare on the basis of the molecular subtype. For example, non-small cell lung cancer can now be subdivided into smaller molecular subgroups with *KRAS, EGFR*, and *MET* mutations, to name only a few (21). Therapeutic outcomes from small groups of patients are often difficult — and sometimes impossible — to evaluate using the traditional randomized controlled trial design. Complementing data from randomized controlled trials, novel single-arm study designs have been developed to enable treatment selection based on the presence of specific biomarkers (22-25). Such designs must incorporate both somatic and germline genetic information that is relevant to therapeutic decision making, while at the same time ideally also addressing issues of costs, efficiency, statistical power and common endpoints across a diverse range of pathological entities (26). One advantage of these newer designs is that they facilitate trials for the many patients with rare cancer types and syndromes (27, 28), and frequently constitute the only available evidence to evaluate the utility of these novel therapeutics.

An important question remains regarding the level of evidence required to define clinical actionability. Pragmatically, the thresholds for tiers of actionability are tied to the regulatory approval decisions made by agencies such as the FDA. The FDA has increasingly accepted evidence obtained from single-arm trials as sufficient to enable drug approvals, thus recognizing the emergence of biomarker-based therapies (29). The FDA recently acknowledged that data from single-arm studies are acceptable under a defined set of circumstances, including the rarity of the condition under question (and therefore the feasibility of conducting a randomized controlled trial), the degree of unmet need, and the magnitude of the observed benefit (29). Hence, we define the tiers of therapeutic actionability as follows: Tier 1, sufficient evidence of clinical benefit

to gain regulatory approval; Tier 2, similar quality and level of evidence as for Tier 1, but without regulatory approval to date; and Tier 3, any evidence of clinical benefit for selecting a treatment based on the presence of the specified germline variant (TABLE 1). Accordingly, we consider 5.2% (544 of 10,389) of the pathogenic and likely pathogenic (P/LP) and 2.8% (288 of 10,389) of the possibly pathogenic (PP) germline variants to be therapeutically actionable by these criteria (FIGURE 1). Even for Tier 1 genes, we determined only a 57% level of concordance between specific germline alterations and the expected cancer type, thus underlining the unreliability of selecting genes for testing on the basis of tumour histology.

[H3] Therapeutic implications of germline BRCA1 and BRCA2 variants

Germline loss-of-function mutations in *BRCA1* and/or *BRCA2* confer susceptibility to breast and ovarian cancer and have clear therapeutic implications (30). Actionable P/LP germline variant forms of *BRCA1/2* were identified in 1.7% (172 of 10,389) of all cancers included in TCGA, 35% of which were in discordant cancer types (histologies other than breast, ovarian, pancreas or prostate cancer) (31). Germline *BRCA1/2* mutations lead to defective DNA repair by homologous recombination. Thus, DNA damage induced by platinum-containing agents cannot be repaired owing to this defect, resulting in genomic instability and cell death. Knowledge of this mechanism led to the evaluation of platinum-based chemotherapy in patients with *BRCA1/2* mutation-associated breast cancer. Most tumours in patients with germline *BRCA1* mutations have loss of heterozygosity (32), although this effect is less common in those exposed to cisplatin (32), suggesting that cells retaining *BRCA1* function are selected for, owing to the ability to repair platinum-induced adducts (33). In one study, a pathological complete response was observed in an impressive 61% of patients receiving neoadjuvant cisplatin, all of whom had germline *BRCA1* mutations and early stage breast cancer (34). Elsewhere, cisplatin induced 9 complete clinical responses, and 7 partial responses after 6 cycles of treatment in a cohort of 20 women with advanced-stage breast cancer who had a germline *BRCA1* mutation (35). Similarly, the TNT trial compared the efficacy of carboplatin with that of docetaxel in the advanced-stage setting. No differences in response rates were observed in comparisons of the whole groups, although women with germline *BRCA1/2* mutations had a 68% response rate to carboplatin compared with 35% to docetaxel (95% CI 6.3–63.1%; $P = 0.03$) (36, 37). Pathogenic germline *BRCA2* variants in particular are associated with a wider spectrum of cancers, therefore, these findings might also apply outside of patients with breast cancer (38, 39). In a trial involving women with advanced-stage ovarian cancer, those with germline *BRCA1/2* mutations had significantly better clinical (34% versus 4%) and pathological (46% versus 25%) response rates, compared to those with wildtype forms of *BRCA1/2* (32). In a retrospective analysis designed to determine platinum sensitivity in men with castration-resistant, predominantly taxane-refractory prostate cancer treated with carboplatin and docetaxel ($n = 141$), a small subset of men with germline *BRCA2* mutations ($n = 8$) had improved response rates (75% versus 17% respectively) (40).

