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Trends in Cancer

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Opinion

When breaks get hot: inflammatory signaling in *BRCA1/2*-mutant cancers

Marcel A.T.M. van Vugt [1,*] and Eileen E. Parkes[2,*]

Genomic instability and inflammation are intricately connected hallmark features of cancer. DNA repair defects due to *BRCA1/2* mutation instigate immune signaling through the cGAS/STING pathway. The subsequent inflammatory signaling provides both tumor-suppressive as well as tumor-promoting traits. To prevent clearance by the immune system, genomically instable cancer cells need to adapt to escape immune surveillance. Currently, it is unclear how genomically unstable cancers, including *BRCA1/2*-mutant tumors, are rewired to escape immune clearance. Here, we summarize the mechanisms by which genomic instability triggers inflammatory signaling and describe adaptive mechanisms by which cancer cells can 'fly under the radar' of the immune system. Additionally, we discuss how therapeutic activation of the immune system may improve treatment of genomically instable cancers.

Highlights

Inflammatory signaling and genomic instability are hallmarks of cancer.

Cancer-associated DNA repair defects, including defects in homologous recombination repair, lead to cytoplasmic DNA.

Genomic instability fuels inflammatory signaling, triggering both tumor-suppressive as well as tumor-promoting traits.

Through largely unknown mechanisms, tumor cells rewire inflammatory signaling to prevent immune clearance.

Unique tumor microenvironment features of genomically unstable tumors may promote immunotherapy resistance

Therapeutic targeting of immune-suppressive mechanisms in genomically instable cancer may potentiate immune checkpoint inhibition.

BRCA1/2 and genomic instability in cancer

Genomic instability (see Glossary) is a trait of tumor cells that is observed in the majority of cancers. However, the level of genomic instability ranges significantly between tumors. High levels of genome instability are associated with hereditary or somatic mutations in DNA repair genes and oncogene-induced replication stress [1]. In particular, defective repair of double-stranded DNA breaks (DSBs) and DNA crosslinks due to mutations in **homologous recombination (HR)** genes [e.g., ***Breast cancer 1, early onset (BRCA1)*** and ***Breast cancer 2, early onset (BRCA2)***] or **Fanconi anemia (FA)** genes yields tumors with extensive genomic instability (Figure 1A). These tumors are characterized by focal genomic deletions and amplifications as well as complex genomic rearrangements [2,3]. Notably, genomic instability drives intratumor heterogeneity and enables rapid acquisition of the genomic aberrations that drive therapy failure [4].

BRCA1 and BRCA2 are key regulators of DNA maintenance through HR [5]. BRCA1 functions in the initiation of HR by controlling DNA-end resection at DNA breaks, whereas BRCA2 functions downstream in HR, in loading the RAD51 recombinase to facilitate the actual recombination process (Figure 1B) [6]. Besides these canonical roles, BRCA1 and BRCA2 function in DNA crosslink repair as part of the FA complex [7] and were shown to protect nascent DNA at stalled replication forks from nucleolytic degradation (Figure 1B) [8,9].

The relevance of BRCA1 and BRCA2 for genome maintenance and cellular viability became evident from genetic studies in mice. Specifically, loss of *Brca1* or *Brca2* leads to accumulation of DNA lesions, a consequent cell cycle arrest, and early embryonal death (Figure 1C) [10–15]. These findings were in apparent contrast with the observed tumor predisposition of *BRCA* mutation carriers and the full loss of *BRCA1* or *BRCA2* in the ensuing tumors (Figure 1A) [16]. This BRCA paradox was partly explained when *Trp53* was conditionally inactivated along with *Brca1/2*, which allows cells to survive with damaged DNA and ultimately promotes tumor onset [2,17,18]. The resulting tumors showed basal-like characteristics, including recurrent genomic

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features such as *MYC* amplification and *RB1* loss [2]. Moreover, these tumors displayed the complex genomic rearrangements that resemble those of human *BRCA1/2*-mutant cancers [2,19–22].

Loss-of-function mutations in *BRCA1/2*, as well as mutations in other HR genes, lead to a profound defect in genome maintenance [23], generically termed **BRCAness**. Defective HR yields aberrant genomes that are enriched for several mutational signatures, including base substitution signatures (SBS3, SBS8), indel signatures (ID6, ID8), and rearrangement signatures (RS1, RS3, and RS5) [3,24–26]. These genomic alterations can be explained by the usage of alternative, nonconservative DNA repair mechanisms to repair DNA DSBs (Figure 1C) [3,27]. In particular, the usage of non-homologous end-joining, polymerase Theta (POLQ)-mediated end-joining (also referred to as alternative end-joining), or single-strand annealing leads to deletions and translocations that are characteristic of HR-deficient cancers [3,24,25,28,29].

In addition to the effects of defective DSB repair, genome integrity in *BRCA1/2*-mutant cancers is affected by defective protection of stalled replication forks [8]. This defect is independent of the HR defect [30] and leads to fork collapse and persistent DNA breaks. Specific to *BRCA1*-mutant cells, re-replication of tracts of DNA adjacent to stalled forks leads to tandem duplications [31], which are a recurrent feature of *BRCA1*-mutant cancers [28,32,33]. The effects of *BRCA1/2* mutation on genome integrity will likely extend beyond the effects of impaired HR and fork protection, as BRCA1 and BRCA2 (and multiple other HR genes) have been described to perform multiple other functions in tumor suppression, including roles for BRCA1 in regulating gene expression and chromatin remodeling [34–36].

Recently, inflammatory signaling was identified as a consequence of genomic instability, including in *BRCA1/2*-mutant cancers. Tumor-cell intrinsic inflammatory signaling can activate immune cells in the tumor microenvironment (TME) and has been linked to responses to immune checkpoint inhibitors. Although important features to respond to cancer immunotherapy are clearly present in genomically instable cancers, patients with such cancers paradoxically only minimally benefit from immune checkpoint inhibitors. Apparently, genomically instable cancers somehow evade immune eradication. To expand benefit of cancer immunotherapy to patients with genomically instable cancers, it is crucial to understand how these cancers have adapted to inflammatory signaling and to investigate if and how these adaptive mechanisms can be therapeutically targeted. We review the mechanisms that drive inflammatory signaling in response to genomic instability caused by *BRCA1/2* inactivation. We also discuss alterations in these tumors to evade immune clearance and options to improve treatment of these cancers.

BRCA1/2 mutation, inflammatory signaling, and immune activation

In line with their DNA repair defects and consequent **tumor mutational burden (TMB)**, *BRCA1/2* mutation status in high-grade serous ovarian cancer associates with a predicted higher neoantigen load and improved overall survival [37]. In this study, neoantigen load correlates with HR deficiency, even in non-*BRCA* mutations. Similarly, *BRCA1/2* mutation in breast cancer associates with increased TMB and increased expression of the PD1/PDL-1 immune checkpoint components, albeit only in *BRCA1*-mutant cancers [38]. Even so, this increased TMB does not reach the scale of that observed in tumors classically responsive to immune checkpoint blockade: microsatellite unstable colorectal cancer, melanoma, and non-small cell lung cancer [39]. It is likely that the increased immunogenicity of *BRCA1/2*-mutant cancers cannot solely be attributed to a moderately elevated TMB, as HR mutations are predictors of response to immune checkpoint inhibitors, independent of TMB [40].

Glossary

BRCAness: defective homologous recombination repair, phenocopying *BRCA1* or *BRCA2* mutation.
Breast cancer 1, early onset (BRCA1): DNA repair protein, involved in initial steps of HR repair. Germline mutations in the *BRCA1* gene lead to predisposition to early onset breast and ovarian cancer.
Breast cancer 2, early onset (BRCA2): DNA repair protein, involved in recruitment of Rad51 in HR repair. Germline mutations in the *BRCA2* gene lead to predisposition to early onset breast and ovarian cancer.
Cyclic GMP-AMP synthase (cGAS): sensor of cytoplasmic DNA and activator of STING.
Fanconi anemia (FA): inherited disorder caused by mutation of one of the FA genes, leading to a defect in the repair of DNA crosslinks.
Genomic instability: defects in genome maintenance leading to progressive accumulation of structural and numerical changes to the genome.
Homologous recombination (HR): pathway for the repair of DNA DSBs and stalled replication forks using a homologous template DNA. HRR acts predominantly in S/G2 phase of the cell cycle.
IFNα: type I interferon-α; a cytokine that activates transcription in response to triggers of the innate immune response.
Interferon regulatory factor-3 (IRF3): transcription factor that, upon activation by TBK1, transactivates cytokines and type-1 interferon genes.
JAK/STAT: signal transduction pathway, involving Janus kinases (JAK) and STAT transcription factors, controlling immunity, proliferation, and cell survival.
Micronucleus: entire chromosome or fragment thereof that forms separate nuclear structure upon missegregation during mitosis.
Retinoic acid-inducible gene-I (RIG-I): cytosolic pattern recognition receptor, responsible for the type-1 interferon response.
Stimulator of interferon genes (STING): adaptor protein that is activated by cyclic GMP-AMP and enhances TBK1 activity.
TANK-binding kinase 1 (TBK1): protein kinase downstream of STING to trigger innate immune responses.

Beyond mutational burden, inflammatory signaling appears to strongly associate with immunogenicity, likely in part because it is a direct read-out of immune cell activation. *BRCA1/2* mutation in ovarian cancer associates with expression of immune-related genes, including **type II interferon gamma (IFNγ)** and TNFR [37]. *BRCA1* mutation is associated with the immunoreactive subtype of high-grade serous ovarian cancers, which are characterized by high levels of tumor-infiltrating immune cells [41]. Also, *BRCA1/2* status is associated with increased abundance of $CD3^+$ and $CD4^+$ **tumor-infiltrating lymphocytes** [42] and increased expression of the immune checkpoint molecules PD1/PDL1 [37,43]. In a separate study only focusing on *BRCA1* status, increased levels of DNA damage in *BRCA1*-mutant ovarian cancers were associated with elevated **stimulator of interferon genes (STING)** levels, increased STAT1 signaling, and increased T cell infiltrate [44]. Moreover, the tumor-associated inflammation in *BRCA1*-mutant tumors is a favorable prognostic feature [45,46].

