The complementarity of DDR, nucleic acids and anti-tumour immunity

https://doi.org/10.1038/s41586-023-06069-6

Received: 19 February 2021

Accepted: 11 April 2023

Published online: 19 July 2023

Check for updates

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Immune checkpoint blockade (ICB) immunotherapy is a first-line treatment for selected cancers, yet the mechanisms of its efficacy remain incompletely understood. Furthermore, only a minority of patients with cancer benefit from ICB, and there is a lack of fully informative treatment response biomarkers. Selectively exploiting defects in DNA damage repair is also a standard treatment for cancer, spurred by enhanced understanding of the DNA damage response (DDR). DDR and ICB are closely linked—faulty DDR produces immunogenic cancer neoantigens that can increase the efficacy of ICB therapy, and tumour mutational burden is a good but imperfect biomarker for the response to ICB. DDR studies in ICB efficacy initially focused on contributions to neoantigen burden. However, a growing body of evidence suggests that ICB efficacy is complicated by the immunogenic effects of nucleic acids generated from exogenous DNA damage or endogenous processes such as DNA replication. Chemotherapy, radiation, or selective DDR inhibitors (such as PARP inhibitors) can generate aberrant nucleic acids to induce tumour immunogenicity independently of neoantigens. Independent of their functions in immunity, targets of immunotherapy such as cyclic GMP–AMP synthase (cGAS) or PD-L1 can crosstalk with DDR or the DNA repair machinery to influence the response to DNA-damaging agents. Here we review the rapidly evolving, multifaceted interfaces between DDR, nucleic acid immunogenicity and immunotherapy efficacy, focusing on ICB. Understanding these interrelated processes could explain ICB treatment failures and reveal novel exploitable therapeutic vulnerabilities in cancers. We conclude by addressing major unanswered questions and new research directions.

It is now well appreciated that aberrantly misplaced DNA generated from faulty DDR or excessive exogenous-induced DNA damage, independent of mutational burden, can induce anti-tumour immunity through the activation of primitive pathogen pattern recognition receptors (for example, cGAS–STING) in cancer cells[1]. Here we review mechanisms for the generation of immunogenic nucleic acids, such as DNA, that can stimulate anti-tumour immunity, focusing on how cells manage double strand DNA breaks, the most lethal form of DNA damage. We discuss therapeutic strategies, including DDR inhibitors, that can induce immunogenic nucleic acid accumulation and highlight cancer cell counter-mechanisms that limit nucleic acid immunogenicity. The recent insights linking DDR, nucleic acids and anti-tumour immunity have prompted clinical interest in—and approvals by the US Food and Drug Administration (FDA) for—treatment strategies that combine DNA-damaging therapies with ICB. We review recent advances in this area and discuss specific DDR-based strategies to enhance the efficacy of combinatorial ICB treatment.

DNA undergoes billions of replication steps in trillions of cells throughout life, inevitably producing replication errors, and is also subject to DNA damage from environmental or endogenous insults[2]. The resulting DNA lesions must be repaired for cellular homeostasis and organismal survival. Generally, the DDR recognizes DNA damage and initiates DNA repair mechanisms through an intricate network of damage sensors and repair molecules that cooperate to maintain genomic integrity[3]. DNA damage resulting in mutations in protein-coding genes is a fundamental mechanism driving cellular transformation, but remarkably, this same cancer-initiating mechanism can generate novel immunogenic molecules (neoantigens) that are capable of stimulating anti-tumour immunity[4,5]. Carcinogen-induced immunodeficient mouse models and studies of immunosuppressed individuals demonstrate the immune system's exquisite capability of detecting and specifically eliminating nascently transformed cells[6], a process termed cancer immunosurveillance[7].

Although mutations resulting from a defective DDR can generate immunogenic neoantigens[8], spontaneous immune rejection of clinically apparent cancers is rare, as tumours avoid, reduce or subvert all elements of immune surveillance through multiple mechanisms[9] (Fig. 1), including by promoting the expression of inhibitory immune checkpoint molecules such as PD-L1. The therapeutic application of antibodies that block PD-1, PD-L1, LAG3 or CTLA4 is termed ICB[10,11], and works by improving the function of tumour-specific $CD8^+$ T cells, among other mechanisms[12,13]. Cancers with high tumour mutational

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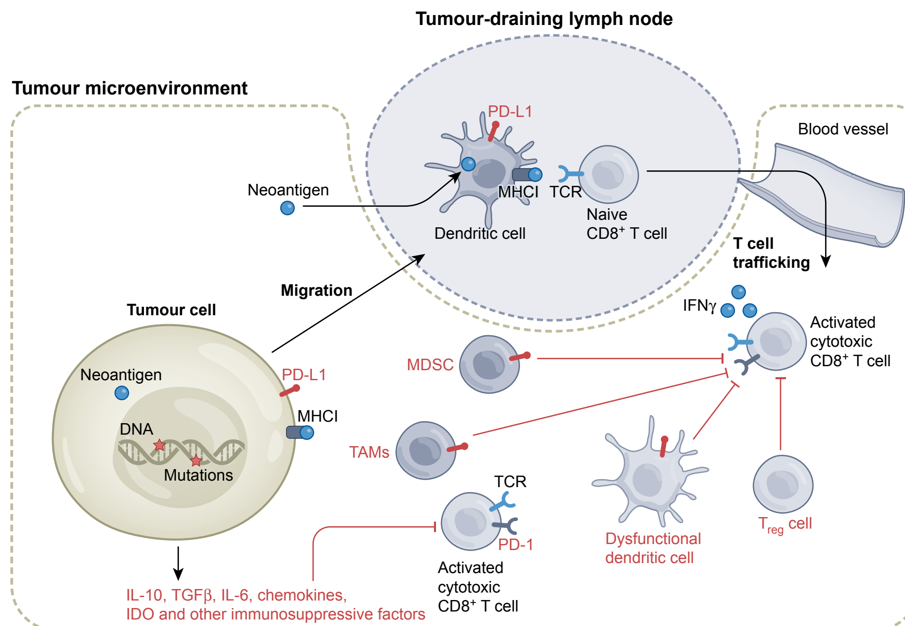

Fig. 1 | Anti-tumour immunity. The defence against evolving cancers by tumour immune surveillance is mediated primarily by adaptive (antigen-specific) lymphocytes, notably $CD8^+$ T cells, and by immune cytokines, notably interferon-γ (IFNγ). Antigen-agnostic innate immune cells such as natural killer cells also participate. Cancer neoantigens loaded onto MHCI molecules mediate highly specific recognition of malignant cells by adaptive immunity, particularly $CD8^+$ cytotoxic T cells. For effective tumour control, neoantigens must be released into the microenvironment, captured by antigen-presenting cells (such as dendritic cells) and presented to naive T cells in tumour-draining lymph nodes or in the tumour itself. Once activated, $CD8^+$ cytotoxic T cells must traffic into the tumour microenvironment and eliminate tumours by producing interferon-γ, perforin, granzymes and other effector molecules. Historically, the paradigm for effective anti-tumour immunity has proposed that the presence of $CD8^+$ cytotoxic T cells, increased by positive regulators such as neoantigen availability and antigen presentation, predicts the ability to control tumours. However, the tumour microenvironment reduces the efficacy of anti-tumour immune cells through myriad mechanisms. Immune cells in draining lymph nodes can become dysfunctional as a direct result of tumour cells migrating there, or indirectly by the tumour altering their function before they migrate to the lymph node. Negative regulators of $CD8^+$ cytotoxic T cell function (shown in red) such as PD-1, PD-L1, CTLA4, LAG3, T regulatory (T_{reg}) cells, tumour-associated macrophages (TAMs), MDSCs (myeloid-derived suppressor cells) or secreted factors are at least as important for controlling anti-tumour immunity as the presence of increased numbers of $CD8^+$ cytotoxic T cells. This is evidenced by the remarkable efficacy of ICB inhibitors (antibodies blocking CTLA4, PD-1, PD-L1 or LAG3) in humans, providing a mechanism for tumour immune escape. Note that the immune cells shown are depicted as suppressing cytotoxic T cells through PD-L1 for simplicity, whereas there are many potential inhibitory immune checkpoints in the tumour microenvironment. TCR, T cell receptor.