Synthetic lethality, a mechanism for tumour-selective effects that exploits the dependence on residual functional pathways of cells with DNA repair defects, forms the basis for the use of poly [ADP-ribose] polymerase (PARP) inhibitors in *BRCA1/2* deficient cancers. Inhibition of PARP causes DNA strand breakage as well as the formation of cytotoxic PARP–DNA complexes, which are lethal to BRCA-deficient cells (30, 41). In germline *BRCA1/2* mutation-associated ovarian cancer, these agents have become the standard of care (42-45), with both olaparib and rucaparib approved by the FDA for use in the treatment-refractory setting. Niraparib and olaparib have also proven effective as maintenance treatments in patients with platinum-sensitive recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancers, irrespective of *BRCA* mutation status (46, 47). Olaparib maintenance therapy yielded a 2.8-month improvement in median progression-free survival (PFS) and reduced the risk of disease progression or death relative to chemotherapy (HR 0.58, 95% CI 0.43–0.80; P <0.001) in women with metastatic breast cancer with a germline *BRCA1/2* mutation (48). In the same setting, another randomized trial revealed an improved objective response rate (63% versus 27%) and improvement in PFS (8.6 versus 5.6 months; HR for disease progression or death 0.54; 95% CI 0.41–0.71; P <0.001) for patients receiving talazoparib compared with the physician's choice of standard chemotherapy (49). In men with advanced-stage prostate cancer, 16 of 49 (33%) patients receiving olaparib had an objective response (50). A homologous recombination defect (in *BRCA1/2, ATM*, Fanconi anaemia genes, *CHEK2*) was identified in 16 patients, of whom 14 (88%) had an objective response. This included all 7 patients with *BRCA2* loss, 3 germline and 4 of the 5 patients with *ATM* mutations, 2 germline (50, 51). Routine use of PARP inhibitors in the treatment of prostate cancer awaits the results of ongoing trials (52, 53). Impressively, the germline mutational burden of non-*BRCA* homologous recombination-associated genes comprises an additional 2.2% (227 of 10,389) of tumours included in TCGA (TABLE 1). These include *ATM, BRIP1, PALB2* and the Fanconi anaemia genes, for which loss-of-function mutations provide variable evidence of actionability (50, 54-56). Germline variants in nucleotide excision repair (NER) genes (*ERCC1, XPA, XPD, XPG*) also appear to confer cisplatin sensitivity across a range of tumour types (57).

[H3] Therapeutic implications of germline PTCH1 and SMO variants.

Basal cell carcinoma nevus syndrome (BCCNS) is most frequently caused by a germline deletion of one copy of patched 1 (*PTCH1*), leading to activation of the hedgehog signalling pathway (58). BCCNS is associated with an increased risk of multiple basal cell carcinomas (BCCs) with a potential for progression to advanced stage disease, as well as medulloblastoma (59). Mutations in *PTCH1* lead to activation of smoothened (*SMO*), and uninhibited nuclear localization of glioma-associated factor (GLI) and its downstream effectors. Two drugs have been tested in this setting. Firstly, vismodegib, a first-in-class oral selective SMO inhibitor, was found to reduce both the incidence of new BCCs and the size of existing BCCs in patients with BCCNS (60). In an open-label trial, treatment with vismodegib induced a 67% response rate in patients with locally advanced disease, and a 38% response rate in the metastatic setting (61). Secondly, a modified form of itraconazole, an antifungal with anti-hedgehog properties (62, 63), is currently being evaluated for efficacy in

patients with BCCNS (64). In an interim analysis, investigators reported a reduction in tumour burden in 8/13 participants, and that this agent was well tolerated (65). Germline suppressor of fused homolog (*SUFU*) variants are implicated in ~5% of all patients with Gorlin syndrome (also known as nevoid basal cell carcinoma syndrome) and confer a substantially increased risk of medulloblastoma (66). Inhibition of *SMO* is associated specifically with responses in patients with the 'sonic hedgehog' (SHH) molecular subtype of medulloblastoma owing to the presence of germline *PTCH1* mutations upstream of *SMO* (67-69) while those located downstream, such as *SUFU*, do not (69). In the TCGA cohort, a total of five pathogenic germline *PTCH1* variants were reported, all in different cancer histologies (70). Whether or not patients with non-BCCNS tumours will respond to inhibitors of the hedgehog pathway currently remains uncertain.