Tumor-infiltrating lymphocytes: lymphocyte-derived immune cells that reside in the tumor microenvironment.
Tumor mutational burden (TMB): total amount of DNA mutations present in the genome of a cancer.
Type II interferon gamma (IFNγ): cytokine critical for immune activation and defense against viral infection.

Analysis of gene expression in patient samples with *BRCA1/2* or FA mutations yielded a gene set signature, which was highly enriched for genes involved in immune signaling [47]. Lymphocyte infiltration only partially explains the expression of immune-related genes, pointing towards cell intrinsic inflammatory signaling [47]. Subsequent studies have revealed that inactivation of *BRCA1*, *BRCA2*, or *FANCD2* results in tumor-cell intrinsic inflammatory signaling, involving secretion of proinflammatory cytokines CXCL10, CCL5, and TNF-α and attraction of immune cells

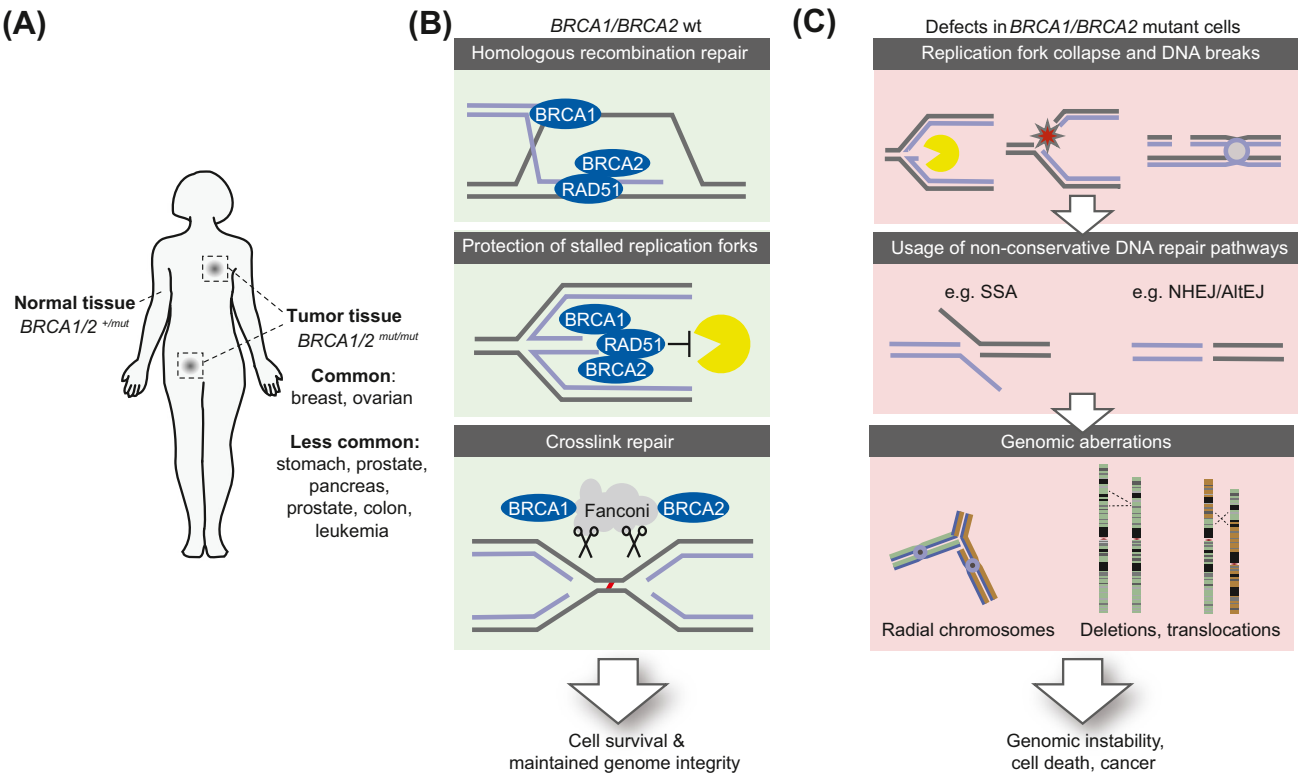

Figure 1. Cancer predisposition and cellular defects associated with *BRCA1/2* mutation. (A) Inherited *BRCA1/2* mutations confer predisposition to a range of cancers, predominantly breast and ovarian cancer. (B) Genome maintenance functions of BRCA1/2. Beyond their canonical role in homologous recombination repair, BRCA1 and BRCA2 are involved in the protection of stalled replication forks and repair by the Fanconi anemia pathway. (C) BRCA1/2 inactivation leads to defective DNA repair and consequent usage of error-prone DNA repair pathways, collapse of stalled forks, and defective completion of crosslink repair. Abbreviations: AltEJ, alternative end-joining; NHEJ, non-homologous end-joining; SSA, single-strand annealing.

[48,49]. Inflammatory signaling in these cells likely stems from the DNA lesions that arise due to their defective DNA maintenance, as similar effects are observed in response to DNA damaging agents [48,50,51]. These findings are in line with observations in genetically engineered mouse models. Combined tissue-specific loss of *Trp53* and *Brca1* leads to development of cancers with signatures associated with higher levels of Th2 cells, T-regulatory cells (T_{regs}), central memory cells, and exhausted T cells, as well as elevated expression of immune checkpoint genes, Pd1 and Ctla4 [22].

Whereas *BRCA1/2* mutation and other HR deficiencies are all characterized by severe genomic instability, not all HR deficiencies are equal in terms of subsequent effects on the TME. For instance, gene expression analysis revealed that ovarian cancers with mutant or hypermethylated *BRCA1* but not *BRCA2*-mutant cancers are associated with the immunoreactive subtype [41]. In a study of *BRCA*-mutant breast cancers, distinct features of the immune TME of *BRCA1*- versus *BRCA2*-mutant were identified, with increased immunosuppressive tumor-associated macrophages in *Brca1*-null mouse models and increased immune checkpoint expression in *BRCA1*-mutant breast cancer [52]. A pan-cancer study of *BRCA1*- versus *BRCA2*-mutant cancers treated with immune checkpoint blockade suggests benefit in *BRCA2*-mutant disease only [53]. Whether similar patterns could be observed in response to other treatments such as chemotherapy is not known.

Some studies have instead observed *BRCA1/2*-mutant cancers to be poorly immunogenic compared with *BRCA1/2*-wild type. Analysis of breast cancers in patients with a germline *BRCA* mutation or clonal loss of heterozygosity found these tumors to have a lower immune gene expression score compared with *BRCA1/2*-wild type or tumors with subclonal loss of heterozygosity, correlating with reduced immune infiltration on immunohistochemical analysis of germline *BRCA1*-mutant breast cancers [54]. These observations add granularity to the association of *BRCA1/2* mutation and immune infiltration, indicating that detailed molecular analysis is needed to clearly understand this complex relationship. Moreover, intratumoral heterogeneity plays a role in enabling immune evasion in *BRCA*-mutant cancers. Using digital pathology to define morphological diversity in ovarian cancer, a significant association between low *BRCA1* expression and increased diversification was noted [55]. Further morphological examination revealed a lack of tumor invasion by CD3+ T cells in morphologically diverse regions, with a suggestion of active exclusion of these cells from tumor nests by upregulation of the immune checkpoint galectin-3 [55]. The relationship between *BRCA1/2* mutation and a favorable immune environment is far from straightforward, with genetic and spatial heterogeneity contributing to a spectrum of immunogenicity in genomically unstable cancers.

Mechanisms of inflammatory signaling in response to genomic instability

BRCA1/2-mutant cancers almost invariably harbor inactivating *TP53* mutations [19], therefore, these cells have incomplete cell cycle control and frequently transmit DNA damage into mitosis [56,57]. These mitotic DNA lesions are positive for the replication stress marker FANCD2 [56] and likely represent 'joint DNA molecules', either due to incomplete DNA replication or unresolved intermediates of repair attempts. Consequently, *BRCA1/2* inactivation leads to elevated numbers of mitotic chromatin bridges and ultrafine DNA bridges [56,58] and results in 53BP1 nuclear bodies in the subsequent G1 cells [57].

Unresolved mitotic DNA damage frequently results in **micronuclei** [59,60], which are small DNA-containing structures surrounded by a single lipid bilayer and are not part of the main nucleus (Figure 2). Micronuclei that originate from DNA lesions that are transmitted into mitosis typically contain acentric chromosome fragments. Chromatin in micronuclei is unable to support faithful

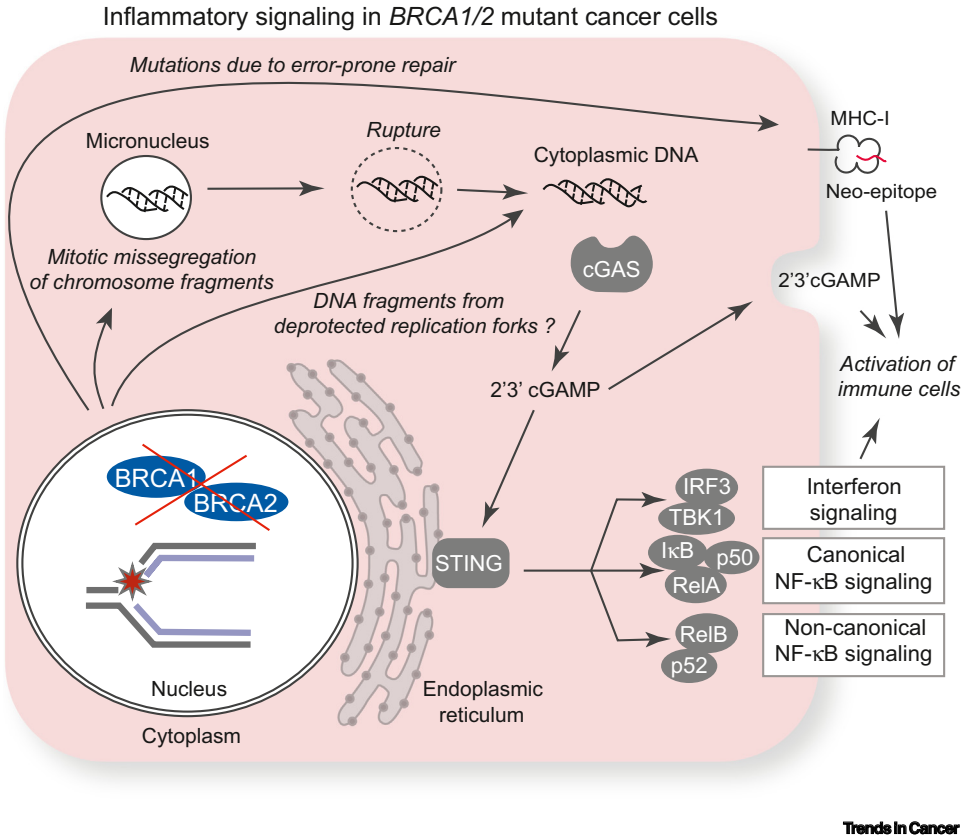

Figure 2. Mechanisms of inflammatory signaling in response to BRCA1/2 inactivation. A schematic overview is provided of the various potential routes by which defective homologous recombination can lead to cytoplasmic DNA and trigger downstream inflammatory signaling pathways and immune cell activation.

