ISSN: 2640-1037

Clinics of Oncology

Review Article

The DNA Damage Repair Response

Black HE and Mills KI*

Department of Medicine, The Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, UK

Volume 3 Issue 1- 2020

Received Date: 18 June 2020

Accepted Date: 27 June 2020

Published Date: 03 July 2020

2. Keywords

DNA damage; Reactive Oxygen Species; Mismatch Repair

1. Abstract

Each day the genome is subjected to thousands of DNA damaging events from diverse sources which can have potentially deleterious consequences. In order to maintain genome integrity eukaryotic cells have evolved a highly complex and multifaceted response network called the DNA damage response, or 'DDR'. The DDR encompasses an intricate network of DNA damage sensor, transducer and effector proteins which elegantly coordinate their activity with cycle progression. DNA damaging events trigger lesion-specific responses to ensure accurate and non-deleterious repair. These responses must occur in concert with chromatin rearrangement and cell cycle checkpoint activation, highlighting the complexity and fine balance of the DDR. This review focuses on the arsenal of lesion-specific repair mechanisms available to the cell and how the DDR harmonizes with the cell cycle and its checkpoints.

3. Main Text

The transmission of genetic information from parent to daughter cells is a fundamental process of life, however this process must occur in the midst of a constant assault on the DNA from both endogenous and exogenous sources, and the number of lesions induced can surpass 10,000 each day [1-3]. In order to combat these events, the body has evolved a complex and varied response system, collectively termed the 'DNA Damage Response' (DDR). This intricate network is responsible for damage recognition, signalling and repair. A range of specialised pathways are activated depending on the DNA lesion in question and these are highly conserved across species. It is critical that DNA lesions are dealt with appropriately to mitigate mutagenic events which could cause cancerous transformation of the cell.

4. Sources of DNA Damage

The stability of DNA is challenged by a host of different biological factors which arise during normal cell function [4]. Problems arising during DNA replication are one of the biggest contributors, for example it's common for base mismatches to arise in S phase and DNA strand breaks are also likely due to aberrant activity of Topoisomerase I and II enzymes [3]. Furthermore, Reactive Oxygen Species (ROS) are formed daily as by-products of normal oxidative respiration and response to infection [5, 6]. ROS are highly damaging, introducing DNA adducts which cause further replication issues such as improper base pairing and replication fork stalling [3-5]. DNA damage can also arise from exogenous sources such as UV radiation from sunlight, remarkably this can induce in excess of 100,000 lesions per hour. Additional sources include naturally occurring radioactivity, chemotherapeutic agents, and tobacco products which all produce Ionizing Radiation (IR) [3, 7].

DNA lesions are predominantly single stranded DNA breaks (SSBs), which are problematic, but the cell is accustomed to dealing with them swiftly. On the contrary, double stranded DNA breaks (DSBs) occur less frequently but are highly deleterious, often toxic, lesions which pose a more significant challenge for the cell's repair machinery [1, 3, 8].

5. Dealing with Simple Lesions

5.1. Direct Repair of DNA

Certain base irregularities can be dealt with directly, without the need for excision from the DNA helix, the most common of which is base alkylation O6-methylguanine caused by simple alkylating agents [8,9]. The enzyme tasked with this is methylguanine DNA Methyltransferase (MGMT). MGMT, a small suicide protein, directly reverses the DNA adduct by permanently transferring the wayward methyl group to a cysteine group located within the enzyme's own active site. MGMT will then disconnect from the repair site and is irrevocably inactivated, hence the term 'suicide protein' [8-10]. Subsequently, cells defective in MGMT are hypersensitive to alkylating agents [2].

Most damage must be repaired through indirect mechanisms involving sequential catalytic events performed by specialised proteins. There are at least five primary repair pathways available to the cell and these will be discussed in greater detail below.