burden (TMB) occur as a result of exposure to carcinogens, such as in melanoma, lung and bladder cancer[8], or from a deficiency in DNA mismatch repair (MMR) proteins (such as MLH1 or MSH6) and/or owing to microsatellite instability[14]. Tumours with MMR deficiency or high TMB respond better to ICB than tumours that are MMR-proficient or have low TMB[15,16], presumably owing to higher levels of (immunogenic) neoantigens. Conversely, enhanced expression of DNA repair proteins (for example, MSH2) can correlate with reduced tumour infiltration by $CD8^+$ T cells and ICB response failure, as demonstrated in melanoma[17]. Historically, the FDA has approved therapies on the basis of tumour histology and not molecular signatures, but the anti-PD-1 antibodies pembrolizumab, nivolumab, and dostarlimab are now FDA-approved for MMR-deficient and/or TMB-high tumours regardless of histology[18,19]. Additionally, clinical studies of several cancers demonstrating paradoxical synergy between chemotherapy, which is considered immunosuppressive, and ICB, which is immune-stimulatory (Table 1), further suggest a beneficial link between DDR or DNA repair and anti-tumour immunity that can be exploited therapeutically.

Although faulty DDR and consequently increased TMB correlate with improved responses to ICB[10,15,16], many MMR-deficient[15,20] or TMB-high[19] cancers are largely unresponsive to ICB. Furthermore, there is no direct evidence for the idea that MMR deficiency dictates tumour immunogenicity in humans by increasing neoantigens. Given the rapid clinical response to combination chemotherapy and ICB relative to the slower rate of chemotherapy-induced accumulation of mutations in tumours, the hypothesis that exogenous induction of DNA damage enhances ICB efficacy and anti-tumour immunity by simply increasing the number of cancer-associated neoantigens remains controversial[21]. Further, carcinogen-driven cancers such as skin (melanoma), lung and bladder cancers can be MMR-proficient with variable TMB yet respond durably to ICB[22,23]. These observations implicate additional pathways intimately linking DDR or DNA repair and anti-tumour immunity beyond TMB or mismatch repair status alone. Understanding this complex interplay may help identify previously undefined ICB response factors, explain development of treatment resistance and improve rational combinatorial ICB approaches.

Immunogenic DNA accumulation in cancer

Damaged nuclear DNA can be immunogenic when fragments spill into the cytoplasm, mimicking viral infection[24]. The cytosolic innate immune mediator cGAS binds to these self-DNA species and stimulates STING-dependent production of type I interferon to improve anti-tumour immunity primarily by activating and recruiting tumour-specific $CD8^+$ T cells[1,25] (Fig. 1). Cancer cells with inherently defective DDR (Fig. 2a) or those exposed to exogenous DNA damage accumulate immunogenic cytoplasmic DNA fragments, but can simultaneously inhibit cGAS–STING through various mechanisms to avoid immune detection[26] (detailed in 'Antagonization of DNA immunogenicity'). These observations support a role for cytoplasmic DNA sensing by cGAS–STING in immune surveillance and suggest a critical link between DNA damage and anti-tumour immunity beyond TMB. The double

Table 1 | Landmark phase III clinical trials testing the efficacy of concurrent chemotherapy plus ICB

Study	Tumour	Design	Endpoints	Biomarkers
IMpassion-130 (ref. 133)	Previously untreated metastatic TNBC	Atezolizumab plus nanoparticle albumin-bound (nab)-paclitaxel versus placebo plus nab-paclitaxel	PFS, failed to reach OS in final analysis[134]	PFS and OS further enhanced in PD-L1⁺ subgroup (defined as PD-L1 expression on ≥1% of tumour-infiltrating immune cells)
Keynote-355 (ref. 135)	Previously untreated metastatic or recurrent TNBC	Pembrolizumab plus investigator's choice chemotherapy (nanoparticle nab-paclitaxel, paclitaxel or gemcitabine plus carboplatin) versus placebo plus chemotherapy	PFS, OS	OS benefit observed with CPS >10 but not in the CPS >1 subgroup (a composite score of PD-L1 expression on tumour-infiltrating immune cells and/or tumour cells)
Checkpoint-648 (refs. 136,137)	Untreated, unresectable advanced, recurrent or metastatic oesophageal squamous cell carcinoma	Nivolumab plus fluorouracil and cisplatin versus fluorouracil and cisplatin	PFS, OS	PFS and OS further enhanced in PD-L1⁺ subgroup (defined as PD-L1 expression on ≥1% of tumour cells)
CASPIAN[138]	Extensive-stage small cell lung cancer	Durvalumab plus platinum-etoposide versus platinum-etoposide	OS	TMB was not predictive of treatment response
IMpower-133 (ref. 139)	Extensive-stage small cell lung cancer	Atezolizumab plus carboplatin and etoposide versus carboplatin and etoposide	PFS, OS	PD-L1 status not assessed, TMB assessed but not predictive of treatment response
Keynote-189 (ref. 140)	Metastatic non-squamous NSCLC without sensitizing *EGFR* or *ALK* mutations	Pembrolizumab plus platinum-pemetrexed versus platinum-pemetrexed	PFS, OS	OS enhanced regardless of PD-L1 status (defined as percentage of membranous PD-L1 expression in tumour cells)
IMpower-150 (ref. 141)	Metastatic non-squamous NSCLC with or without sensitizing *EGFR* or *ALK* mutations	Atezolizumab plus bevacizumab, carboplatin and paclitaxel versus bevacizumab, carboplatin and paclitaxel	PFS, OS	PFS and OS enhanced in combination group regardless of PD-L1 expression, effector T cell gene signature, or *EGFR* or *ALK* status
CheckMate-9LA[142]	Advanced non-small-cell lung cancer	Nivolumab, ipilimumab plus short course (2 cycles) of platinum doublet chemotherapy versus platinum doublet chemotherapy	PFS, OS	OS enhanced regardless of PD-L1 expression or histology (squamous or non-squamous)

CPS is the combined PD-L1 score, calculated as the sum of PD-L1⁺ tumour cells, lymphocytes or macrophages divided by the total number of viable tumour cells, multiplied by 100. NSCLC, non-small-cell lung cancer; OS, overall survival; PFS, progression-free survival, TNBC, triple-negative breast cancer.

strand break (DSB), a lethal form of DNA damage, is a major precursor lesion for cGAS-activatable DNA fragments in cancer. DSB repair mechanisms involved in immunogenic DNA production include error-prone non-homologous end joining (NHEJ) or template-dependent homologous recombination (HR)[27] (Box 1).