DNA replication and DNA repair, leading to additional DNA damage, including local chromosome shattering (i.e., 'chromotrypsis') [61–63]. The nuclear lamina of micronuclei is not properly organized and frequently ruptures, leading to the release of micronucleus DNA into the cytoplasm (Figure 2) [64].

To respond to microbial pathogens, cells have evolved an innate immune response, involving cytosolic DNA and RNA sensors, including the DNA sensor **cyclic GMP-AMP synthase (cGAS)**, the RNA sensor **retinoic acid-inducible gene-I (RIG-I)** and the Toll-like DNA/RNA receptors [65,66]. Like microbial DNA, 'self' DNA that ends up in the cytosol following missegregation of chromosomes during mitosis is also recognized by cGAS [67–69]. Activated cGAS subsequently catalyzes the production of cyclic 2′3′ GAMP, which triggers STING-dependent inflammatory signaling, including the production of type-1 IFNs [65]. Under physiological conditions, cytoplasmic DNA can be effectively degraded by the TREX1 nuclease [70]. Additionally, DNA:RNA hybrids can be processed by RNase H1/H2, whereas genome-embedded ribonucleotides can be hydrolyzed by RNAseH2, which prevents the accumulation of cytoplasmic nucleic acids and activation of the cGAS/STING pathway [68,71–73]. When the amount of cytoplasmic DNA is overwhelming, for instance, when a micronucleus is induced upon *BRCA1/2* inactivation, the cGAS/STING pathway is triggered (Figure 2) [48,49,74]. Micronucleus formation upon missegregation of chromosome fragments leads to recruitment and activation of cGAS [67]. Micronucleus rupture strongly enhances this process, as was elegantly shown by cGAS recruitment along with leakage of cytoplasmic markers

into micronuclei and the loss of nuclear markers out of micronuclei [68]. In line with this observation, a split-GFP reporter for cGAS shows highest activity in micronuclei [75], and cGAS can be activated directly by purified micronuclei [70]. However, additional sites of cGAS activation may exist (see Outstanding questions) and various layers of regulation are emerging that control cGAS activity.

Chromatin [76–80] and the post-translational modification status of cGAS [75] are increasingly recognized to determine the ability of cGAS to be activated by DNA. In addition, it was recently suggested that cGAS-dependent inflammatory signaling requires DNA stretching at mitotic chromatin bridges, rather than micronucleus formation *per se* [81]. However, these findings were done using antimitotic drugs or spindle-assembly checkpoint perturbation instead of genomic instability due to defective DNA repair. Yet, mitotic chromatin bridges are also frequently observed in HR-deficient cancer cells [56,58] and these structures may contribute to cGAS/STING activation.

Cytoplasmic DNA can also arise from deprotected stalled replication forks, as observed in SAMHD1-deficient cells [82]. Whether DNA fragments from stalled replication forks in *BRCA1/2*-mutant cells also migrate into the cytoplasm is currently unclear. More recently, DNA fragments derived from R-loops were shown to end up in the cytoplasm of repair-deficient pancreatic cells [83]. The observation that DNA repair defects in differentiated tissues lead to cytoplasmic DNA fragments suggests that undergoing mitosis is not required to yield cytoplasmic DNA and trigger inflammatory responses [83].

Once STING is activated, canonical NF-κB signaling is instigated through the IkK complex, leading to RelA/p50 translocation to the nucleus, where it transactivates canonical NF-κB targets, predominantly involved in immune responses [84] (Figure 2). Additionally, STING activates noncanonical NF-κB signaling, through the p100/RelB complex. Processing of p100 into p52, which associates with RelB, leads to a transcriptional response that is mostly linked to increased survival, through transactivation of antiapoptotic genes and epithelial-to-mesenchymal transition [84]. Finally, STING signaling leads to **TANK-binding kinase 1 (TBK1)**-dependent **interferon regulatory factor-3 (IRF3)** phosphorylation and subsequent transactivation of IRF3 target genes. Prominent among IRF3 target genes are type-1 IFNs, which induce autocrine and paracrine signaling. Type-1 (α and β) or type-2 (γ) IFNs induce pleiotropic effects on the physiology of cells, through the transactivation of a broad repertoire of target genes, collectively called 'interferon-stimulated genes' (ISGs). A major downstream consequence of IFN signaling is widespread immunomodulation. Binding of IFNs to the ubiquitously expressed **IFNα/β** receptor (IFNAR) ultimately results in phosphorylation and activation of the **JAK/STAT** pathway. Whereas JAK-mediated phosphorylation of the STAT1 transcription factor predominantly leads to growth suppressing and proapoptotic effects, STAT3 promotes proliferation, while it prevents apoptosis [85]. The repertoire of STAT expression thus determines the ultimate outcome of IFN signaling. Early on, IFNs were shown to stimulate multiple aspects of the innate immune system, including activation of macrophages and natural killer (NK) cells [86,87] and maturation of dendritic cells (DCs) [88]. Of note, through production of various soluble factors, NK cells interact with other immune cells, to promote efficient adaptive immune responses, beyond their role in innate immunity [89,90]. IFNs also directly support the proliferation and activity of certain T cell subsets, including the proliferation of $CD8^+$ T cells [91] and development of T-helper (TH)-1 cells, while restraining TH2 cell development [92]. As such, type-1 IFNs, among other innate cytokines, are considered important signals in shaping the effector and memory T cell pool.

Tumor-intrinsic adaptation mechanisms to escape immune clearance

Constitutive cGAS activation in *BRCA1/2*-mutant cancer presents a challenge to tumor development, as cGAS/STING pathway activation typically results in IFN signaling and, thus, immune-mediated

tumor cell clearance. Therefore, *BRCA1/2*-mutant tumor cells need to dampen the IFN response that is triggered by genomic instability to sustain their growth and evade the immune system. Although our knowledge on how exactly cancer cells evade clearance by the immune system is incomplete, a number of mechanisms have been described (Figures 3 and 4). Understanding these mechanisms and the subsequent tumor-promoting effects may enable future stratification of cGAS/STING-targeting therapies in the clinical setting.

A key mechanism that suppresses cGAS/STING signaling is the same mechanism by which HR-defective cells limit their genome instability: restoration or rewiring of DNA repair will prevent the generation of cytoplasmic DNA and will decrease the cues that trigger inflammatory signaling. For instance, POLQ-mediated repair [93] is upregulated in HR-defective tumors and was recently established as a therapeutic vulnerability of *BRCA1/2*-mutant cancers [94]. POLQ inhibition yields micronuclei and IFN signaling [95], illustrating that utilization of alternative repair pathways in

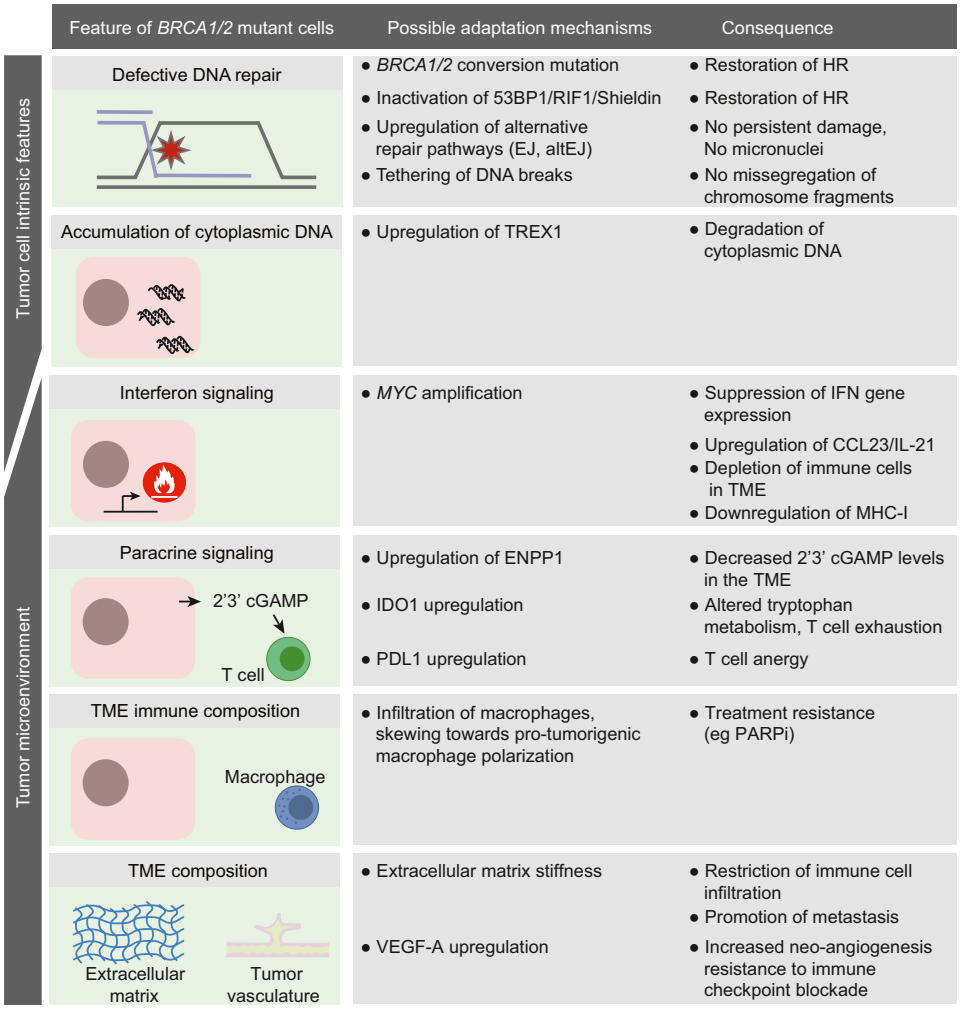

Figure 3. Adaptation mechanisms of *BRCA1/2*-mutant cancer cells to survive inflammatory signaling. An overview is provided of tumor-cell intrinsic and extrinsic mechanisms by which *BRCA1/2*-mutant cells evade clearance by the immune system. Abbreviations: altEJ, alternative end-joining; EJ, end-joining; HR, homologous recombination; TME, tumor microenvironment.