[H3] *Therapeutic implications of germline defects in mismatch repair genes*

Immunotherapies have dramatically improved the outcomes of a subset of patients with certain types of cancers, although a consistent predictive biomarker is currently lacking. Lynch syndrome and other related microsatellite instability syndromes result from germline defects in DNA mismatch repair (MMR) genes (*MSH2, MLH1, PMS2, MSH6, POLE*) (71) and are characterized by an increased risk of colorectal, endometrial, and other cancers (72). Pathogenic germline variants in MMR genes comprised 6% (52 of 853) of the germline mutational burden in TCGA, with only 31% in colorectal and endometrial cancers (31). Mismatch repair deficient (dMMR) colorectal cancers (CRCs) express many key immunomodulatory proteins (antigen chaperone molecules, pro-inflammatory cytokines and cytotoxic mediators), suggesting that MMR deficiency might sensitize tumours to immune-checkpoint inhibition (ICI) (73-75). In a pivotal study, the efficacy of pembrolizumab was evaluated in three separate patient cohorts — those with dMMR CRCs (n = 11, of which 9 had Lynch syndrome), those with MMR proficient (pMMR) CRCs (n = 21) and a group of non-CRC, dMMR tumours (n = 9, of which 4 had Lynch syndrome). Both the dMMR–CRC and non–CRC cohorts had dramatically improved response rates (40% and 71%, respectively) and median PFS (not reached and 5.4 months) compared to the group with pMMR tumours (response rate 0% and 2.2 months). MMR-deficient tumours had a much higher somatic mutation burden (mean of 1,782 per tumour) compared with pMMR tumours (mean of 73 per tumour), and this higher mutational load was associated with longer PFS durations (75). These benefits were observed regardless of whether the driver mutations were germline or somatic (76). On the basis of these data, the FDA approved pembrolizumab for the treatment of dMMR cancers, irrespective of tumour type — the first drug approval based entirely on genomics rather than cancer histology (29).

Biallelic MMR deficiency (bMMR) is a rare cancer predisposition syndrome resulting from homozygous germline mutations in MMR genes (77). Individuals with bMMR typically develop cancers before reaching adulthood, most commonly malignant gliomas, haematological malignancies and gastrointestinal cancers (78). bMMR tumours have a high tumour mutational burden (mean of 1,589 per tumour), which is unusual for

paediatric cancers, and suggests sensitivity to ICI (77). Two siblings with bMMR-associated glioblastomas were treated with nivolumab, with dramatic benefits described in a case report (77). Accordingly, bMMR is an opportunity for ICI in pediatric patients with cancer.

Germline mutations in the genes encoding DNA polymerases (*POLD1* and *POLE*) are most commonly associated with susceptibility to CRCs, as well as endometrial cancers (79). Pathogenic germline variant forms of these genes comprised 2% (16 of 853) of the germline mutational burden in TCGA, mostly in cancer types not typically associated with these variants (80). Mutations in these genes confer an exceptionally high tumour mutational burden (estimated to be approximately 4,500 mutations per primary tumour) (81). Case reports have described patients with other cancer histologies - a glioblastoma in the setting of a pathogenic germline *POLE* mutation and an endometrial cancer in the setting of a somatic *POLE* mutation demonstrating a high tumour mutational burden and a prompt, durable objective response to pembrolizumab (81, 82). With pathogenic germline *POLE* and *POLD1* variants seen in a diverse range of tumour histologies, it will be interesting to see whether these variants predict sensitivity to ICI.

[H3] Therapeutic implications of germline oncogene variants

Germline transmission of gain-of-function mutations in oncogenes is uncommon, presumably because constitutive activation of signalling pathways interferes with development. However, because many cancer drugs target kinases with oncogenic effects, identifying germline oncogenic variants is of clinical interest. Gastrointestinal stromal tumours (GISTs) are mesenchymal tumours affecting the gastrointestinal tract, 90% of which harbour a somatically acquired *KIT* mutation (83). *PDGFRA* mutations are present in 30–40% of *KIT*-negative GISTs and are mutually exclusive to *KIT* mutations (84). Imatinib and sunitinib are potent broad-spectrum tyrosine kinase inhibitors (TKIs) of *PDGFRA/B* and *KIT* that are widely used in treating patients with sporadic GISTs (85, 86). Familial GISTs most commonly arise from germline gain-of-function mutations in *KIT* (exon 11) (87), or infrequently, germline *PDGFRA* mutations (88, 89). Familial disease tends to be associated with the development of multiple GISTs and these are frequently unresectable, thus increasing the importance of systemic therapies in patients with these tumours. In a case report describing two related individuals with a germline *KIT* mutation in exon 11, treatment with imatinib resulted in a durable partial response in one patient (83). Interestingly, given the theoretically constitutive presence of the oncogenic signal, no adverse events were attributed to inhibition of *KIT* in noncancerous tissues, similar to the experience with crizotinib in two paediatric patients with neuroblastomas associated with a germline *ALK* variant (83, 90). How imatinib and sunitinib should be used in patients with familial GISTs associated with pathogenic germline variations in *KIT*, *PDGFRA, NF1, SDHB* or *SDHC* (91), which comprise an additional 0.25% of TCGA (26 of 10,389) remains unclear.

In addition to GISTs, germline variant forms of *SDHB* and *SDHC* confer an increased risk of malignant phaeochromocytomas and paragangliomas (92). Therefore, the presence of a germline *SDHB* variant has both

prognostic and predictive implications. Such variants are often associated with MGMT promoter methylation, which in turn is hypothesized to confer sensitivity to temozolomide (93, 94). A retrospective analysis of a cohort of patients with these cancers treated with temozolomide demonstrated a significantly longer PFS for those with a germline *SDHB* variant, (19.7 months compared to 2.9 months, HR 0.16, 95% CI 0.04–0.72; $P = 0.007$) (92). However, the retrospective nature of the available evidence limits our ability to differentiate between the prognostic and predictive value of such variants.