Exogenous DNA damage from large genotoxic insults like therapeutic radiation can sever chromosomal DNA, forming numerous DSBs and thus directly generating cGAS-activatable double stranded DNA (dsDNA) fragments. NHEJ (Fig. 2b) attempts to ligate DSBs rapidly, independent of cell cycle stage, but can inadvertently produce aberrantly rearranged chromosomal fragments[28]. These structurally abnormal fragments mis-segregate during anaphase, forming micronuclei that rupture and release genomic dsDNA into the cytoplasm, which activates cGAS–STING[29]. Micronuclei harbouring immunogenic DNA could similarly form without exogenous DNA damage in cells exhibiting baseline chromosomal instability[30], a common outcome of DDR (for example, caused by *ATM* mutations[31]) or DSB repair (for example, caused by *BRCA2* mutations) mutant gene cancers[32]. Since formation of DSB-induced micronuclei increases with accelerated mitotic progression, rational targeting of the cell cycle could improve cancer immunotherapy[24]. Failure to eliminate DSBs eventually induces apoptosis, which classically provokes chromosome fragmentation[33]. These apoptosis-dependent dsDNA fragments can be circularized by DNA ligase III, forming extrachromosomal episomes[34]. Circular dsDNA triggers production of type I interferon more potently than linear DNA in normal cells[34] but the effects in cancer cells remain undescribed.

DNA damage can also induce the accumulation of cytosolic single stranded DNA (ssDNA), which activates cGAS–STING when DSBs undergo end resection, a key initiating and regulatory step for HR (Box 1). End resection is promoted by BRCA1 and mediated by nucleases such as EXO1 and MRE11 (ref. 27), which resect the DSB to yield a 3′ ssDNA overhang (Fig. 2b). Breast cancer cells co-depleted of EXO1 and the end resection co-factor BLM do not accumulate cGAS-activating cytosolic ssDNA during S and G2 phases (when HR is most active), either basally or following irradiation[35]. Although DSBs can form from exogenous genotoxic insults, DNA replication forks encountering DNA lesions (for example, crosslinks), transcription machinery or depleted nucleotide pools can stall and ultimately collapse, forming one-ended DSBs[36]. These endogenous replication fork-associated DSBs require HR-mediated repair[27]. Cancer cells experiencing significant DNA replication stress and/or nuclease driven hyper-resection of unprotected DSBs can accumulate around tenfold more cytoplasmic ssDNA than dsDNA[31,37]. The recent discovery that MLH1 protects endogenously derived DSBs from hyper-resection by EXO1 to prevent cytosolic DNA accumulation[37] provides a molecular mechanism for cGAS–STING activation and intrinsic ICB sensitivity in MMR-defective tumours independent of TMB[38,39]. Replication forks that encountering the transcription machinery can also yield R-loops, RNA–DNA hybrids that are cGAS substrates when cytoplasmic[40] and are increased in rapidly dividing cells[41]. A gene signature for DNA replication stress correlated with increased type I interferon expression and ICB responsiveness in several human clinical trials[42], suggesting that agents targeting DNA replication could enhance ICB efficacy.

Immunogenic DNA fragments may also be derived from telomeres (chromosome ends resembling DSBs). Aberrant telomere maintenance—particularly in the approximately 15% of cancers that are dependent on the alternative lengthening of telomere (ALT) pathway for telomere maintenance—can generate cytoplasmic single and double stranded extrachromosomal telomere repeat DNA[43]. How these cGAS-activatable DNA substrates accumulate in ALT cells is not fully understood, but several factors associated with HR (such as XRCC3 and NBS1 (ref. 44)

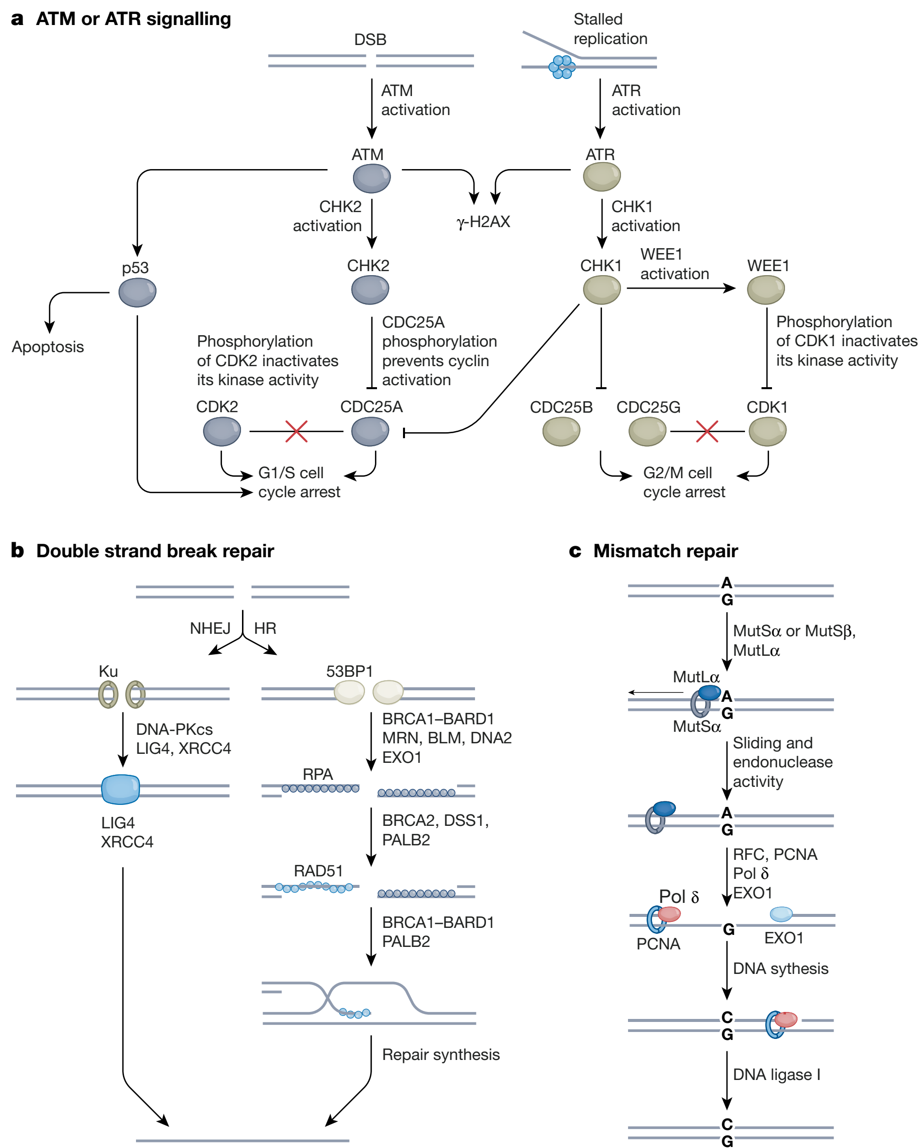

Fig. 2 | Outline of DDR and DNA repair. a, Recognition of DSBs or stalled replication forks begins a kinase-driven cell signalling cascade through ATM or ATR, respectively. ATM and ATR modulate DDR by recruiting DNA repair proteins and regulate cell cycle progression with CHK1 and CHK2. The phosphorylation cascade prevents cyclin activation for cell cycle arrest, allowing time to repair DNA damage. If the DNA damage burden cannot be repaired, p53 phosphorylation can signal apoptosis. **b**, During NHEJ, DSBs are bound by Ku and DNA-PKcs, which tether DNA ends and recruit downstream factors such as XRCC4 that ultimately facilitate ligation of the DSB. NHEJ is fully operational throughout the cell cycle, whereas HR is more restricted to the S and G2 cell cycle phases, when a newly replicated sister chromatid is available to template repair. DNA end resection, facilitated by factors such as EXO1, MRE11, BRCA1 and BLM–DNA2, results in a single-stranded substrate for eventual RAD51-mediated DNA homology search. Strand invasion and repair synthesis results in an error-free product. **c**, Base mismatches are recognized by MSH proteins in humans (homologues of *Escherichia coli* MutS proteins), and subsequent endonuclease activity by MLH1 provides a substrate for EXO1 to remove mismatched bases and flanking DNA sequences. The PCNA sliding clamp promotes fill-in by DNA polymerase δ (Pol δ), with subsequent ligation by DNA ligase I.