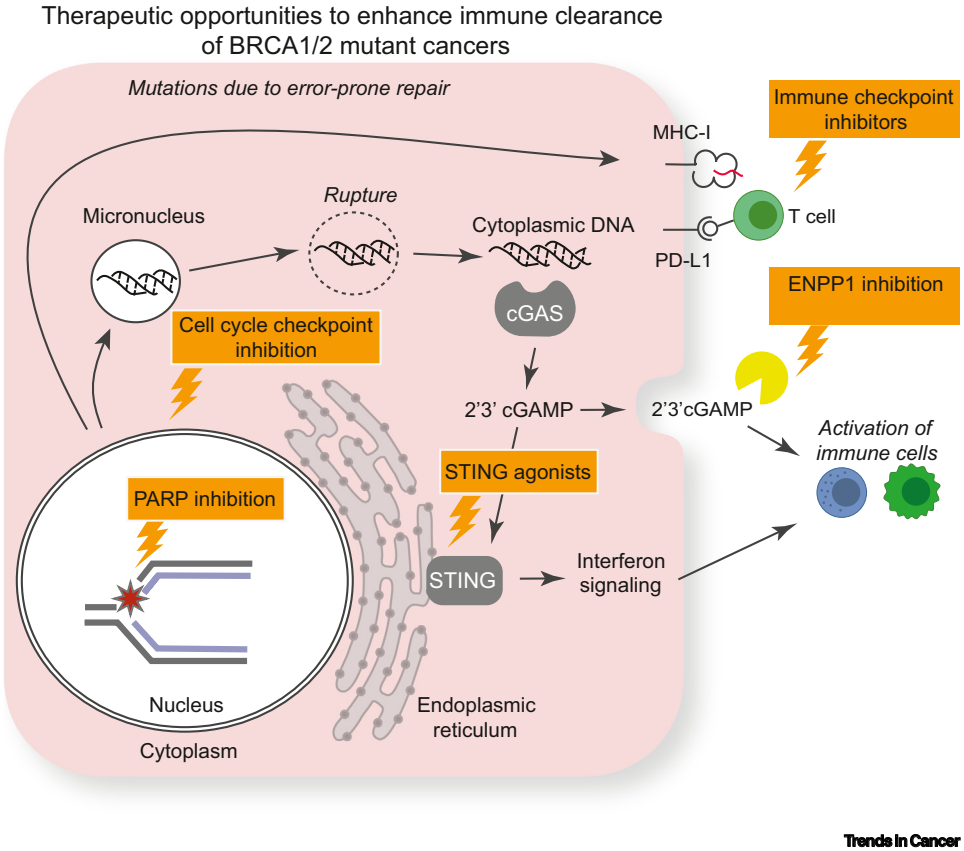

Figure 4. Therapeutic opportunities to enhance immune clearance of *BRCA1/2*-mutant cancers. Several therapeutic strategies are highlighted that could enhance the immune clearance of *BRCA1/2*-mutant cancer cells. Whereas PARP inhibition or cell cycle checkpoint perturbation can be used to enhance the load of cytoplasmic DNA, STING agonist treatment can be used to directly activate inflammatory signaling. Immune checkpoint inhibitors and ENPP1 inhibition can be used to activate immune cells in the tumor microenvironment.

BRCA1/2 mutant cancers prevents excessive missegregation of chromosome fragments and the accumulation of cytoplasmic DNA. Likewise, *BRCA1/2*-mutant cells depend on Cip2A and TopBp1, which form a complex with Mdc1 to tether chromosome fragments during mitosis, preventing the generation of micronuclei [96,97]. In addition, several mechanisms have been described by which HR-deficient cells can manage with defective replication fork protection, including the inactivation of PAXIP1 [98] and EZH2 [99]. Whether restored fork protection in *BRCA1/2*-mutant cancers affects cGAS/STING signaling is unknown.

Besides preventing cytoplasmic DNA, several enzymes degrade cytoplasmic DNA and thereby suppress an IFN response, including TREX1 and RNAseH1. However, TREX1 and RNAseH1-mediated degradation of cytoplasmic DNA does not appear to be a significant compensatory mechanism in *BRCA1/2*-mutant cancers [70,100]. Another mechanism by which IFN signaling could be suppressed is the downregulation of DNA sensors or their effector proteins, such as cGAS and STING. While it is striking that cGAS and STING are rarely mutated across cancers [69,101], promoter methylation of both cGAS and STING results in their downregulation in many solid tumors [101]. Treatment of melanoma and ovarian cell lines with demethylating agents results in rescue of cGAS and/or STING expression [102,103]. Moreover, virus-driven cancers, such as human papilloma virus-driven cervical cancers, downregulate cGAS/STING signaling

via direct binding of viral onco-proteins to STING [104]. However, it is not currently known how often these epigenetic or other silencing events occur in *BRCA1/2*-mutant or genomically unstable cancers; whether cGAS/STING signaling persists in *BRCA1/2*-mutant cancers by accident or through active mechanisms remains an important question.

Important cell intrinsic mechanisms that modulate inflammatory signaling involve common tumor suppressor genes and oncogenes. *TP53* mutations affecting the DNA binding or tetramerization domains result in binding of p53 to TBK1, preventing STING/TBK1/IRF3 activation and subsequent IFN signaling [105], reflecting a potential mechanism of immunosuppression in *TP53*-mutant cancers. Additionally, common driver oncogenes may exert their tumorigenic effect through immune suppression. Early on, *N-MYC* expression was linked to downregulation of MHC-1, which possibly prevents neoantigen-mediated immune clearance [106]. More recently, C-MYC, in conjunction with MIZ1, was shown to directly repress IFN gene expression in tumor cells, in a model of *KRAS*/*CMYC*-driven pancreatic cancer [107,108]. C-MYC appears to act as a generic suppressor of inflammatory signaling, as it also suppresses IFN signaling in a model of *BRCA1*-mutant breast cancer [109]. C-MYC-mediated suppression of IFN signaling likely reflects a clinically relevant mechanism, with *C-MYC* being frequently amplified in *BRCA1/2*-mutant cancers [110]. In addition to these cell intrinsic changes, oncogene expression has been linked to extensive modulation of the TME.

Modulation of the microenvironment to escape immune clearance

The TME of *BRCA1/2*-mutant tumors is closely linked to the native environment in which a tumor arises, illustrated by site-specific characteristics of tissue-resident macrophages [111]. However, a common characteristic across sites is the increased immune infiltration in breast, ovarian, and prostate *BRCA1/2*-mutant tumors [23,112,113]. The composition of this immune infiltrate plays a crucial role in tumor progression and therapeutic response. Like other cancers, *BRCA1/2*-mutant tumors can modulate their TME to avoid immune clearance and harness tumor-promoting inflammation.

Tumor cells actively modify the TME to suppress immune responses, through secretion of immune-suppressive cytokines. For instance, production of immune-suppressive cytokines (e.g., IL-10) prevents maturation of DCs [114]. Also, amplification of *C-MYC* promotes the secretion of CCL9 and IL-23, leading to suppression of immune responses in the local microenvironment [115]. Conversely, *C-MYC* overexpression leads to suppression of IFN signaling, along with decreased secretion of proinflammatory cytokines [107–109]. Consequently, *C-MYC* overexpression depletes immune cells from the TME of *BRCA1*-mutant tumors and prevents killing of organoids derived from *BRCA1*-mutant cancers by T cells [109].

A role of constitutive cGAS/STING activation in recruiting immune-suppressive cells has been described in various cancers, although it is currently not characterized in *BRCA*-mutant cancers. However, it has been reported that radiation therapy results in STING-dependent recruitment of myeloid-derived suppressor cells (MDSCs) [116], suggesting a potential role in genomically unstable tumors. In addition, STING activation is required for PD-L1 upregulation in response to DNA damaging treatment [48,117,118] and STING agonists have been reported to upregulate PD-L1 [119,120], suggesting that the increased PD-L1 expression observed in *BRCA1/2*-mutant cancers could be related to cGAS/STING pathway activation.

Constitutive activation of the cGAS/STING pathway appears to result in rewiring of downstream signaling, with preferential activation of the noncanonical NF-κB/RelB pathway as opposed to TBK1/IRF3 signaling. Specifically, activation of RelB is associated with a paucity of IFN production in chromosomally unstable cells [69]. Similarly, STING-dependent RelB signaling results in

suppression of IFN production in DCs following radiation treatment and antagonizes IRF3/canonical NF-κB pathways [121]. Earlier reports suggested that the TNF receptor-associated factor TRAF3 could mediate noncanonical NF-κB activation downstream of STING in response to cytosolic DNA [122]. However, the role of TRAF3 in downstream modulation of the STING pathway remains unclear. Noncanonical NF-κB activation itself has both pro- and antitumorigenic effects, leading to chronic tumor-promoting inflammation and mediating the development of tertiary lymphoid structure development, associated with an improved response to immune checkpoint blockade [123–125]. Additionally, it remains unknown how chronic STING-mediated RelB activation shapes the TME and modifies host immune responses. Given the central role of cGAS/STING signaling in the tumor immune microenvironment and response to immune-targeting therapies, identifying the event(s) responsible for pathway choice downstream of STING in genomically unstable cancer remains a key question for the field.