Germline loss-of-function variants in *NF1* are associated with an increased risk of a range of malignancies. The loss of neurofibromin 1 activity increases RAS activity and induces downstream activation of the MAP kinase and PI3K–mTOR signalling pathways in a distinct fashion to sporadic cases (95). A phase I study investigating the safety and tolerability of the MEK1/2 inhibitor selumetinib demonstrated a 71% partial response rate in children with unresectable plexiform neurofibromas (96). This led to the FDA approval of this agent as a designated orphan drug in this setting, for patients with neurofibromatosis type 1. Clinical trials designed to assess the efficacy of selumetinib in NF1-associated cancers are currently underway. Of particular interest are the central nervous system tumours, which often have limited levels of surgical accessibility and few systemic therapy options available (97).

Germline variations in *RET* are associated with multiple endocrine neoplasia (MEN) type 2, which includes several tumour syndromes associated with the endocrine glands. Hereditary medullary thyroid carcinoma (MTC) is associated with MEN type 2A and 2B; 95% of patients with MEN2B have a point mutation in exon 16 (codon 918) of *RET* (98). Pathogenic germline variants in *RET* comprise 2.5% of the germline mutational burden in TCGA, most of which were observed in discordant cancer types (98). Vandetanib, a small-molecule inhibitor of VEGFR2, EGFR and RET, induces responses in approximately half of all patients with either sporadic or hereditary MTCs (99, 100). Treatment with cabozantinib, compared with placebo, has been shown to result in a significant improvement in response rates (28% versus 0%) and median PFS (11.2 months versus 4.0 months, HR 0.28, 95% CI 0.19–0.40; $P < 0.001$) in patients with metastatic MTCs (101). *RET* mutations were present in half of all patients, with 6% originating in the germline. Both vandetanib and cabozantinib have received FDA approval for the treatment of advanced-stage MTCs, independent of *RET* mutation status (98, 102).

NSCLCs associated with somatic mutations in *EGFR* are usually sensitive to treatment with EGFR–TKIs, although presence of the T790M mutation usually confers resistance. A germline form of $EGFR^{T790M}$ has been identified in a family with multiple bronchoalveolar carcinomas. The presence of $EGFR^{T790M}$ was confirmed in two of five tumours (from two patients) available for analysis, while the other three tumours had additional somatic activating mutations in *EGFR* that typically confer sensitivity to EGFR inhibition (a missense L858R mutation in two tumours and a L747–T751del in the third). These tumours however, were resistant to gefitinib, suggesting the dominance of the germline T790M variant (103). Similarly, *ALK* translocations

predict responsiveness to crizotinib, a competitive inhibitor of ALK and MET (104). Activating variations in ALK are known to cause hereditary neuroblastomas (105). In a phase I dose-finding study designed to investigate the safety and activity of crizotinib, an objective response was observed in 14 of 79 paediatric patients (median 10.1 years of age) with refractory tumours of various types. Among the 14 responders, 13 harboured an *ALK* translocation, mutation or amplification. Two patients with germline *ALK*-associated neuroblastoma participated in this study, with one patient deriving clinical benefit through a notable improvement of symptoms and the other having a complete response (90). In a subsequent study, investigators analyzed the frequency of germline and somatic *ALK* variations in a cohort of 1,530 patients with neuroblastoma, of whom 126 had a variant form of *ALK* with 88 also having a matched nontumour DNA sample available. A total of seven germline *ALK* mutations were identified, with varying levels of in vitro sensitivity to crizotinib, including germline *ALK* variants that are predictive of inherent resistance (106).

Hereditary activating variants in *MET* are associated with papillary renal cell carcinomas (RCC) (107). Three patients in TCGA harboured germline *MET* variants, all of whom had concordant papillary RCCs (108). Foretinib is a multikinase inhibitor of *MET, VEGF, RON, AXL* and *TIE-2*. Five of 10 patients with germline *MET*-mutation-positive RCCs had an objective response to foretinib, compared with 5 of 57 patients who lacked detectable germline alterations (109). Whether germline *MET* variants are implicated in other tumour types that are known to harbour *MET* alterations, such as gastric cancer, CRC or NSCLC, and will also derive benefit from MET inhibition, is currently unclear (110-112).

[H3] Therapeutic implications of germline mutations in the PI3-kinase pathway

Germline variations in *PTEN* result in PTEN hamartoma tumour syndrome. *PTEN* acts through the PI3K–mTOR signalling pathway. In preclinical studies, rapamycin counteracts the activation of PI3K–mTOR signalling and antagonizes its downstream effectors (113). In a case report, a patient with a germline c.507delC *PTEN* variant receiving rapamycin appears to have had a disease response and improved symptoms (114). Sirolimus has also shown promising effects in patients with hamartoma syndromes such as tuberous sclerosis complex and Peutz–Jeghers syndrome, which is associated with inactivating variations in *TSC1* and/or *TSC2* and *STK11*, respectively (115, 116). Genes within this pathway comprise 3% (26 of 853) of the burden of germline alterations in TCGA.