and NHEJ (such as Ku70–Ku80 (ref. 45), hereafter referred to as Ku) factors are implicated. Nonetheless, cancers that are dependent on the ALT pathway could produce immunogenic cytosolic DNA and elicit cGAS–STING-dependent anti-tumour immunity[43,46,47] thereby rendering them sensitive to ICB. Since telomere protection is enhanced in rapidly dividing cancer cells compared with normal cells, inducing telomere dysfunction by inhibiting telomerase (a reverse transcriptase) with nucleotide chain terminators such as 6-thio-2-deoxyguanosine could improve ICB efficacy while limiting unwanted toxicity[47]. Dysfunction of upstream regulators of extratelomeric DNA production such as Tousled-like kinases 1 and 2 enhance cGAS–STING-mediated immunity independently of DNA replication[48] and merit investigation for their therapeutic potential.

The mitochondrion also harbours potentially immunogenic self-DNA[49]. Mitochondrial DDR and how mitochondrial DNA becomes cytoplasmic remain poorly understood, although canonical nuclear DDR or DNA repair proteins could influence the immunogenicity of mitochondrial DNA. For instance, ATM can regulate the transcription of genes preserving mitochondrial integrity, and ATM inhibition increases cytoplasmic mitochondrial DNA in mice[50]. HR proteins such as RAD51, BRCA1 and MRE11 localize to mitochondria, suggesting roles in maintaining mitochondrial DNA integrity[51–53]. Similar to nuclear DNA replication forks, DSBs from collapsed mitochondrial DNA replication forks can be hyper-resected by MRE11 to generate cGAS-activatable DNA substrates[54]. The relative immunogenicity of each cGAS substrate (Fig. 3) probably depends on its relative abundance, subcellular source,

Box 1

Fundamentals of DSB repair

Aberrant DSB repair is a major driver of immunogenic DNA accumulation in cancer cells. To deal with DSBs, cells initiate cascades of phosphorylation, ubiquitylation and PARylation at DSB sites to initiate cell cycle slowing[143] and recruit the DNA repair machinery[144,145] (Fig. 2a). DSBs are repaired conservatively by HR or by error-prone NHEJ[27]. HR is normally predominant in S and G2 cell cycle phases when a homologous DNA repair template is available[146]. DNA end resection is the key initiating step of HR and is facilitated by many factors, including the MRE11–RAD50–NBS1 complex (MRN), BRCA1, BARD1, CtIP, DNA2 and EXO1 (ref. 27). For HR to occur, the 5′ strand of a DSB end is resected to yield a 3′ ssDNA overhang of considerable length[27]. The ssDNA tail is rapidly coated by RPA, which is subsequently replaced with the RAD51 recombinase, with the RPA–RAD51 interaction being mediated by the BRCA2–DSS1 complex[147]. The RAD51–ssDNA nucleoprotein complex catalyses the search for a homologous template and DNA strand invasion to prime repair DNA synthesis, ultimately restoring the injured chromosome to its original form[148]. By contrast, NHEJ predominates in G1 phase and relies on 53BP1 to physically occlude the HR end resection machinery at DSB ends[149] (Fig. 2b). Driven by specific factors such as DNA-PKcs, Ku and XRCC4, NHEJ can rapidly ligate DSB ends with the potential introduction of insertion or deletion (indel) mutations[28]. Additionally, entire chromosomal translocations can occur when error-prone NHEJ aberrantly ligates DSB ends on separate chromosomes, a characteristic feature of chromosome-unstable cancers. DSBs are generally lethal forms of DNA damage if not resolved[27]. When NHEJ or HR are defective or become dysregulated, potentially immunogenic fragmented dsDNA, ssDNA or entire chromosome arms can accumulate. Erroneously inserted, deleted or misincorporated bases during DNA replication are repaired by DNA mismatch repair (Fig. 2c). Recent evidence suggests that functional crosstalk of MMR proteins occurs in DSB repair processes such as end resection[37]. DSBs thus appear to be the central DNA lesion from which immunogenic nucleic acids are created in the setting of faulty DDR (for example, owing to ATM defects), DNA repair (for example, MMR defects) or excessive DNA damage (for example, caused by radiation).

specific secondary structures, the inciting DNA-damaging insult and genetic context (for example, MMR or BRCA deficiency) among other considerations, but is little studied.

Antagonization of DNA immunogenicity

Mounting anti-pathogen immunity while restraining autoimmunity triggered by host nucleic acids is a delicate balance. Mammalian cells have evolved mechanisms to prevent cGAS–STING-mediated auto-inflammation from self-DNA. For example, nucleosomes[55] or other DNA-binding proteins such as BAF[56] physically protect chromatin from cGAS binding when nuclear envelope integrity is lost during normal physiologic processes such as cell division. Cancer cells attempt to diminish DNA immunogenicity by reducing cytosolic nucleic acid content and cGAS–STING activity (Box 2), or by rewiring type I interferon signalling to avoid immune elimination. Understanding these mechanisms could explain the development of resistance to treatment with ICB and DNA-damaging agents.

For example, mRNA levels of *CGAS* and *TMEM173* (the gene encoding STING) in tumours predicted ICB response in human MMR-deficient colorectal cancers[39] Analysis of the The Cancer Genome Atlas (TCGA) pan-cancer database revealed a gene mutation rate[57] for *CGAS* and *TMEM173* below 1%, suggesting epigenetic downregulation of *CGAS* or *TMEM173* mRNA. In support, a study of KRAS-mutated lung cancers revealed *TMEM173* epigenetic silencing through loss of liver kinase B1 (ref. 58). Pharmacological inhibition of epigenetic regulators such as EZH2 restores STING activation to potentiate ICB in ICB-refractory mouse and human prostate cancers[59]. Thus, despite accumulating cytosolic immunogenic DNA, suppression of cGAS–STING expression could be a strategy for immune evasion in cancers with defective DDR or DNA repair.

cGAS–STING activation promotes MHCI-restricted antigen presentation and tumour-specific $CD8^+$ T cell recruitment via canonical NF-κB transcription of type I interferons to augment anti-tumour defences. Nonetheless, type I interferons can paradoxically promote tumour progression. Chronic exposure to type I interferon and persistent cGAS–STING activation enhanced spread of non-canonical NF-κB-driven metastatic breast cancer[30]. How cancer cells rewire type I interferon signalling to become pro-tumorigenic or immunosuppressive remains an important, unanswered question—this could depend on the timing and chronicity of DNA damage-driven cytosolic DNA accumulation.