An intriguing mechanism of TME modulation downstream of STING involves the transmembrane pyrophosphatase ENPP1. ENPP1 is a negative regulator of cGAS signaling and was found to be upregulated in chromosomally unstable tumors [126]. ENPP1 hydrolyzes cyclic dinucleotides, including 2′3′cGAMP, resulting in pA(3′5′)pG and subsequent AMP production [127]. Further breakdown of AMP by NT5E (CD73) generates immunosuppressive adenosine within the TME [126]. Therefore, ENPP1 upregulation results in restriction of 2′3′cGAMP transfer to host immune cells, preventing STING activation and IFN signaling. The increased adenosine levels amplify this immunosuppressive effect and promote tumor metastasis [128]. The expression of ENPP1 in cancers with genomic instability due to *BRCA1/2* mutation requires further investigation and represents a potential therapeutic target [129,130].

Constitutive cGAS/STING activity and PD-L1 upregulation in *BRCA1/2*-mutant tumors could render them highly sensitive to immune checkpoint blockade. In addition, current evidence based on TMB [53] and DNA repair deficiency [40] suggests that increased response rates could be expected in *BRCA1/2*-mutant tumors. In contrast, the majority of patients with *BRCA1/2*-mutant tumors do not derive any clinical benefit from immune checkpoint blockade. Indeed, prospective trials of PARP inhibition and anti-PD-1 in platinum-resistant ovarian cancer [131] and advanced triple-negative breast cancer (TNBC) [132] have not demonstrated improved responses in *BRCA1/2*-mutant tumors, although patient numbers were small. Potential reasons for this outcome may include treatment at an advanced stage, with weakened immune responses in pretreated disease [133,134]. Additionally, TME features play an important role in intrinsic immunotherapy resistance.

Myeloid and lymphoid cells in the BRCA-mutant TME

BRCA1-mutant breast cancers contain increased tumor-promoting immunosuppressive macrophages compared with *BRCA*-wild-type disease [135]. Single-cell analysis of *BRCA1*-mutant TNBCs additionally demonstrated increased infiltration of CD4+ T_{regs} and exhausted (PD-1+) T cells, indicating an immunosuppressed TME [135]. Other studies have similarly reported increased infiltration of T_{regs} in *BRCA1*/2-mutant breast cancers [136]. Moreover, in a study of *BRCA2*-mutant prostate cancer, increased T_{regs} were identified in early-stage disease compared with BRCA-wild type [137]. In general, genomic instability arising from HR deficiency promotes immune evasion via 'M2'-like immunosuppressive polarization of macrophages and influx of T_{regs}, particularly as instability progresses towards aneuploidy with chromosomal arm or whole chromosomal copy number alterations [138]. cGAS/STING pathway activation in genomically unstable cancers may play a role in this immunosuppressive skewing of the TME via upregulation of ENPP1 and subsequent increased adenosine levels [126]. Additionally, downstream STING-mediated responses, such as upregulation of CCL2, CCL7, and CCL12, result in infiltration of

MDSCs, which promote immunosuppression and therapeutic resistance [116]. Thus, TME features of *BRCA1/2*-mutant cancers tip the balance in favor of immune-resistance and may account for the poor response rates to immune checkpoint blockade observed in these tumors. As immune editing in genomically unstable cancers is an iterative process, it is not clear how the immune microenvironment of early-stage *BRCA1/2*-mutant cancers compares with metastatic disease. The studies cited earlier have primarily been conducted on early-stage disease (where resection specimens and genetic data are readily available). Further longitudinal analysis of *BRCA1/2*-mutant primary cancers and metastases with a focus on the immune TME would enable design of immune-targeting strategies in both early- and late-stage genomically unstable cancers.

Metabolic and microenvironment immune evasion in BRCA-mutant cancers

BRCA1/2-mutant tumors also alter immunometabolism to suppress immune responses. In DNA repair-deficient breast cancers with constitutive cGAS/STING pathway activation, indoleamine 2,3-dioxygenase 1 (*IDO1*) was found to be among the top upregulated genes [47,48]. Consistently, in high-grade TNBC, IDO1 is coexpressed in 70% of tumor cell PD-L1 positive cases, with a trend towards increased coexpression in *BRCA1/2*-mutant cancer [139]. IDO1 catabolizes tryptophan, resulting in anergy, cell cycle arrest, or apoptosis of T cells [140]. Additional immunosuppressive effects include IL6 production and expansion of protumorigenic MDSCs [141]. STING activity in the TME induces IDO1, suggesting a targetable mechanism of immune escape in *BRCA1/2*-mutant tumors. Enthusiasm for IDO1 inhibition was dampened in the clinical trial setting following a Phase III study that did not demonstrate improved responses using combination IDO1 inhibitor and anti-PD-1 treatment, compared with PD-1 targeting alone [142]. However, stratification of patients, selecting those with STING-active, *BRCA1/2*-mutant tumors, may be fruitful for future studies using combination IDO1-targeting approaches.

Tumors are complex collections of cells, comprising not just tumor and immune cells. Modifications within the tumor vasculature, such as vascular endothelial growth factor (VEGF)-driven angiogenesis, promote tumor growth and immunosuppression by promotion of T_{reg} and MDSC activity [143]. VEGF-A upregulation further correlates with the presence of *BRCA1/2* mutations [144]. A direct link between *BRCA1* loss, STING activity, and VEGF-A upregulation was recently reported, whereby a *Brca1*-knockout model of ovarian cancer demonstrated increased sensitivity to combination immune checkpoint blockade in a *Sting*-null background, associated with reduced VEGF activity [44]. In contrast, acute stimulation of STING using exogeneous agonists resulted in reduced CD31+ vessel density, demonstrating the differing effects of constitutive versus acute STING pathway stimulation [145]. Recently, the combination of PARP inhibition and the anti-VEGF agent bevacizumab was approved as maintenance treatment for *BRCA*-mutant or homologous recombination deficiency ovarian cancer [146]. Taken together, treatment of inflamed *BRCA1/2*-mutant tumors with antiangiogenics may overcome intrinsic STING-mediated immunosuppression, permitting response to immune targeting agents.

Mechanical properties of the TME can influence chromosomal instability. Cellular stresses caused by 2D cell culture induces increased chromosomal instability, which is rescued in 3D model systems and is dependent on integrins [147]. Additionally, cancer cells cultured using stiff hydrogels have increased chromosomal instability [148]. The composition of the extracellular matrix, which determines microenvironmental characteristics of the tumor, such as stiffness, is mediated by cancer-associated fibroblasts (CAFs) [149]. Potential variations in fibroblast phenotypes between *BRCA*-wild type and *BRCA1/2*-mutant cancers are not clearly defined. Recent data suggests there are key alterations between fibroblasts in *BRCA*-wild type and *BRCA*-mutant pancreatic cancers, with the latter containing increased clusterin-expressing CAFs

associated with inflammatory gene expression [150]. However, potential links between STING signaling and subsequent effects on the mechanical TME are unclear. Given the close relationship between immune cell exclusion and extracellular matrix composition [151], the mechanical properties of the TME represent a further barrier to immunotherapy for exploration in genomically unstable cancers.

These direct links between genomic characteristics of tumor cells and subsequent immune TME support a layered approach to patient stratification for combination immune targeting treatments and may be beneficial in the development of personalized immunotherapy. Understanding the immunosuppressive mechanisms adopted by cGAS/STING active cancers may enable the identification of novel therapeutic strategies for clinical exploration.

Clinical implications

In line with a profound role in modulating immune function, tumor-cell intrinsic IFN signaling is a key determinant in the response to immune checkpoint inhibitors. Functional genetic CRISPR/Cas9 screens in cocultures of tumor cells and T cells identified multiple IFN signaling components to be required for effective immune checkpoint inhibitor response [152,153]. Conversely, a common mechanism to evade immune clearance, including for *BRCA1/2*-mutant tumors, involves suppression of IFN signaling [154]. Consequently, approaches to therapeutically increase inflammatory signaling to increase benefit of immune checkpoint inhibitors have been proposed and preclinically tested.

PARP inhibitors exacerbate the accumulation of cytoplasmic DNA, the subsequent activation of cGAS/STING, and immune reactivity in *BRCA1*-mutant or otherwise HR-deficient tumor models [66,155]. Notably, the therapeutic effects of PARP inhibition were in large part dependent on T cells, stressing the involvement of immune cells in the *in vivo* effects of PARP inhibition BRCA-deficient tumors [155]. As a logical follow-up approach, the combination of PARP inhibitors with immune checkpoint inhibitors was tested. In agreement with earlier findings, PARP inhibition in a *BRCA1*-deficient humanized mouse TNBC xenograft model resulted in increased T cells in the TME and elevated IFN signaling [156]. Of note, synergistic effects were also observed in HR-proficient models in this study.

Approaches combining PARP inhibition with immune checkpoint blockers have been evaluated in clinical trials involving patients with *BRCA1/2*-mutant breast cancer, with ongoing studies in *BRCA2*-mutant castration-resistant prostate cancer (NCT02484404)[i], *BRCA2*-mutant bladder cancer (NCT02553642[ii], NCT01928394[iii], NCT02108652)[iv], and *BRCA1/2*-mutant ovarian cancer (NCT02657889)[v]. Interestingly, the activity of PD1/CTLA4 inhibitors is also under investigation in the absence of PARP inhibitors in HR-deficient cancers (NCT02985957)[vi]. These approaches aim to prime local immune responses, employing the targeted effects of PARP inhibition, to enable subsequent improved responses to immunotherapy. However, in a similar manner to radiotherapy priming for immune responses, it is likely that optimization of scheduling and dose of PARP inhibition will be indicated. Of note, several approaches have been explored to increase the therapeutic effects of PARP inhibitors, including cell cycle checkpoint inhibitors. Targeting of the WEE1, ATR, and CHK1 kinases promotes entry into mitosis with unresolved DNA lesions and exacerbates the effects of PARP inhibition in *BRCA1/2*-mutant cancer cells [56,157–159]. Combined ATR and PARP inhibition further promotes IFN signaling in *BRCA1/2*-mutant cancer cells [160], although the *in vivo* consequences of such combination approaches need to be explored.