6. Indirect Repair of DNA Single Stranded Breaks

6.1. Mismatch Repair Pathway

The base Mismatch Repair (MMR) system is crucial for resolving simple base mismatches and insertion or deletion loops that arise

***Corresponding Author (s):** Ken Mills Department of Medicine, Patrick G Johnstone Centre for Cancer Research, Queen's University Belfast, UK, Tel: +44 28 9097 2786; E-mail: k.mills@qub.ac.uk*

Citation: Mills KI, The DNA Damage Repair Response. Clinics of Oncology. 2020; 3(1): 1-14.

during DNA replication [3]. MMR is activated when MutSα and MutSβ (recognition factors) bind to the affected genomic region, this is followed by recruitment of MutL, which mediates protein-protein interactions in an ATP-dependent manner at the site of mismatch. Next, the affected DNA strand is excised by exonuclease-mediated degradation and the sequence is restored by DNA polymerase. Sealing of DNA nicks by DNA ligase enzymes concludes MMR [11]. MMR is summarised in (Figure 1A).

6.2. Base Excision Repair

The Base Excision Repair (BER) pathway is evolved to deal with spontaneous pre-mutagenic base distortions primarily arising from endogenous agents such as ROS and deamination [12]. In brief, BER is initiated when DNA glycosylase enzymes recognize the afflicted base and catalyse its hydrolytic removal. Left behind is an abasic site where an incision is made by nuclease enzymes, generating a larger gap known as a 'repair patch'. Repair patches demonstrate significant heterogeneity in terms of their size and the proteins involved, these are termed 'short patch' or 'long patch' repair intermediates. The sequence homology is restored via repair synthesis by DNA polymerases and is concluded by the action of DNA ligase [12-15]. BER corrects relatively simple errors which have little to no impact on the structure of the DNA helix, therefore single base damage does not pose immediate threat to the genome if BER is functional (summarised in Figure 1B).

6.3. Nucleotide Excision Repair

The Nucleotide Excision Repair (NER) pathway is responsible for dealing with more complex lesions such as DNA adducts and Inter/Intra-Strand Crosslinks (ICLs) which cause distortion of the DNA helix. Improper helix configuration can result in incorrect base pairing and impedes progression of the replication fork. This type of damage is mainly caused by exogeneous sources and NER represents the main pathway for resolution of Ultraviolet (UV)-induced damage [13, 14]. NER can operate via two distinct routes, transcription-coupled NER, which recognises DNA lesions disrupting the progression of RNA polymerases, and global-genome NER which surveys the entirety of the genome for helix disruptions [3, 16]. Briefly, NER begins with the recognition of the DNA lesion by a protein complex composed of XPA, RPA and XPC-hHR23B, although XPC-hHR23B is unessential for repair in the instance of transcription-coupled NER. Next, the DNA surrounding the site of damage is loosened, in an ATP-dependent fashion, reliant on Transcription Factor II H (TFIIH). NER differs from BER in that the damaged DNA is excised as part of a 22-32 base single-stranded oligonucleotide. Excision occurs via cleavage of the damaged DNA at its 3' end by XPG and at its 5' end by ERCC1-XPF. The resulting gap is filled by DNA synthesis catalysed by DNA polymerases δ and ε-holoenzyme, which are dependent on PCNA, and the gap is sealed by DNA ligase 8 [14, 16, 17]. Defects in NER are associated with hypersensitivity to UV radiation and are also responsible for diseases including xeroderma pigmentosum and Cockayne syndrome [16]. The process of NER is summarised in Figure 1C.