[H3] VEGF inhibitors in clear cell RCCs associated with Von Hippel–Lindau syndrome

Germline variations in *VHL* result in Von Hippel–Lindau (VHL) syndrome, which is implicated in 80% of patients with clear cell RCC (117-119). The presence of this mutation results in excess VEGF production. With activation of the VEGF signalling pathway originally identified on the basis of this germline defect, activation of the corresponding somatic pathway provides the rationale for treatment with a range of VEGF-

inhibitors (including sunitinib, pazopanib and axitinib) that are effective in the treatment of advanced-stage RCC (120).

[H3] Other applications of genomics in patient management

The composition of a patient's germline genome clearly influences the tolerance, and efficacy of conventional therapies. Pharmacogenetic information can be used to explain or predict the emergence of toxicities in response to cytotoxic agents, including *DPYD* deficiency and 5-fluorouracil-related toxicities, *UGT1A1* and irinotecan-related toxicities, and *CYP2D6* testing for optimized tamoxifen dosing (121-125). Conditions such as ataxia telangiectasia and Fanconi anaemia are associated with increased early or late effects of ionizing radiation, along with germline variations in *ATM, TP53, RAD51, XRCC1, ERCC2* (126). Similarly, germline variants might predict intrinsic resistance to both targeted therapies (103, 127-129) and standard chemotherapy regimens (130, 131). Evidence is also emerging suggesting that sensitivity to ICI might vary according to HLA genotype (132).

Importantly, germline information is also relevant in the setting of curative treatment. The presence or absence of known pathogenic germline variants can influence surgical decisions, including the choice between breast-conserving surgery or mastectomy in women with *BRCA1/2* mutations (133-135) or the selection of bone marrow transplantation instead of systemic therapy in patients with a germline pathogenic variant of a Fanconi anaemia-related gene (136, 137). The immediate implications for definitive standard therapy will increasingly justify the earlier integration of genetics referrals and the entry of genetics into widespread clinical use, as exemplified by efforts to introduce routine *BRCA1/2* testing in breast and ovarian cancer clinics (138, 139).

Other applications of information on germline genetic variants include chemoprevention in those with an underlying cancer predisposition, as exemplified by the prophylactic use of aspirin in patients with Lynch syndrome (140, 141). Given the sensitivity of patients with defective MMR to ICI, we can envision the potential for the use of an immune-checkpoint inhibitor as chemoprevention in patients with Lynch syndrome. Evaluation of such trials will require measure of public health and economic impacts as well as clinical endpoints.

[H1] Conclusions

The massive expansion and application of genome sequencing has revealed a rich landscape of germline variants across a range of tumour histologies and much of this information is therapeutically actionable. This clinical relevance, combined with the clonal nature of germline alterations, makes them potentially useful predictive biomarkers. Among heritable cancer susceptibility genes, alterations in DNA repair genes influence the emergent somatic tumour profile, an observation that is highly relevant to treatment with both

immunotherapies and agents that are reliant on synthetic lethality. Interestingly, the development of most cancer drugs typically migrates from the advanced-stage to the adjuvant setting — and even into the preventative space. In the future, agents such as immunotherapies might have an important role in chemoprevention in individuals with suitable hereditary cancer predisposition syndromes, such as Lynch syndrome. Finally, a growing appreciation of the therapeutic implications of germline variations is likely to affect the level of demand for germline testing and its clinical interpretation, thus creating an interesting tension between whether or not a variant that might reach a threshold for therapy, but not risk management should be reported to the patient and/or their family. The time has come for clinicians to fully consider and exploit the therapeutic potential latent within the germline genome.

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S.T. researched data for the article, all authors made a substantial contribution to discussions of content, S.T. and D.M.T. wrote the manuscript and all authors edited and/or reviewed the manuscript prior to submission.

Competing interests
There are no competing interests.

Key points

- Expanded application of genomic sequencing has revealed a significant burden of germline variants across a range of tumour histologies
- The relevance of germline variation with regards to therapy selection is only now being realised
- The clonal nature of germline alterations makes them ideal predictive biomarkers
- A growing appreciation of the therapeutic relevance of germline variations is likely to increase the demand for germline testing and its clinical interpretation
- There is an added complexity to the clinical interpretation of germline variants-variants may reach a threshold for therapy, but not risk management