STING agonists have been proposed as effective treatments for *BRCA1/2*-mutant cancers, by restoring IFN signaling in the TME. In a preclinical model of *Brca1*-deficient breast cancer,

STING agonist treatment led to increased IFN-dependent recruitment of cytotoxic T cells and subsequent tumor response [161]. STING agonists are typically analogs of 2′3′cGAMP, delivered intratumorally to activate local immune responses. While STING agonism may be effective in *BRCA1/2*-mutant tumors due to intrinsic inflammation, chronic adaptation to inflammatory signaling may present additional barriers to therapeutic efficacy. Preventing generic activation of STING in the TME by using next-generation STING agonists targeted to antigen-presenting cells [162] may overcome these barriers. However, these approaches do not yet address factors preventing recruitment of immune cells to the TME for activation.

Concluding remarks

Similar to other defining characteristics of cancer, phenotypes related to *BRCA1/2* mutation exist on a spectrum, with further complexity added by site-specific TME features. This complexity prevents an uncomplicated correlation between *BRCA1/2* mutations, cGAS-STING activation, the immune TME, and treatment response. However, insight into the immune-evading mechanisms common to STING-active, *BRCA1/2*-mutant tumors is improving apace with evolving techniques of immune phenotyping of tumors. Combined, these new insights will enable personalized combination approaches of immune-targeting agents, alongside treatments targeting resistance mechanisms, to ultimately provide the greatest future improvements in patient outcomes (see Outstanding questions).

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Declaration of interests

No interests are declared.

Resources

[i]https://ClinicalTrials.gov/show/NCT02484404

[ii]https://ClinicalTrials.gov/show/NCT02553642

[iii]https://ClinicalTrials.gov/show/NCT01928394

[iv]https://ClinicalTrials.gov/show/NCT02108652

[v]https://ClinicalTrials.gov/show/NCT02657889

[vi]https://ClinicalTrials.gov/show/NCT02985957

References

1. Negrini, S. *et al.* (2010) Genomic instability an evolving hallmark of cancer. *Nat. Rev. Mol. Cell Biol.* 11, 220–228
2. Liu, X. *et al.* (2007) Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like breast cancer. *Proc. Natl. Acad. Sci. U. S. A.* 104, 12111–12116
3. Davies, H. *et al.* (2017) HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat. Med.* 23, 517–525
4. Gerlinger, M. and Swanton, C. (2010) How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. *Br. J. Cancer* 103, 1139–1143
5. Wyman, C. *et al.* (2004) Homologous recombination-mediated double-strand break repair. *DNA Repair* 3, 827–833
6. Chen, C.-C. *et al.* (2018) Homology-directed repair and the role of BRCA1, BRCA2, and related proteins in genome integrity and cancer. *Annu. Rev. Cancer Biol.* 2, 313–336
7. Bunting, S.F. *et al.* (2012) BRCA1 functions independently of homologous recombination in DNA interstrand crosslink repair. *Mol. Cell* 46, 125–135
8. Schlacher, K. *et al.* (2011) Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell* 145, 529–542
9. Schlacher, K. *et al.* (2012) A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. *Cancer Cell* 22, 106–116
10. Sharan, S.K. *et al.* (1997) Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* 386, 804–810
11. Hakem, R. *et al.* (1996) The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell* 85, 1009–1023
12. Tutt, A. *et al.* (1999) Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification. *Curr. Biol.* 9, 1107–1110
13. Hakem, R. *et al.* (1998) Developmental studies of Brca1 and Brca2 knock-out mice. *J. Mammary Gland Biol. Neoplasia* 3, 431–445
14. Liu, C.Y. *et al.* (1996) Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early post-implantation development. *Genes Dev.* 10, 1835–1843
15. Gowen, L.C. *et al.* (1996) Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat. Genet.* 12, 191–194
16. Elledge, S.J. and Amon, A. (2002) The BRCA1 suppressor hypothesis: an explanation for the tissue-specific tumor development in BRCA1 patients. *Cancer Cell* 1, 129–132

Outstanding questions

Which aspects of defective DNA repair in BRCA1/2-mutant cancers trigger inflammatory signaling? Research involving separation-of-function mutants of *BRCA1/2* and combinations of mutations that rescue specific repair defects is warranted to address this.

Which DNA/chromatin features determine cGAS/STING activation and inflammatory signaling? It remains incompletely clear what the exact cue is for inflammatory signaling in HR-deficient cells and what the contribution is of micronuclei, micronucleus rupture, mitotic chromosome bridges, or mitosis-independent routes.

To what extent do different sources of genome instability lead to specific or distinct inflammatory signaling profiles? A comprehensive analysis involving various genome maintenance defects, including DNA repair defects, CIN, and telomere erosion is required.

What are the key differences between acute versus chronic inflammatory signaling? What are the downstream events that determine dominance of IRF3 or noncanonical NF-κB pathway signaling?

What is the contribution of the various DNA sensing pathways in driving inflammatory signaling in cancers? It remains unclear how the different DNA sensors (cGAS, RIG-I, inflammasome, TLRs) collaborate or are active in different contexts.

Can immune checkpoint therapy be used to clear early-stage cancer lesions with genome instability?

Does *BRCA1/2* haploinsufficiency trigger inflammatory signaling, and does anticancer immune clearance play a role in tumor development?

To what extent are the mechanisms that *BRCA1/2*-mutant tumors employ to evade the immune system reversible?

How can inflammatory signaling be reinstated therapeutically, in a way that promotes antitumor immunity?

17. Jonkers, J. *et al.* (2001) Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat. Genet.* 29, 418–425
18. Xu, X. *et al.* (1999) Conditional mutation of Brca 1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat. Genet.* 22, 37–43
19. Curtis, C. *et al.* (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 486, 346–352
20. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. *Nature* 490, 61–70
21. Holstege, H. *et al.* (2010) Cross-species comparison of aCGH data from mouse and human BRCA1- and BRCA2-mutated breast cancers. *BMC Cancer* 10, 455
22. Hollern, D.P. *et al.* (2019) A mouse model featuring tissue-specific deletion of p53 and Brca1 gives rise to mammary tumors with genomic and transcriptomic similarities to human basal-like breast cancer. *Breast Cancer Res. Treat.* 174, 143–155
23. Lord, C.J. and Ashworth, A. (2016) BRCAness revisited. *Nat. Rev. Cancer* 16, 110–120
24. Alexandrov, L.B. *et al.* (2020) The repertoire of mutational signatures in human cancer. *Nature* 578, 94–101
25. Hwang, T. *et al.* (2020) Defining the mutation signatures of DNA polymerase θ in cancer genomes. *NAR Cancer* 2, zcaa017
26. Nguyen, L. *et al.* (2020) Pan-cancer landscape of homologous recombination deficiency. *Nat. Commun.* 11, 5584
27. Stok, C. *et al.* (2021) Shaping the BRCAness mutational landscape by alternative double-strand break repair, replication stress and mitotic aberrancies. *Nucleic Acids Res.* 49, 4239–4257
28. Nik-Zainal, S. *et al.* (2016) Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* 534, 47–54
29. Póti, Á. *et al.* (2019) Correlation of homologous recombination deficiency induced mutational signatures with sensitivity to PARP inhibitors and cytotoxic agents. *Genome Biol.* 20, 240
30. Daza-Martin, M. *et al.* (2019) Isomerization of BRCA1–BARD1 promotes replication fork protection. *Nature* 571, 521–527
31. Willis, N.A. *et al.* (2017) Mechanism of tandem duplication formation in BRCA1-mutant cells. *Nature* 551, 590–595
32. Menghi, F. *et al.* (2018) The tandem duplicator phenotype is a prevalent genome-wide cancer configuration driven by distinct gene mutations. *Cancer Cell* 34, 197–210
33. Menghi, F. and Liu, E.T. (2016) Reply to Watkins *et al.*: Whole-genome sequencing-based identification of diverse tandem duplicator phenotypes in human cancers. *Proc. Natl. Acad. Sci. U. S. A.* 113, E5259–E5260
34. Savage, K.I. *et al.* (2014) Identification of a BRCA1-mRNA splicing complex required for efficient DNA repair and maintenance of genomic stability. *Mol. Cell* 54, 445–459
35. Filipponi, D. *et al.* (2013) Wip1 controls global heterochromatin silencing via ATM/BRCA1-dependent DNA methylation. *Cancer Cell* 24, 528–541
36. Zhu, Q. *et al.* (2011) BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. *Nature* 477, 179–184
37. Strickland, K.C. *et al.* (2016) Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. *Oncotarget* 7, 13587–13598
38. Wen, W.X. and Leong, C.-O. (2019) Association of BRCA1- and BRCA2-deficiency with mutation burden, expression of PD-L1/PD-1, immune infiltrates, and T cell-inflamed signature in breast cancer. *PLoS One* 14, e0215381
39. Lawrence, M.S. *et al.* (2013) Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 499, 214–218
40. Hsiehchen, D. *et al.* (2020) DNA repair gene mutations as predictors of immune checkpoint inhibitor response beyond tumor mutation burden. *Cell Reports Med.* 1, 100034
41. George, J. *et al.* (2013) Nonequivalent gene expression and copy number alterations in high-grade serous ovarian cancers with BRCA1 and BRCA2 mutations. *Clin. Cancer Res.* 19, 3474–3484
42. McAlpine, J.N. *et al.* (2012) BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. *Mod. Pathol.* 25, 740–750
43. Wieser, V. *et al.* (2018) BRCA1/2 and TP53 mutation status associates with PD-1 and PD-L1 expression in ovarian cancer. *Oncotarget* 9, 17501–17511
44. Bruand, M. *et al.* (2021) Cell-autonomous inflammation of BRCA1-deficient ovarian cancers drives both tumor-intrinsic immunoreactivity and immune resistance via STING. *Cell Rep.* 36, 109412
45. van Verschuer, V.M.T. *et al.* (2015) Tumor-associated inflammation as a potential prognostic tool in BRCA1/2-associated breast cancer. *Hum. Pathol.* 46, 182–190
46. de Boo, L. *et al.* (2020) Tumour-infiltrating lymphocytes (TILs) and BRCA-like status in stage III breast cancer patients randomised to adjuvant intensified platinum-based chemotherapy versus conventional chemotherapy. *Eur. J. Cancer* 127, 240–250
47. Mulligan, J.M. *et al.* (2014) Identification and validation of an anthracycline/cyclophosphamide-based chemotherapy response assay in breast cancer. *J. Natl. Cancer Inst.* 106, djt335
48. Parkes, E.E. *et al.* (2017) Activation of STING-dependent innate immune signaling by S-phase-specific DNA damage in breast cancer. *J. Natl. Cancer Inst.* 109, djw199
49. Heijink, A.M. *et al.* (2019) BRCA2 deficiency instigates cGAS-mediated inflammatory signaling and confers sensitivity to tumor necrosis factor-alpha-mediated cytotoxicity. *Nat. Commun.* 10, 100
50. Sistigu, A. *et al.* (2014) Cancer cell–autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nat. Med.* 20, 1301–1309
51. Legrier, M.E. *et al.* (2016) Activation of IFN/STAT1 signalling predicts response to chemotherapy in oestrogen receptor-negative breast cancer. *Br. J. Cancer* 114, 177–187
52. Samstein, R.M. *et al.* (2020) Mutations in BRCA1 and BRCA2 differentially affect the tumor microenvironment and response to checkpoint blockade immunotherapy. *Nat. Cancer* 1, 1188–1203
53. Zhou, Z. and Li, M. (2021) Evaluation of BRCA1 and BRCA2 as indicators of response to immune checkpoint inhibitors. *JAMA Netw. Open* 4, e217728
54. Kraya, A.A. *et al.* (2019) Genomic signatures predict the immunogenicity of BRCA-deficient breast cancer. *Clin. Cancer Res.* 25, 4363–4374
55. Heindl, A. *et al.* (2018) Microenvironmental niche divergence shapes BRCA1-dysregulated ovarian cancer morphological plasticity. *Nat. Commun.* 9, 3917
56. Schoonen, P.M. *et al.* (2017) Progression through mitosis promotes PARP inhibitor-induced cytotoxicity in homologous recombination-deficient cancer cells. *Nat. Commun.* 8, 15981
57. Feng, W. and Jasin, M. (2017) BRCA2 suppresses replication stress-induced mitotic and G1 abnormalities through homologous recombination. *Nat. Commun.* 8, 525
58. Laulier, C. *et al.* (2011) The relative efficiency of homology-directed repair has distinct effects on proper anaphase chromosome separation. *Nucleic Acids Res.* 39, 5935–5944
59. Löbrich, M. and Jeggo, P.A. (2007) The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. *Nat. Rev. Cancer* 7, 861–869
60. Lewis, C.W. and Golsteyn, R.M. (2016) Cancer cells that survive checkpoint adaptation contain micronuclei that harbor damaged DNA. *Cell Cycle* 15, 3131–3145
61. Umbreit, N.T. *et al.* (2020) Mechanisms generating cancer genome complexity from a single cell division error. *Science* 368, eaba0712
62. Crasta, K. *et al.* (2012) DNA breaks and chromosome pulverization from errors in mitosis. *Nature* 482, 53–58
63. Zhang, C.-Z. *et al.* (2015) Chromothripsis from DNA damage in micronuclei. *Nature* 522, 179–184
64. Hatch, E.M. *et al.* (2013) Catastrophic nuclear envelope collapse in cancer cell micronuclei. *Cell* 154, 47–60
65. Chen, Q. *et al.* (2016) Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. *Nat. Immunol.* 17, 1142–1149
66. Chabanon, R.M. *et al.* (2019) PARP inhibition enhances tumor cell-intrinsic immunity in ERCC1-deficient non-small cell lung cancer. *J. Clin. Invest.* 129, 1211–1228