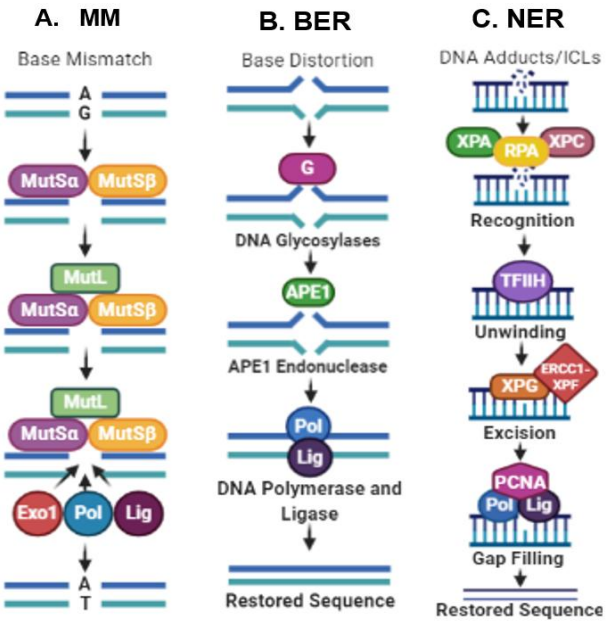

Figure 1: Mechanistic Pathways of Single Stranded Break Repair
A. Mismatch repair resolves simple replication errors. MutSα and MutSβ recognise the lesion, and MutL is recruited to mediate protein interactions. Exonucleases excise the error and DNA polymerase and ligase enzymes restore the strand.
B. Base excision repair resolves base distortions by recruiting DNA glycosylases which recognize and excise the base. Nuclease enzymes act to create a 'short' or 'long' repair patch which is then filled by DNA polymerase, and DNA ligase seals the strand.
C. Nucleotide excision repair resolves helix-distorting lesions. These are recognised by XPA and RPA:XPC-hHR23B complex. The helix is loosened by TFIIH and a single stranded oligo is excised by XPG and ERC1-XPF. The gap is filled by DNA polymerase dependent on the presence of PCNA, and the gap is sealed by DNA ligase.

6.3.1. Dealing with Complex Lesions

Indirect Repair of DNA Double Stranded Breaks

DSBs arise naturally in some circumstances and are necessary for crucial biological processes including V(D)J and Class Switch Recombination (CSR) in immune system development. However, most DSBs arise from exogenous sources and are lethal if not correctly repaired [3]. In some circumstances, just one break can overwhelm the cell leading to apoptosis 18'. Most SSBs should be resolved by MMR, BER or NER, but those that escape their surveillance have the potential to potentiate to DSBs via collisions with replication machinery. Complex lesions require complex repair mechanisms, the mammalian cell possesses two, Non-Homologous End-Joining (NHEJ) and Homologous Recombination (HR) [19, 20].

6.4. Non-Homologous End-Joining

6.4.1. Classical Pathway: NHEJ is used by the cell much more frequently than HR, as it is available throughout the entirety of the cell cycle, in fact over 60% of DSBs induced by exogenous sources are repaired by NHEJ in mammalian cells [19, 21]. However, it repairs

DSBs through the direct ligation of the broken DNA ends, with little to no end processing preceding. Therefore, it's often referred to as an 'error prone' mechanism of repair, resulting in sequence deletions [22]. NHEJ is essential to the cell with defects in pathway components including DNA Ligase IV resulting in embryonic lethality in mice, and mutations in DNA-Dependent Protein Kinase (DNA-PK) have been shown to cause Severe Combined Immunodeficiency (SCID) as well as hypersensitivity to IR [23-27]. NHEJ is a relatively simple repair mechanism with just a few key proteins required to resolve breaks.