Similarly, persistent activation of upstream DDR kinases such as ATM by DNA damage can drive the production of other immunosuppressive molecules (such as IL-6 (refs. 60,61)) through cellular senescence[62] and the senescence-associated secretory phenotype (SASP). Although tumour cell senescence induced by chemotherapy or irradiation appears desirable, these DNA-damaging agents could also paradoxically promote cancer immune evasion through SASP-mediated immunosuppression[63]. Agents that prevent DNA damage-induced tumour cell SASP include small molecule inhibitors of p53 (ref. 64) or mTORC1 (ref. 65) and small molecule senolytics[66], and could enhance anti-tumour immunity. Thus, cGAS–STING-independent pathways that promote or inhibit DNA damage-induced anti-tumour immune responses require further characterization, which could suggest additional therapeutic targets to restore nucleic acid immunogenicity in cGAS–STING-resistant tumours.

cGAS-independent immune activation

DDR can regulate DNA immunogenicity—for instance, independently of cGAS (Fig. 4). Upstream DDR kinases activate DNA damage repair proteins by phosphorylation, but also activate numerous non-DDR pathways including immune signalling[67]. In normal human cells, nuclear ATM drives alternative STING complex formation following etoposide-induced DNA damage, independently of cytoplasmic cGAS[68]. Nuclear DNA repair proteins (such as MRE11 (ref. 69) or MUS81 endonuclease in prostate cancer cells[70]) can translocate to the cytoplasm to activate cytosolic DNA-mediated cGAS-independent STING or type I interferons. In lymphoma cells, cytosolic DNA-PKcs can drive type I interferon production entirely independently of cGAS or STING, suggesting the existence of additional, STING-independent DNA immunogenicity regulators in human tumour cells[71,72].

Remarkably, DDR proteins can also directly regulate RNA immunogenicity (Box 3). The NHEJ factor XRCC4 promotes cytoplasmic RNA sensing through RIG-I by stabilizing RIG-I multimer formation on cytoplasmic RNA molecules during viral infection[73]. As downregulation of cGAS–STING signalling is a major mechanism facilitating tumour immune evasion, identification of novel cGAS or STING-independent mechanisms that restore nucleic acid immunogenicity could provide new ways to overcome DNA-damaging agents and/or resistance to ICB.

DDR mediators can also simultaneously drive tumour microenvironmental immunosuppression. In certain contexts, DDR factors can upregulate PD-L1 expression independently of cGAS–STING or type I

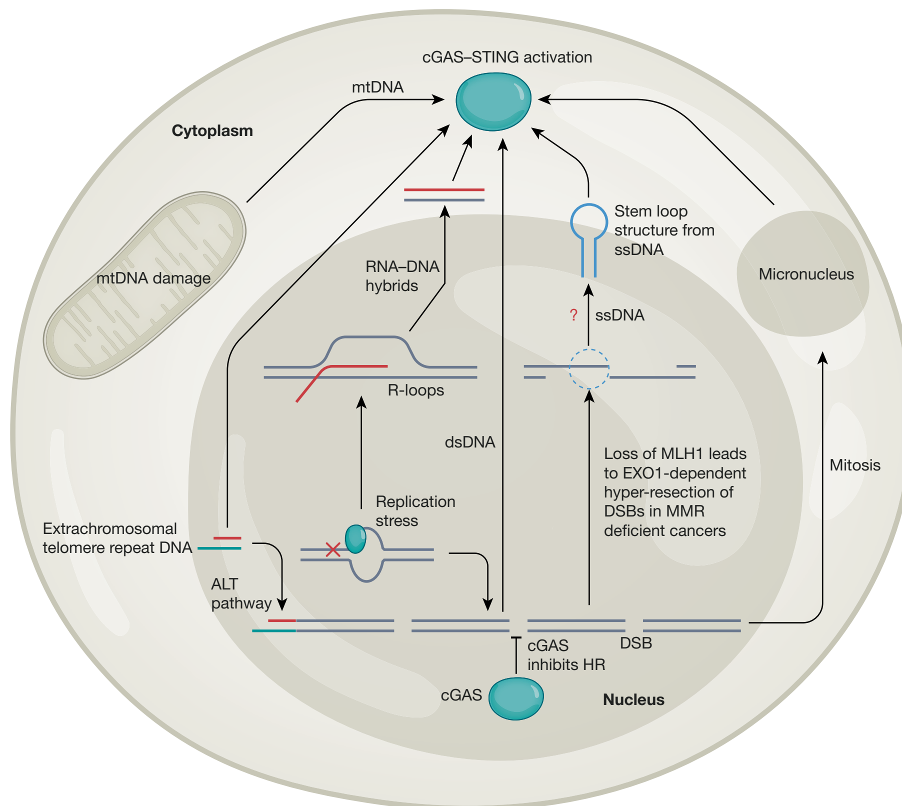

Fig. 3 | Sources of immunogenic DNA. cGAS–STING signalling in cancer cells is classically activated by cytosolic dsDNA, ssDNA that folds to form dsDNA-containing stem-loop structures or RNA–DNA hybrids derived from cellular compartments, such as nucleus or mitochondrion. Nuclear processes contributing to cGAS-activatable DNA accumulation in cancer include excessive exogenous DNA damage (such as that caused by chemotherapy), DNA replication stress, chromosome instability, telomere dysfunction, apoptosis and aberrant transcription (for example, R-loop formation). Nuclear DNA can also be aberrantly introduced into cytoplasm directly or when damaged chromosomes mis-segregate during anaphase, generating dsDNA-containing micronuclei that can subsequently rupture. The source of cytoplasmic DNA that predominantly influences nucleic acid-driven tumour immunogenicity probably depends on the genetic background of the specific tumour cell, histology, cancer stage and tumour microenvironmental context. Although cGAS is classically thought to be a cytoplasmic DNA-activatable protein, it can become nuclear and directly influence nuclear DNA damage repair and replication, highlighting a crosstalk between immune and DDR mediators. mtDNA, mitochondrial DNA.

interferons. For example, ATR–CHK1 regulates tumour PD-L1 expression following irradiation by phosphorylating STAT3, a major transcription factor regulating the expression of *CD274* (refs. 74,75) (which encodes PD-L1) expression. In breast cancer cells, ATM promotes metastases by upregulating IL-8 via NF-κB activation[76], which appears to be independent of DNA damage-induced cytosolic nucleic acids and can attract immunosuppressive neutrophils. The ATR inhibitor AZD6738 counteracted irradiation-induced PD-L1 expression to reduce immunosuppressive regulatory T cell numbers and enhance anti-tumour CD8$^+$ T cell functions in mouse tumour models in vivo[74]. Thus, strategies targeting these DDR molecules will require careful consideration of net effects.

Similarly, DNA repair mediators could have pleiotropic effects on immunity, independent of TMB or DSB-induced cGAS-activatable DNA fragments. The non-MMR mutations—such as *BRCA1* or *BRCA2* mutations in HR—can increase TMB and accumulation of cytosolic self-DNA, as in MMR deficiency, but *BRCA* mutation-associated cancers (such as breast, ovarian and prostate cancers) generally exhibit poor responses to ICB[77,78]. Furthermore, despite the epistatic relationship between BRCA1 and BRCA2 in HR, these proteins fulfil functionally distinct roles during DSB repair. Of note, *BRCA2*-mutated tumours appear to show a better response to ICB than *BRCA1*-mutated tumours in breast cancer models in mouse[79] as well as in human melanomas[80]. Notably, *BRCA2*-mutated breast cancers accumulate anti-tumour CD8$^+$ T and natural killer cells, whereas *BRCA1*-mutated tumours accumulate immunosuppressive PD-L1-expressing macrophages[81]. These observations suggest that BRCA1, BRCA2 and other DNA repair proteins have differential effects on anti-tumour immunity beyond simply influencing TMB or cGAS activation from cytosolic DNA accumulation.