67. Harding, S.M. *et al.* (2017) Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature* 548, 466–470
68. Mackenzie, K.J. *et al.* (2017) cGAS surveillance of micronuclei links genome instability to innate immunity. *Nature* 548, 461–465
69. Bakhoum, S.F. *et al.* (2018) Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* 553, 467–472
70. Mohr, L. *et al.* (2021) ER-directed TREX1 limits cGAS activation at micronuclei. *Mol. Cell* 81, 724–738
71. Shen, Y.J. *et al.* (2015) Genome-derived cytosolic DNA mediates type I interferon-dependent rejection of B cell lymphoma cells. *Cell Rep.* 11, 460–473
72. Mackenzie, K.J. *et al.* (2016) Ribonuclease H2 mutations induce a cGAS/STING-dependent innate immune response. *EMBO J.* 35, 831–844
73. Pokatayev, V. *et al.* (2016) RNase H2 catalytic core Aicardi-Goutières syndrome-related mutant invokes cGAS-STING innate immune-sensing pathway in mice. *J. Exp. Med.* 213, 329–336
74. Reisländer, T. *et al.* (2019) BRCA2 abrogation triggers innate immune responses potentiated by treatment with PARP inhibitors. *Nat. Commun.* 10, 3143
75. Li, T. *et al.* (2021) Phosphorylation and chromatin tethering prevent cGAS activation during mitosis. *Science* 371, eabc5386
76. Zierhut, C. *et al.* (2019) The cytoplasmic DNA sensor cGAS promotes mitotic cell death. *Cell* 178, 302–315
77. Kujirai, T. *et al.* (2020) Structural basis for the inhibition of cGAS by nucleosomes. *Science* 370, 455–458
78. Pathare, G.R. *et al.* (2020) Structural mechanism of cGAS inhibition by the nucleosome. *Nature* 587, 668–672
79. Michalski, S. *et al.* (2020) Structural basis for sequestration and autoinhibition of cGAS by chromatin. *Nature* 587, 678–682
80. Boyer, J.A. *et al.* (2020) Structural basis of nucleosome-dependent cGAS inhibition. *Science* 370, 450–454
81. Flynn, P.J. *et al.* (2021) Chromatin bridges, not micronuclei, activate cGAS after drug-induced mitotic errors in human cells. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2103585118
82. Coquel, F. *et al.* (2018) SAMHD1 acts at stalled replication forks to prevent interferon induction. *Nature* 557, 57–61
83. Chatzidoukaki, O. *et al.* (2021) R-loops trigger the release of cytoplasmic ssDNAs leading to chronic inflammation upon DNA damage. *Sci. Adv.* 7, eabj5769
84. Gilmore, T.D. (2006) Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene* 25, 6680–6684
85. Stephanou, A. *et al.* (2000) Opposing actions of STAT-1 and STAT-3 on the Bcl-2 and Bcl-x promoters. *Cell Death Differ.* 7, 329–330
86. Denis, M. (1991) Interferon-gamma-treated murine macrophages inhibit growth of tubercle bacilli via the generation of reactive nitrogen intermediates. *Cell. Immunol.* 132, 150–157
87. Gidlund, M. *et al.* (1978) Enhanced NK cell activity in mice injected with interferon and interferon inducers. *Nature* 273, 759–761
88. Luft, T. *et al.* (1998) Type I IFNs enhance the terminal differentiation of dendritic cells. *J. Immunol.* 161, 1947–1953
89. Vitale, M. *et al.* (2005) NK-dependent DC maturation is mediated by TNFalpha and IFNgamma released upon engagement of the NKp30 triggering receptor. *Blood* 106, 566–571
90. Morandi, B. *et al.* (2009) NK cells provide helper signal for CD8+ T cells by inducing the expression of membrane-bound IL-15 on DCs. *Int. Immunol.* 21, 599–606
91. Tough, D.F. *et al.* (1996) Induction of bystander T cell proliferation by viruses and type I interferon in vivo. *Science* 272, 1947–1950
92. Huber, J.P. and Farrar, J.D. (2011) Regulation of effector and memory T-cell functions by type I interferon. *Immunology* 132, 466–474
93. Ceccaldi, R. *et al.* (2015) Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair. *Nature* 518, 258–262
94. Zatreanu, D. *et al.* (2021) Polθ inhibitors elicit BRCA-gene synthetic lethality and target PARP inhibitor resistance. *Nat. Commun.* 12, 3636
95. Wang, A. *et al.* (2019) Abstract I14: Polymerase theta synthetic lethal interaction in homologous recombination-deficient pancreatic ductal adenocarcinoma. *Cancer Res.* 79, I14
96. Adam, S. *et al.* (2021) CIP2A is a prime synthetic-lethal target for BRCA-mutated cancers. *bioRxiv* Published online February 8, 2021. https://doi.org/10.1101/2021.02.08.430060
97. De Marco Zompit, M. *et al.* (2021) The CIP2A-TOPBP1 complex safeguards chromosomal stability during mitosis. *bioRxiv* Published online February 8, 2021. https://doi.org/10.1101/2021.02.08.430274
98. Ray Chaudhuri, A. *et al.* (2016) Replication fork stability confers chemoresistance in BRCA-deficient cells. *Nature* 535, 382–387
99. Rondinelli, B. *et al.* (2017) EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation. *Nat. Cell Biol.* 19, 1371–1378
100. Yang, Y.-G. *et al.* (2007) Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease. *Cell* 131, 873–886
101. Konno, H. *et al.* (2018) Suppression of STING signaling through epigenetic silencing and missense mutation impedes DNA damage mediated cytokine production. *Oncogene* 37, 2037–2051
102. Falahat, R. *et al.* (2021) Epigenetic reprogramming of tumor cell-intrinsic STING function sculpts antigenicity and T cell recognition of melanoma. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2013598118
103. De Queiroz, N.M.G.P. *et al.* (2019) Ovarian cancer cells commonly exhibit defective STING signaling which affects sensitivity to viral oncolysis. *Mol. Cancer Res.* 17, 974–986
104. Lau, A. *et al.* (2015) DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway. *Science* 350, 568–571
105. Ghosh, M. *et al.* (2021) Mutant p53 suppresses innate immune signaling to promote tumorigenesis. *Cancer Cell* 39, 494–508
106. Bernards, R. *et al.* (1986) N-myc amplification causes down-modulation of MHC class I antigen expression in neuroblastoma. *Cell* 47, 667–674
107. Muthalagu, N. *et al.* (2020) Repression of the type I interferon pathway underlies MYC- and KRAS-dependent evasion of NK and B cells in pancreatic ductal adenocarcinoma. *Cancer Discov.* 10, 872–887
108. Sodir, N.M. *et al.* (2020) MYC instructs and maintains pancreatic adenocarcinoma phenotype. *Cancer Discov.* 10, 588–607
109. Zimmerli, D. *et al.* (2021) MYC promotes immune-suppression in TNBC via inhibition of IFN signaling. *bioRxiv* Published online February 24, 2021. https://doi.org/10.1101/2021.02.24.432659
110. Annunziato, S. *et al.* (2019) Comparative oncogenomics identifies combinations of driver genes and drug targets in BRCA1-mutated breast cancer. *Nat. Commun.* 10, 397
111. Ham, S. *et al.* (2020) The impact of the cancer microenvironment on macrophage phenotypes. *Front. Immunol.* 11, 1308
112. Soslow, R.A. *et al.* (2012) Morphologic patterns associated with BRCA1 and BRCA2 genotype in ovarian carcinoma. *Mod. Pathol.* 25, 625–636
113. Calagua, C. *et al.* (2021) A subset of localized prostate cancer displays an immunogenic phenotype associated with losses of key tumor suppressor genes. *Clin. Cancer Res.* 27, 4836–4847
114. Matsuda, M. *et al.* (1994) Interleukin 10 pretreatment protects target cells from tumor- and allo-specific cytotoxic T cells and downregulates HLA class I expression. *J. Exp. Med.* 180, 2371–2376
115. Kortlever, R.M. *et al.* (2017) Myc cooperates with Ras by programming inflammation and immune suppression. *Cell* 171, 1301–1315
116. Liang, H. *et al.* (2017) Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. *Nat. Commun.* 8, 1736
117. Grabosch, S. *et al.* (2019) Cisplatin-induced immune modulation in ovarian cancer mouse models with distinct inflammation profiles. *Oncogene* 38, 2380–2393
118. Wang, W. *et al.* (2019) Upregulation of PD-L1 via HMGB1-activated IRF3 and NF-κB contributes to UV radiation-induced immune suppression. *Cancer Res.* 79, 2909–2922
119. Fu, J. *et al.* (2015) STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade. *Sci. Transl. Med.* 7, 283ra52
120. Corrales, L. *et al.* (2016) The host STING pathway at the interface of cancer and immunity. *J. Clin. Invest.* 126, 2404–2411
121. Hou, Y. *et al.* (2018) Non-canonical NF-κB antagonizes STING sensor-mediated DNA sensing in radiotherapy. *Immunity* 49, 490–503