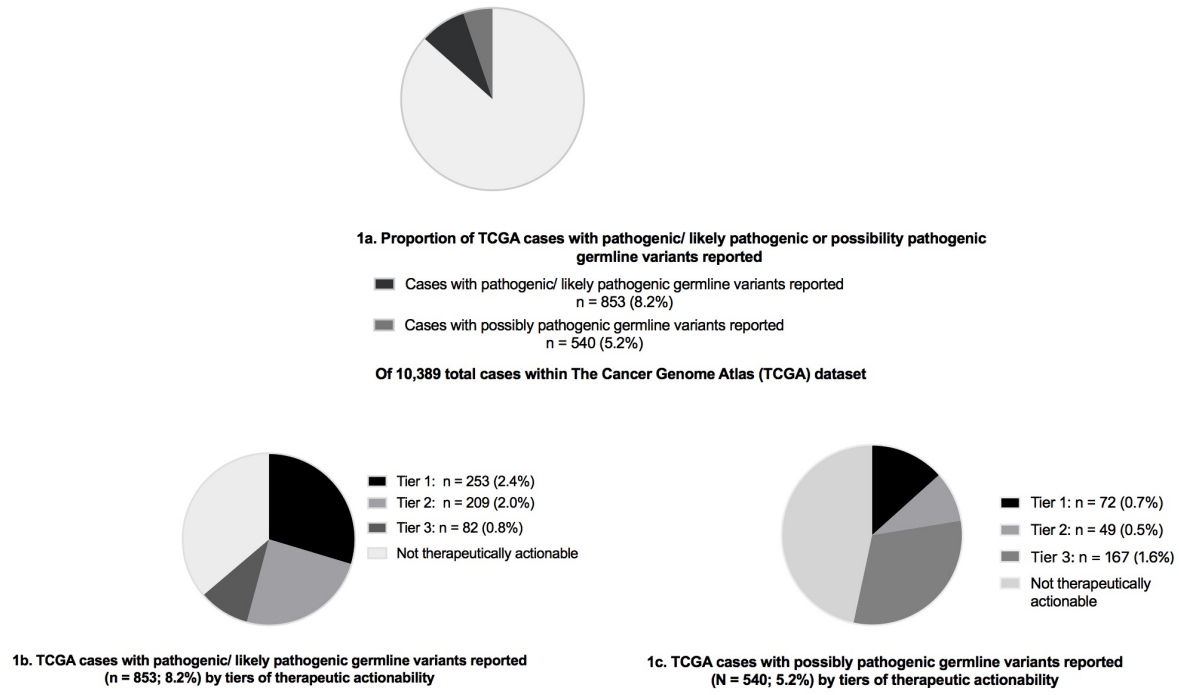

Figure 1. Results of the quantification analysis for germline variations with therapeutic actionability

Table 1. Summary of evidence for germline variations with therapeutic actionability

Family/ genes	**Evidence by Tiers of Actionability**			☐ Tier 1 – sufficient evidence of clinical benefit to gain regulatory approval ☐ Tier 2 – similar quality of evidence as Tier 1, but without regularly approval to date ☐ Tier 3 – any evidence of clinical benefit for selecting a treatment based on the specified germline variant		
1. HRR	**Study type**	**n**	**Cancer type**	**Genotype**	**Treatment**	**Response**
BRCA1 *BRCA2*	Clinical trial	376	Advanced BC	56 (15%) *BRCA1/2* carriers	carboplatin	ORR 68% in carriers vs. 33% in non-carriers (P=0.03) (36, 37)
	Retrospective cohort	190	OC	34 *BRCA1, 1 BRCA2* carrier	carboplatin	Significantly improved cCR in carriers, 12/35 (34%) vs non-carriers, 8/190 (4%) and histopathological responses, 16/35 (46%) vs 42/169 (25%) (32)
	Clinical trial	54	Advanced BC	54 *BRCA1/2* carriers	olaparib	ORR was 41% with 400mg BD dose and 22% with 100mg BD dose (n=20) (42)
	Clinical trial (Ph III)	431	Advanced BC	431 BRCA1/2 carriers	talazoparib	Median PFS and ORR were significantly improved with talazoparib compared with physician's choice standard therapy: 8.6 vs. 5.6 m and 63% vs. 27% respectively (49)
	Clinical trial (Ph II)	33	Advanced OC	33 *BRCA1/2* carriers	olaparib	ORR = 11/33 (33%) (43)
	Clinical trial (Ph II)	63	TNBC & OC, or primary peritoneal	17 *BRCA1/2*	olaparib	An OR was seen in 7/17 (41%) of carriers and in 11/46 (24%) of non-carriers (44)
	Clinical trial (Ph II)	29	Advanced OC, BC, pancreas, prostate	*29 BRCA1/2*	olaparib	RR= 26.2% overall; by site of origin: OC = 60/193 (31.1%); BC 8/62 (12.9%); pancreas = 5/23 (21.7%) and prostate = 4/8 (50.0%) (11)
ATM *BRIP1* *CHEK1* *CHEK2* *BARD1* *FAM175A* *PALB2* *RAD51C* *RAD51D* *NBN* *ATR*	Retrospective cohort	390	1 of 13 HR genes	24% germline and/or 9% somatic in ≥ 1 of these genes	platinum	Mutations (in *BRCA1/2 ATM BARD1 BRIP1 CHEK1/2 FAM175A MRE11A, PALB2, RAD51C/D, or NBN)* highly predictive of primary platinum sensitivity and improved OS = 66m (germline), 59m (somatic), 41m in non-carriers (54). In vitro studies suggest somatic *RAD51/54, RPA1, SNB1, ATR, ATM, CHEK1/2, FANCD2/CA/CC* mutations also confer sensitivity to PARP-inhibition (142).