The seemingly contradictory and dual effects of DDR or DNA repair mediators on immunity makes formulating a simple, grand unifying theory linking DNA damage, nucleic acids and ICB efficacy highly complex. The oncogenic context of the tumour—such as the presence of an overactivated immune suppressive Wnt–β-catenin pathway[82]—could also dictate the final consequence of faulty DNA repair (such as MMR) on anti-tumour immunity, and would require nuanced, personalized characterization in human clinical trials.

Regulation of DDR by immune mediators

Just as DDR mediators control immunity in non-canonical ways, immune mediators also regulate DDR and/or DNA repair independently of anti-tumour immunity. For instance, cGAS can translocate to the nucleus to inhibit HR, killing tumours directly[83], or promote cellular transformation[84]. Genetic cGAS deletion enhanced STING-independent, cell-intrinsic chemotherapy and irradiation sensitivity in established cancers by increasing DNA replication stress[85]. Inhibiting participation of nuclear cGAS in DNA replication or DNA repair while simultaneously facilitating the immunostimulatory effects of cytoplasmic cGAS could be useful in reversing resistance to treatment with DNA-damaging agents, including in STING-deficient tumours.

In contrast to cGAS, STING globally altered gene expression regulating reactive oxygen species homeostasis rather than directly affecting

Box 2

Antagonization of cGAS–STING signalling in cancer cells

Certain exonucleases expressed by cancer cells can deplete the total content of immunogenic nucleic acids to avoid consequent cGAS–STING activation. For instance, upregulation of the DNA exonuclease TREX1 following radiation degrades radiation-induced cytosolic DNA, preventing cGAS–STING-dependent systemic abscopal immunity in mice bearing mammary tumours[150] and is associated with non-response in a phase I clinical trial of anti-PD-1 plus radiation[151]. Cancer cells can also reduce cGAS–STING signalling despite accumulating cytosolic DNA by inhibiting STING activity post-translationally through ubiquitination[152], sumoylation[153,154] or oxidation at cysteine 147 caused by reactive oxygen species associated with DNA damage[155] (such as that caused by radiation). Mutant *TP53*—the most commonly mutated tumour suppressor gene in human cancers[156]—can further antagonize downstream STING signalling by preventing STING from binding to its effector molecule TBK1 (ref. 157). Similarly, phosphatases such as PPM1A[158] can antagonize the TBK1–STING phosphorylation cascade in select cancers. Uncovering the myriad counter-resistance mechanisms deployed by tumour cells against immunogenic self-DNA accumulation and nucleic acid immunogenicity could suggest new therapeutic opportunities and biomarkers for ICB treatment response. For example, post-translational modifications of STING or regulators of downstream STING signalling could be targeted with small molecule inhibitors of specific E3 ligases[159] or proteolysis-targeting chimeras[160], respectively. Parallel treatment strategies include increasing mitochondrial permeabilization[161] with emricasan (a pan-caspase inhibitor) or venetoclax (a BCL2 inhibitor), which can potentiate mitochondrial DNA immunogenicity and tumour response to irradiation in vivo[162]. Furthermore, immunogenic DNA can be released into the microenvironment by dying tumour cells and captured by local immune cells. Dendritic cell cGAS–STING activation following uptake of exogenous tumour-derived DNA is crucial for spontaneous anti-tumour immunity against highly immunogenic tumours in mice[163,164] and responses to irradiation[165]. Thus, tumour-infiltrating immune cells could provide an additional way to drive DNA immunogenicity, particularly in tumours with silenced cGAS–STING signalling cascades. Together, these findings linking DDR, nucleic acid immunogenicity and anti-tumour immunity are influencing the rational use of selective DNA-damaging agents with ICB and offer putative biomarkers for treatment response.

nuclear DNA repair in head and neck cancers[86], suggesting novel uncharacterized mechanisms. STING can thus reduce DNA damage from reactive oxygen species and could be targeted by small molecule STING agonists to increase tumour-intrinsic immune-independent ionizing irradiation sensitivity. Elucidating upstream regulatory factors that dictate the effects of cGAS and STING on anti-tumour immunity versus their dual role in DDR, and understanding the gene-regulatory effects of STING will help in the development of improved nucleic acid-targeting therapies.

DSB induction increases PD-L1 expression in affected cells[75], suggesting PD-L1 involvement in DDR and/or DNA repair. Tumour cell-intrinsic PD-L1 promotes HR in human and mouse cancer models independent of PD-L1 immune checkpoint functions[87]. Genetic PD-L1 depletion, but not surface PD-L1 blockade with anti-PD-L1 ICB, rendered tumours that were resistant to PARP inhibition sensitive to PARP inhibitors, both in vitro and in vivo[87]. Distinct from canonically surface-expressed PD-L1, nuclear or cytoplasmic PD-L1 in cancer cells can directly bind DNA or RNA to regulate transcription or mRNA stability of genes involved in DDR[88,89]. These cell-intrinsic PD-L1 signals could promote DNA-damaging chemoresistance via multiple mechanisms, including direct suppression of nucleic acid immunogenicity, and could explain some failures of ICB treatment[90].

The cytidine deaminase APOBEC3B—originally discovered as an innate immune cytoplasmic viral restriction factor[91]—promotes tumour immunogenicity and ICB response when in the nucleus by increasing DNA or RNA point mutations that generate immunogenic neoantigens[92]. APOPEC3B can also suppress cancer cell DDR independent of its deaminase activity, augmenting DNA damage, cGAS–STING activation and cancer progression[93]. The HIV-1 restriction factor SAMHD1, a nucleoside triphosphohydrolase, could theoretically prevent cGAS activation by inhibiting reverse transcription of endogenous retroviruses to DNA in the cytoplasm from depleted cellular dNTP pools. Similar to APOPEC3B, SAMHD1 can crosstalk with DDR pathways by acting at stalled replication forks to potentiate ATR–CHK1 DDR checkpoint activation[94].

In sum, there is significant crosstalk between DDR, mechanisms that generate cytosolic nucleic acids and immune modulators. Why these immune and DDR modulators evolved functional intersections remains unexplained but these intersections highlight the importance of such molecules in carcinogenesis and/or cancer progression. Although immune mediators such as cGAS or PD-L1 could be exploited to enhance anti-tumour immunity (for example, ICB or cGAS–STING agonists), they could also simultaneously increase intrinsic cancer cell resistance to DNA-damaging agents[90,95,96]. Defining the totality of functional crosstalk in these networks could improve combinatorial DDR-targeting strategies and efficacy of cancer immunotherapies or predict treatment responses.

Finally, it is well known that DNA damage elicits immunity, but less appreciated that immune cells or their effector cytokines can extrinsically damage DNA or regulate cancer cell-intrinsic DDR processes. For instance, macrophage-generated reactive oxygen or nitrogen species are DNA-damaging[97]. Similarly, T cell-derived cytokines such as interferon-γ can induce DNA-damaging reactive oxygen species[98] or regulate DDR gene transcription, similar to type I interferons[97]. Thus, cytokine treatments could improve the efficacy of cytotoxic chemotherapy[99]. In summary, the composition of the tumour microenvironment, including the relative cytokine milieu adds additional layers to the mechanisms regulating the intersection of DDR, nucleic acids and anti-tumour immunity.