The process of end joining is initiated by the proteins Ku and the large 470kDa protein kinase DNA-PKcs, collectively forming the DNA-PK complex, a key player in NHEJ [28]. Ku is a heterodimer of the polypeptides Ku70 and Ku80 (70kDa and 80kDa respectively) which has a very high affinity for DNA ends, making it the first protein to bind. The toroidal structure of Ku promotes its loading at the break site, followed by signalling to DNA-PKcs [29, 30]. DNA-PKcs is the catalytic subunit of DNA-PK, upon its recruitment it will stabilise break ends and inhibit their resection. Upon contact with break ends the serine/threonine kinase activity of DNA-PKcs is activated, promoting its autophosphorylation at the six-residue cluster of T2609, this leads to a conformational change in the protein, destabilizing its binding to DNA and increasing accessibility for additional repair factors at the break site. Further autophosphorylation at the five- residue S2056 cluster helps to limit unnecessary processing of break ends [30-32]. These phosphorylation events serve further purposes with $T2609^{P}$ important for promoting repair by HR when NHEJ fails and $S2056^{P}$ is necessary for inhibition of HR by preventing break resection [30-33].

The concomitant assembly of the Ku heterodimer and DNA-PKcs at breaks is followed by the recruitment of XRCC4 and DNA Ligase IV (XRCC4:LIG4) which is responsible for ligating the broken ends, concluding NHEJ [28, 32, 34]. XRCC4 and LIG4 are tightly complexed and work interdependently, although XRCC4 is crucial for the stabilization of LIG4 and for increasing its ability to ligate DSB ends. The protein XLF is also required at this stage to enhance the ligation of breaks by XRCC4:LIG4 in the presence of the biological Mg^{2+} gradient [35]. Since homology is not a pre-requisite for NHEJ repair, the high degree of flexibility of XRCC4:LIG4 is important; the complex can perform strand ligation independent of the second strand in the break, it can seal breaks across nucleotide gaps and is also capable of gluing incompatible DNA ends [36].

Limited processing of break ends may be carried out by the protein Artemis when DSBs are otherwise irreparable. Artemis is an endonuclease enzyme which, upon interaction with, is phosphorylated by DNA-PKcs in an ATP-dependent manner. This event confers an endonucleolytic activity to Artemis, promoting the trimming of 5' and 3' DSB overhangs [30, 32, 37]. The entirety of the classical NHEJ response is summarised in Figure 2A.

6.4.2. Alternative Pathway: Many studies have now demonstrated that there are mechanistically distinct sub-pathways of NHEJ which operate in the absence of classical end-joining repair factors. These include, but are most likely not limited to, alternative-NHEJ (alt-NHEJ) and microhomology-mediated end-joining (MMEJ) [38-42]. MMEJ is often used interchangeably with alt-NHEJ to refer to non-classical end-joining, although several studies have shown there are distinct differences in their modes of repair [41, 42].

Alt-NHEJ was discovered through studies of repair in cells defective for classical-NHEJ (c-NHEJ) and it is used primarily as a fail-safe repair mechanism [43]. However, the kinetics of repair are slower via alt-NHEJ and it has been associated with an increased frequency of deletions, inversions and gross chromosomal rearrangements [44, 45]. With this in mind, it has been proposed that alt-NHEJ is distinct from c-NHEJ in three key aspects: it occurs in cells defective for c-NHEJ components, it is highly error-prone and it typically occurs at break junctions displaying excessive deletions and microhomology [39].

The precise mechanisms of alt-NHEJ are not well characterised, but several groups have offered preliminary mechanistic models for this back-up repair system. It is believed that repair is initiated via a Poly(ADP-Ribose)Polymerase 1 (PARP1) dependent mechanism which requires the absence of Ku proteins, but not necessarily DNA-PKcs [46]. The Ku heterodimer may also compete with PARP1 for end binding, directing repair towards either c-NHEJ or alt-NHEJ [47]. Annealing of PARP1 to DNA is thought to be favoured in alt-NHEJ due to the presence of larger regions of microhomology at break ends [41]. It's then proposed that the Mre11-Rad50-NBS1 (MRN) complex is recruited to the break, and in concert with transcription factor CtIP DNA ends are resected [39]. The end resection properties of MRN are of importance in the search for homology at these DSBs, and the subsequent action of DNA Ligases I and III (LIGI/LIGIII) are then required to seal the DNA [39-42, 48]. Figure 2B summarises the alt-NHEJ pathway.