122. Abe, T. and Barber, G.N. (2014) Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF-κB activation through TBK1. *J. Virol.* 88, 5328–5341
123. Yu, H. *et al.* (2020) Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduct. Target. Ther.* 5, 209
124. Cabrita, R. *et al.* (2020) Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature* 577, 561–565
125. Helmink, B.A. *et al.* (2020) B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* 577, 549–555
126. Li, J. *et al.* (2021) Metastasis and immune evasion from extracellular cGAMP hydrolysis. *Cancer Discov.* 11, 1212–1227
127. Kato, K. *et al.* (2018) Structural insights into cGAMP degradation by ecto-nucleotide pyrophosphatase phosphodiesterase 1. *Nat. Commun.* 9, 4424
128. Vijayan, D. *et al.* (2017) Targeting immunosuppressive adenosine in cancer. *Nat. Rev. Cancer* 17, 709–724
129. Carozza, J.A. *et al.* (2020) Structure-aided development of small-molecule inhibitors of ENPP1, the extracellular phosphodiesterase of the immunotransmitter cGAMP. *Cell Chem. Biol.* 27, 1347–1358
130. Baird, J. *et al.* (2018) MV-626, a potent and selective inhibitor of ENPP1 enhances STING activation and augments T-cell mediated anti-tumor activity in vivo. *J. Immunother. Cancer* 6, P410
131. Konstantinopoulos, P.A. *et al.* (2019) Single-arm phases 1 and 2 trial of niraparib in combination with pembrolizumab in patients with recurrent platinum-resistant ovarian carcinoma. *JAMA Oncol.* 5, 1141–1149
132. Vinayak, S. *et al.* (2019) Abstract PD5-02: durability of clinical benefit with niraparib + pembrolizumab in patients with advanced triple-negative breast cancer beyond BRCA: (TOPACIO/Keynote-162). *Cancer Res.* 79, PD5-02
133. Hiam-Galvez, K.J. *et al.* (2021) Systemic immunity in cancer. *Nat. Rev. Cancer* 21, 345–359
134. Shaked, Y. (2019) The pro-tumorigenic host response to cancer therapies. *Nat. Rev. Cancer* 19, 667–685
135. Mehta, A.K. *et al.* (2021) Targeting immunosuppressive macrophages overcomes PARP inhibitor resistance in BRCA1-associated triple-negative breast cancer. *Nat. Cancer* 2, 66–82
136. Li, Y. *et al.* (2021) Comprehensive analysis of regulatory factors and immune-associated patterns to decipher common and BRCA1/2 mutation-type-specific critical regulation in breast cancer. *Front. Cell Dev. Biol.* 9, 750897
137. Jenzer, M. *et al.* (2019) The BRCA2 mutation status shapes the immune phenotype of prostate cancer. *Cancer Immunol. Immunother.* 68, 1621–1633
138. Davoli, T. *et al.* (2017) Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. *Science* 355, eaaf8399
139. Dill, E.A. *et al.* (2018) IDO expression in breast cancer: an assessment of 281 primary and metastatic cases with comparison to PD-L1. *Mod. Pathol.* 31, 1513–1522
140. Munn, D.H. *et al.* (2005) GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2, 3-dioxygenase. *Immunity* 22, 633–642
141. Smith, C. *et al.* (2012) IDO is a nodal pathogenic driver of lung cancer and metastasis development. *Cancer Discov.* 2, 722–735
142. Long, G.V. *et al.* (2019) Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. *Lancet Oncol.* 20, 1083–1097
143. Rahma, O.E. and Hodi, F.S. (2019) The intersection between tumor angiogenesis and immune suppression. *Clin. Cancer Res.* 25, 5449–5457
144. Ruscito, I. *et al.* (2018) Characterisation of tumour microvessel density during progression of high-grade serous ovarian cancer: clinico-pathological impact (an OCTIPS Consortium study). *Br. J. Cancer* 119, 330–338
145. Yang, H. *et al.* (2019) STING activation reprograms tumor vasculatures and synergizes with VEGFR2 blockade. *J. Clin. Invest.* 129, 4350–4364
146. Ray-Coquard, I. *et al.* (2019) Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N. Engl. J. Med.* 381, 2416–2428
147. Knouse, K.A. *et al.* (2018) Chromosome segregation fidelity in epithelia requires tissue architecture. *Cell* 175, 200–211
148. López-Carrasco, A. *et al.* (2020) Impact of extracellular matrix stiffness on genomic heterogeneity in MYCN-amplified neuroblastoma cell line. *J. Exp. Clin. Cancer Res.* 39, 226
149. DeLeon-Pennell, K.Y. *et al.* (2020) Fibroblasts: the arbiters of extracellular matrix remodeling. *Matrix Biol.* 91–92, 1–7
150. Shaashua, L. *et al.* (2021) BRCA mutational status shapes the stromal microenvironment of pancreatic cancer linking CLU+ CAF expression with HSF1 signaling. *bioRxiv* Published online August 19, 2021. https://doi.org/10.1101/2021.08.18.456576
151. Pickup, M.W. *et al.* (2014) The extracellular matrix modulates the hallmarks of cancer. *EMBO Rep.* 15, 1243–1253
152. Manguso, R.T. *et al.* (2017) In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nature* 547, 413–418
153. Patel, S.J. *et al.* (2017) Identification of essential genes for cancer immunotherapy. *Nature* 548, 537–542
154. Bakhoum, S.F. and Cantley, L.C. (2018) The multifaceted role of chromosomal instability in cancer and its microenvironment. *Cell* 174, 1347–1360
155. Pantelidou, C. *et al.* (2019) PARP inhibitor efficacy depends on CD8+ T-cell recruitment via intratumoral STING pathway activation in BRCA-deficient models of triple-negative breast cancer. *Cancer Discov.* 9, 722–737
156. Wang, Z. *et al.* (2019) Niraparib activates interferon signaling and potentiates anti-PD-1 antibody efficacy in tumor models. *Sci. Reports* 9, 1–12
157. Fang, Y. *et al.* (2019) Sequential therapy with PARP and WEE1 inhibitors minimizes toxicity while maintaining efficacy. *Cancer Cell* 35, 851
158. Kim, H. *et al.* (2020) Combining PARP with ATR inhibition overcomes PARP inhibitor and platinum resistance in ovarian cancer models. *Nat. Commun.* 11, 1–16
159. Kim, H. *et al.* (2017) Targeting the ATR/CHK1 axis with PARP inhibition results in tumor regression in BRCA-mutant ovarian cancer models. *Clin. Cancer Res.* 23, 3097–3108
160. Schoonen, P.M. *et al.* (2019) Premature mitotic entry induced by ATR inhibition potentiates olaparib inhibition-mediated genomic instability, inflammatory signaling, and cytotoxicity in BRCA2-deficient cancer cells. *Mol. Oncol.* 13, 2422–2440
161. Pantelidou, C. *et al.* (2021) STING agonism enhances anti-tumor immune responses and therapeutic efficacy of PARP inhibition in BRCA-associated breast cancer. *bioRxiv* Published online January 27, 2021. https://doi.org/10.1101/2021.01.26.428337
162. Jang, S.C. *et al.* (2021) ExoSTING, an extracellular vesicle loaded with STING agonists, promotes tumor immune surveillance. *Commun. Biol.* 4, 497