Clinical applications

Pre-existing MMR-defective cancers or those with high microsatellite instability (MSI) respond better to ICB than microsatellite-stable cancers, regardless of tumour histology[15]. On the basis of the Keynote-158 study, pembrolizumab received FDA approval for high-TMB cancers, defined as those with at least ten mutations per megabase[19]. Remarkably, in a recent phase I clinical trial of locally advanced MSI-high rectal cancer, all 12 patients experienced pathological complete response from dostarlimab[100] (anti-PD-1), underscoring the immense clinical potential of targeting faulty DDR or DNA repair for therapeutic benefit. However, most patients with cancer do not have high-MSI or high-TMB tumours, highlighting the need for alternate strategies to induce or exploit defective DDR and nucleic acid-induced immunogenicity for effective cancer immunotherapy.

We discuss three major ICB-potentiating DNA-damaging modalities below: chemotherapy, radiation and targeted DDR inhibitors.

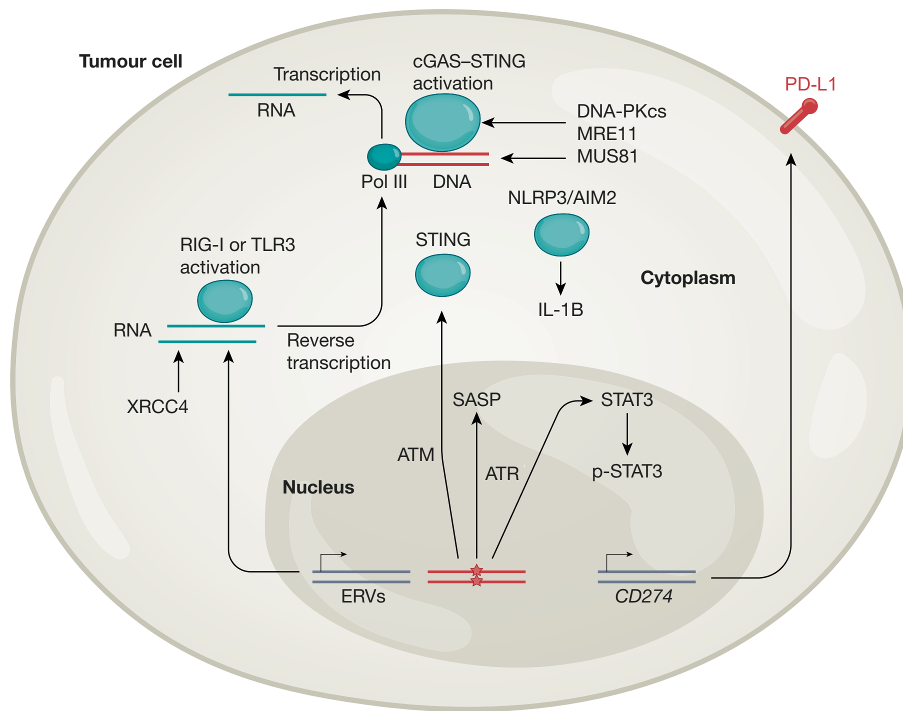

Fig. 4 | cGAS-independent nucleic acid immunogenicity. The DDR and DNA repair pathways can alter anti-tumour immunity independently of the direct generation of cGAS-activatable cytosolic DNA. For instance, ATR activation following irradiation-induced DSBs can induce STAT3-mediated PD-L1 upregulation or activate SASP independently of DNA damage. ATM can activate the SASP that suppresses anti-tumour immunity via an IL-6 driven mechanism. Damaged DNA in the nucleus can trigger ATM-mediated STING activation, bypassing cytosolic DNA-mediated cGAS activation. When present in cytosol, DNA repair factors such as MRE11, DNA-PKcs, XRCC4 or MUS81 can potentiate cytoplasmic DNA or RNA immunogenicity independently of cGAS. dsDNA can activate the NLRP3/AIM2 inflammasome or be converted by RNA polymerase III (pol III) to single stranded RNA in the cytoplasm, which can trigger production of TLR3 and type I interferons. Endogenous retroviruses or retro-transposable elements can trigger RIG-I–MAVS-mediated production of type I interferon.

Cytotoxic chemotherapy

Systemic cytotoxic drugs should theoretically counteract anti-tumour immunity through bone marrow suppression but chemotherapy paradoxically improved ICB efficacy in several landmark phase III trials, independently of PD-L1 or TMB status (Table 1). Many clinical questions regarding overall survival effect, individual agent contributions to ICB efficacy and full mechanisms remain unanswered[101]. The timing of cytotoxic agent application also warrants further investigation. ICB could be more efficacious if it is applied after chemotherapy than before chemotherapy, as suggested by recent trials of adjuvant nivolumab (anti-PD-1) in muscle-invasive bladder[102] and oesophageal[103] cancer.

As DNA damage from cytotoxic agents occurs over brief intervals, we suspect that their immune-potentiating properties with ICB are unlikely to arise from increasing TMB, and are more likely to be owing to alternative immunogenicity mechanisms, such as nucleic acid-driven cGAS–STING activation. Additional immunogenic cell death mechanisms include upregulation of tumour calreticulin expression and consequent macrophage-mediated anti-tumour immunity in response to specific chemotherapies[104] (such as doxorubicin). Uncovering immune-potentiating effects of specific cytotoxic agents in combination with ICB will require profiling of cell signalling and tumour microenvironmental changes in humans in specialized window-of-opportunity clinical trials[105] or sequential biopsies of tumour tissues during treatment[106].

Irradiation

Similar to chemotherapy, irradiation is a standard DNA-damaging treatment modality and could augment local tumour immunogenicity by activating the cGAS–STING–type I interferon pathway or release of tumour antigens to activate antigen-presenting cells[107]. These immune-potentiating properties could explain irradiation-induced abscopal effects in humans when used in combination with ICB, whereby local irradiation treats distant tumours by eliciting systemic anti-tumour immunity, as observed in ICB-treated tumour-bearing mice[108] and patients with melanoma[109]. In contrast to chemotherapy, an overall survival benefit of ICB plus irradiation in humans has yet to be reproducibly demonstrated in prospective randomized controlled trials in any setting. In JAVELIN-100, a phase III trial of unresectable locally advanced oesophageal cancer, avelumab (anti-PD-L1) did not extend progression-free survival when combined with chemoradiation[110]. Of note, participants in JAVELIN-100 received a lead-in dose of avelumab prior to chemoradiation. By contrast, maintenance nivolumab (anti-PD-1) administered after neoadjuvant chemoradiation in the CHECKMATE-577 trial of resected, locally advanced oesophageal cancer improved disease-free survival (22.4 versus 11.0 months), leading to FDA approval in this setting[103]. Interestingly, durvalumab (anti-PD-L1) maintenance after chemoradiotherapy increased 24-month overall survival from 55.6% to 66.3% compared with placebo in the PACIFIC trial follow-up study of unresectable stage III NSCLC[111]. The overall mixed results in these clinical trials raise questions regarding the clinical importance of ICB timing (before or after irradiation), warranting extensive further investigation.