	Clin trial (Ph II)	50	CRPC	Tumour/ normal HR analysis	olaparib	16 patients had an OR; 14 of 16 (88%) showed a HR defect – 7 *BRCA2* loss (4 biallelic somatic, 3 germline); 4 *ATM* variants (2 germline) (50)
2. FA *FANCA-M*	Clinical trial	61	Advanced cancers	Somatic FA defect	velaparib +/- MMC	OR in 6/61 pts (56)
3. NER *ERCC1* *XPA* *XPB* *XPD* *XPG*	Retrospective cohort	836	NSCLC	*ERCC1* expression	platinum	RR significantly improved in tumours with high ERCC1 expression compared to low ERCC1 (OR = 0.48). Germline XPA (A23G variant) in NSCLC, XPD (variant at codons 313 and 751) in esophageal cancer, XPD (codon 751) in OC, ERCC1 (codon 118) in CRC pts and XPG (46His/His variant) in NSCLC all showed cisplatin sensitivity (143) (57)
4. MMR *MLH1* *PMS2* *MSH6*	Clin trial (Ph II)	41	Advanced CRC	MMR dMMR 10 pMMR 18	pembro	For dMMR cancers, immune-related ORR= 40% (4 of 10) and immune-related PFS= 78% (7 of 9) vs. pMMR CRCs, immune-related ORR=0% (0 of 18) and immune-related PFS = 11% (2 of 18). HR for PFS = 0.10; HR for death = 0.22 (75)
	Expansion of above study	86	Advanced cancer pts, 12 tumour histologies	MMR deficient	pembro	ORR was 53%, 21% achieved a CR and responses were durable. Functional assays in responding pts showed expansion of neoantigen-specific T-cell clones that were reactive to mutant neopeptides within the tumour in vivo (76)
PMS1	Possible Lynch syndrome, but clinical significance remains uncertain. In a family with a germline PMS1 mutation, a germline MSH2 mutation was later demonstrated, questioning the predisposition conferred by the PMS1 alone (144, 145)					
POLD1	Retrospective cohort	34 0	NSCLC	High TMB, *POLE* and *POLD1*	pembro	Exome sequencing demonstrated that a high TMB significantly improved durable clinical benefit (83% vs 22%) and PFS (not yet reached vs. 3.4 m). *POLE* and *POLD1* variants were implicated in 2 of 3 responders with the highest TMB; including a never-smoker with a