Optimal irradiation doses and schedules based on specific tumour histologies, anatomical compartments and tumour stage to maximize tumour immunogenicity while minimizing immunosuppression are also incompletely defined[112]. A recent study showed that low-dose abdominal radiotherapy in immunologically cold, advanced-stage ovarian cancer activated a type I interferon response, promoting immune cell recruitment in humans and in and mouse models, potentially

Box 3

Mechanisms of RNA immunogenicity in cancer

Similar to DNA, RNA can also be immunogenic in cancer cells and elicit anti-tumour immunity following cytosolic RNA sensing via the RIG-I–MAVS–type I interferon pathway[166]. RIG-I-activable RNA can be induced by DNA damage when RNA polymerase III transcribes RNA from cytoplasmic DNA fragments[167]. Other sources of immunogenic RNA include endogenous retroviruses (ERVs) that can become cytoplasmic double stranded RNA (dsRNA) during their life cycle. ERVs can trigger the innate immune RNA sensors RIG-I and TLR3 directly, or cGAS–STING when ERV RNA is reverse transcribed. In human small cell lung cancers, direct correlation of ERV expression with an immunological 'hot' (T cell-infiltrated) microenvironment has been observed[168]. Regulation of ERV expression involves epigenetic silencing by DNA methyltransferase (DNMT)-induced CpG methylation in multiple carcinomas[169]. DNMTs can be inhibited with low-dose 5-azacytidine and 5-aza-2′-deoxycytidine, which have immunomodulatory properties and reactivate ERV expression and enhance ICB efficacy, as shown in ovarian cancer in vivo[170]. Whereas DNMTs can silence ERV transcription epigenetically, covalent RNA modifications such as N^6-methyladenosine, 2′-O-methylation or pseudouridine could alter nucleic acid immunogenicity post-transcriptionally by directly blocking nucleic acid sensing by RIG-I or other cytoplasmic RNA sensors[171–173]. Other RNA species such as long non-coding RNA can enhance tumour immunogenicity independently of RIG-I, TLR3 and type I interferon by increasing MHCI gene transcription in *cis*[174], activating anti-tumour immunity. The relative importance of RNA nucleic acids driving tumour immunogenicity will be context-specific and could facilitate personalized ICB.

rendering these highly immunotherapy-resistant tumours sensitive to ICB[113]. Irradiation producing high linear energy transfer (such as α-particles or protons) might also be especially efficacious at ICB senitization[114].

Selective DDR inhibitors

Whereas chemotherapy induces DNA damage irrespective of DDR status, DDR inhibitors can selectively induce DNA damage in cancer cells with pre-existing DNA repair defects, resulting in synthetic lethality and limiting unwanted toxicity in normal cells. PARP inhibitors have been approved by the FDA as therapy for HR-deficient ovarian, breast, prostate, and pancreatic cancers harbouring mutations in *BRCA1* or *BRCA2* (ref. 115). In response to PARP inhibition, BRCA-deficient tumour cells accumulate cytosolic DNA that promotes anti-tumour immunity by activating cGAS–STING to induce inflammatory chemokines such as CCL5 and CXCL9 locally, as well as interferon-β to activate immune cells[116,117]. PARP inhibitors can also elicit DNA damage-induced immune effects in BRCA wild-type tumours—for example, in small cell[21] and ERCC1 (a nucleotide excision repair molecule)-deficient NSCLCs[118]. Nonetheless, clinical trials testing combined ICB and PARP inhibition have demonstrated only limited treatment efficacy overall[119,120]. However, type I interferon signalling and T cell activation were enriched in responders to the combined treatment, suggesting that cytosolic nucleic acid sensing may be a determinant of treatment response[119,121]. Several phase III trials testing the efficacy of combinations of ICB plus PARP inhibitors in breast, ovarian and lung cancer are ongoing (for example, ClinicalTrials.gov identifiers: NCT03602859, NCT03522246, NCT04380636 and NCT03598270) and could identify additional response biomarkers beyond HR status, although a clinical trial in prostate cancer (ClinicalTrials.gov identifier: NCT03834519) was recently terminated for futility.

Apart from FDA-approved PARP inhibitors, small molecule DDR inhibitors, including those inhibiting DNA-PKcs, ATR, CHK1, ATM, CHK2 and WEE1 are currently in cancer trials[122,123]. These appear to require specific mutations or pre-existing DDR vulnerabilities to generate immunogenic DNA. For example, CHK2 inhibition increases cytosolic DNA in ARID1A-deficient but not in ARID1A wild-type ovarian cancer to improve the response to anti-PD-L1 in mouse models[124]. ARID1A, independent of its chromatin remodelling activity, promotes MMR and ATR–CHK1 signalling[124]. Thus, impaired DDR functionality can occur in tumours without mutations in known DDR or DNA repair genes, demonstrating the need for a detailed understanding of tumour genetics to optimise specific treatments. However, inhibition of CHK1 and CHK2 could poison $CD8^+$ T cell efficacy in mice[125]. Nonetheless, a phase I trial of anti-PD-L1 plus prexasertib (an inhibitor of CHK1 and CHK2) showed activity in cyclin E-amplified ovarian cancer and activated peripheral blood $CD8^+$ T cells, highlighting the potential of DDR inhibitors for enhancing anti-tumour immunity[126].

Additional strategies

Any of the above approaches could potentially be combined with other immunotherapies such as bi-specific molecules or engineered cytokines, or with adoptive cell therapies such as chimeric antigen receptor T cell therapy[127]. Combining DDR inhibition with adoptive $CD8^+$ T cells could be effective, as the efficacy of PARP inhibition is at least partially dependent on $CD8^+$ T cells[128]. RIG-I or STING activation by synthetic oligonucleotides or non-oligonucleotide agonists enhances survival and augments anti-PD-1 efficacy in select cancer models[129] but may be toxic owing to global immune activation. STING-mediated immune potency and clinical efficacy of small molecule STING agonists has been modest, perhaps owing to poor bioavailability, low specificity, generalized immune activation with autoimmunity[130], lack of biomarkers for optimal patient stratification, and/or differences in STING activation in human compared to mouse[131], among other factors. Adding local agents such as oncolytic viruses for abscopal stimulation shows promise in pre-clinical studies and could treat solitary or distant lesions[132]. Selective enhancement of STING-mediated type I interferon activation by targeting specific DDR cancer cell vulnerabilities (for example, BRCA1) using DDR inhibitors is a promising strategy for enhancing tumour immunogenicity and ICB efficacy while limiting systemic autoimmune toxicity of global STING agonism, but further studies to understand the efficacy of DDR inhibitors and treatment resistance mechanisms are needed (Box 2).

Concluding remarks

The DDR and immunity are deeply intertwined in many ways that remain incompletely understood. A deeper understanding of how specific DDR molecules affect immune responses will inform studies on tumour immune escape and approaches to overcome it. Knowledge from such endeavours will provide valuable information regarding how to combine DDR inhibitors or inducers of DNA damage with immunotherapy modalities for optimal therapeutic efficacy. Since DDR and DNA repair molecules can either enhance or inhibit anti-tumour immunity, careful considerations of individual and net effects of agents interfering in these pathways is warranted. Reliable treatment response biomarkers to facilitate patient stratification in combinatorial therapies are largely lacking. Creative clinical trial designs based on mechanistic understanding will help maximize the informative value of data and minimize patient numbers in trials. DNA repair and the effects of DDR in immune cells require further exploration. Tumour immunogenicity through specific pathways (for example, HMGB1, calreticulin, necrosis

and pyroptosis) and immunogenicity from immunogenic neoantigens as related to the DDR will need to be thoroughly interrogated to help drive the design of new therapeutic regimens and for optimal efficacy versus safety.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-023-06069-6.

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Acknowledgements We acknowledge the following funding sources: P.S. (R35 CA24180), T.J.C. (R01 CA268641, CA05415), A.V.R.K. (F30 CA239390) and C.M.R. (American Cancer Society PF-22-034-01-DMC).

Author contributions A.V.R.K. and T.J.C. conceived the idea and wrote and edited the manuscript. C.M.R. and P.S. assisted with writing specific sections and editing the article.

Competing interests The authors declare no competing interests.

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Peer review information *Nature* thanks the anonymous reviewer(s) for their contribution to the peer review of this work.
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