						POLD1 E374K mutation (146)
POLE	Case report	1	EC	*POLE* (and high TMB)	pembro	Patient achieved a PR (81). *POLE* mutations occur in 10% of ECs and are associated with high TMB (even > than MSI-high tumours) and a larger repertoire of immune signature genes. Similarly, germline *POLD1* and *POLE* has been associated with an increased risk of colorectal adenomas/ cancers, ECs with high TMB (79)
	Case report	1	GBM	Germline *POLE*	pembro	Patient achieved an OR (82)
5. Hedge-hog *PTCH1*	Clinical trial	41	Advanced basal cell carcinoma	BCCNS	vismodegib	Pts with BCCNS were randomised to placebo (n=15) or vismodegib (n=26). The rate of new BCCs was lower with vismodegib than placebo: 2 vs. 29 (P<0.001) as was size (% change from baseline): -65% vs. -11% (P=0.003) (60)
	Clin trial (Ph IIb)	13	Advanced basal cell carcinoma	BCCNS	SUBA-intraconazole	An interim analysis after pts completed at least 16 weeks of treatment demonstrated a >30% reduction of tumour burden in 62% of pts (8 of 13) (64)
6.Onco-genes *RET*	Clin trial (Ph III)	331	Advanced MTC	33 hereditary MTC	vandetanib	Pts randomly assigned (2:1) to vandetanib or placebo. Statistically significant PFS, ORR, disease control rate, and biochemical response with vandetanib. A pt with a G691S *RET* mutation developed PD (99)
	Cli trial (Ph I/II)	16	Children adolescent	15 germline M918T *RET*	vandetanib	Amongst children (aged 5-12) and adolescents (aged 13-18), 47 % of demonstrated a PR (100)
	Clin trial (Phase III)	330	Advanced MTC	20 hereditary MTC	cabozantinib	There was an improved median PFS with cabozantinib: 11.2 vs. 4.0 m with placebo (HR 0.28). PFS was prolonged across all subgroups including age, prior TKI treatment, and *RET* mutation status (hereditary or sporadic) (101)
MET	Clin trial (Phase II)	67	Papillary RCC	10 germline *MET*	foretinib	In the 10 pts with germline mutation (5 had *MET H1094R*); 5 achieved a PR and the remaining 5 pts, SD. In contrast, responses were seen in 5 of 57 (9%) of those without a germline mutation (109)
KIT	Case report	2	GIST	germline *KIT*	imatinib	Treatment with half-dose imatinib in one of two affected resulted in at least a PR, with the pt remaining on treatment for ≥ 1 year (83)
PDGFRA	Case report	7	GIST	5 germline *PDGFRA*	imatinib	Germline variants (*Asp846)* were present in 5 of 7 members in a family affected by GISTs (88, 91)
EGFR	Case report (family)	5	NSCLC	Germline *EGFR T790M*	EGFR inhibitors	A germline *EGFR T790M* variant conferred intrinsic resistance despite 3 of 5 having somatic activating mutations (L858R, delL747-T751) which normally confers sensitivity. Highlights the germline's dual influence on cancer susceptibility and treatment resistance (103)
ALK	Ph I	36	Refractory cancers and neuroblastomas	*2 germline ALK*	crizotinib	Pts (aged 12 months to 22 years) with refractory cancers enriched for *ALK* variants (n=25) and neuroblastomas. 2 of the 11 neuroblastoma pts had germline variants: one achieved a CR after 3 cycles and the other derived clinical benefit with resolution of ^{123}I-MIBG avidity after 7 cycles (90)
	In vitro	88	Neuroblastoma	*7 germline ALK*	crizotinib	In vitro studies demonstrate that the exact activating germline variant confers differential sensitivity to crizotinib – R1275Q, R1060H, I1183T, L1204F, R1231Q and I1250T (106)
7. MTOR *TSC1* *TSC2*	Retrospective cohort	39	Various tumour types	mTOR pathway	everolimus	FFPE tumor/ normal pairs from pts treated with everolimus, 22 with exceptional clinical benefit, 17 without. Activating genomic variation in mTOR signaling (*MTOR, TSC1, TSC2, NF1, PIK3CA* and *PIK3CG*) were seen in 45% (10 of 22) of pts with clinical benefit (115)
STK11	In vivo		Cohort of mice	*Lkb1* $^{+/-}$	rapamycin	A large cohort of mice treated with rapamycin demonstrated a reduction in tumour burden (116)
NF1	Case report	1	GIST	*NF-1*	sunitinib	Unclear whether pathogenicity in *NF-1* associated GISTs are *KIT*-independent. A patient with a *NF1*-associated GIST demonstrated a PR to sunitinib (91, 147)
	Clinical trial Ph I		plexiform NFs	*NF-1*	selumetinib	71% of children with unresectable plexiform neurofibromas achieved a PR with associated clinical benefit (96)
PTEN	Case report	1		*c.507delC PTEN*	rapamycin	A patient treated with rapamycin showed clear evidence of clinical and cellular benefit (114)
8. Other *SDHB* *SDHC*	Retrospective cohort	15	PPGLs	*10 germline SDHB*	TMZ	Median PFS was longer in those pts with a germline a *SDHB* variant, 19.7 m compared to 2.9 m, in those without (92)
CDK4	Case report	1	Melanoma	Germline *CDK4, MC1R*	BRAF inhibitor	A patient with multiple primary melanomas with germline *CDK4* and *MC1R* variants and somatic *BRAF* mutations in 9 of 10 melanomas showed only a transient clinical benefit followed by marked PD, suggesting intrinsic resistance conferred by the germline variant (129)
VHL	Clin trial Ph II	116	Advanced RCC	*VHL*	bev	Treatment with bev vs placebo in metastatic RCC demonstrated an improved TTP, HR 2.55. No OS benefit (118)
	Clin trial Ph III	732	Metastatic clear cell RCC	*VHL*	bev	The addition of bev to IFN compared to IFN alone improved median PFS to 8.5 m (compared to 5.2 m) with improved RR=25.5% vs. 13.1% (148)

Abbreviations: BC – breast cancer, BCC – basal cell carcinoma, BCCNS – basal cell carcinoma naevus syndrome, BD – twice daily, cCR – clinical complete response, Clin – clinical, CR – complete response, CRC – colorectal cancer, CRPC – castrate resistance prostate cancer, EC – endometrial cancer, FA – Fanconi's anaemia, FFPE – formalin-fixed, paraffin-embedded, GBM – glioblastoma multiforme, GIST – gastrointestinal stromal tumours, HR – homologous recombination repair, IFN – interferon, m – months, MMC – mitomycin C, MMR – mismatch repair, dMMR – MMR deficient, pMMR –MMR proficient, MTC – medullary thyroid carcinoma, NSCLC – non-small cell lung cancer, OC- ovarian cancer, ORR – objective response rate, OR – objective response, OS – overall survival, PARP - poly ADP ribose polymerase, pembro – pembrolizumab, Ph – phase, pts – patients, PFS – progression-free survival, PPGLs – phaeochromocytomas & paragangliomas, PR – partial response, RCC – renal cell carcinoma, RR – response rate, SD – stable disease, TKI – tyrosine kinase inhibitors, TMB – tumour mutational burden, TNBC – triple negative breast cancer, TTP – time to progression.