6.5. Homologous Recombination

Unlike NHEJ, the HR pathway is high-fidelity, promoting error free repair via the use of a homologous sister chromatid repair template [49]. The requirement for a homologous template means that HR is isolated to S and G2 phases of the cell cycle when DNA has been replicated. HR has several sub-pathways, but each is always instigated by the generation of ssDNA, and all are characterised by homology search and invasion of the template strand [3, 21, 50]. One sub-pathway is Single-Strand Annealing (SSA), which repairs DSBs via ligation of complementary DNA sequences lying on both sides of the lesion [49].

The first step in HR is to clean up the ends of the DSB by nucleolytic

resection in the 5' to 3' direction [49-51]. This is carried out by the MRN complex, which is also employed in NHEJ. Each component of MRN serves a different purpose, Mre11 has an inherent endonuclease activity, as well as 3' to 5' exonuclease activity, RAD50 is believed to promote the unwinding of DNA through its ATPase action and Nbs1 may be important for relaying signals from damage sensors to the MRN complex [52, 53]. To aid with break resection BRCA1 and CtIP are also recruited [54]. BRCA1 binds CtIP via its BRCT domains located at the C terminus of the protein which promotes the binding of BRCA1 to MRN, whilst CtIP also binds Nbs1 directly. The MRN: BRCA1: CtIP complex forms in a cell-cycle dependent manner, requiring the activity of CDKs. Therefore, this complex may be important for initiation of DSB repair by HR [55].

Resection of the DSB generates a 3' ssDNA overhang. ssDNA in any capacity is highly unstable and is quickly bound by two proteins, RAD52 which binds at ssDNA ends, and Replication Protein A (RPA), which binds the ssDNA overhangs [56-59]. The defining step of HR is strand invasion, catalysed by the RAD51 protein (mammalian homolog of yeast RecA) [50]. RPA binds with high-affinity, whereas RAD51 has a low-affinity for ssDNA, therefore RAD51 must compete for the binding of 3' overhangs [60]. RAD51 paralogs RAD51B, RAD51C, RAD51D and XRCC2 are recruited to promote the displacement of RPA and loading of RAD51 [61, 62]. Another protein crucial to this process is the breast cancer associated gene, BRCA2 which directly binds RAD51 via a series of internal BRCT repeats [54, 63].

Once loaded, RAD51 forms a nucleoprotein filament which invades the homologous repair template, and BRCA2 performs the unique function of directing nucleofilament formation to the junction where ssDNA meets dsDNA at the resected break [50, 61]. Sister chromatids are preferentially used as templates because although homologous chromosomes can be used, repair this way would result in loss of heterozygosity [64, 65]. When the search for sequence homology has concluded RAD51 catalyses strand exchange with the help of RAD51 paralogs, displacing one strand to form a displacement loop (D-loop) [61, 66]. DNA synthesis is then initiated by a DNA polymerase enzyme which extends the 3' end of the invading filament [50, 61, 67]. The second 3' overhang can then anneal, initiating further strand synthesis, in a process known as second-end capture [68]. The result is a four-way complex joined by two Holliday Junctions (HJ) which are duplexes of homologous DNA, formed through strand exchange [50, 69, 70]. Double HJs (dHJs) are primarily resolved through the action of BLM helicase-TopoisomeraseIIIα-RMI1-RMI2 (BTR) complex [71]. BTR catalyses the dissolution of dHJs and suppresses further crossover events [72]. Any HJs which escape resolution by BTR will be acted on by HJ resolvase enzymes (GEN1 and MUS81-EME1) at a later stage in the cell cycle [71-74]. An additional route of break resolution following D-loop formation is Synthesis-Dependent Strand Annealing (SDSA). The 3' invading strand detaches following extension and synthesis and is subsequently ligated by DNA Ligase I with the second end of the break to exclusively form non-crossover products [75, 76]. The new sequence has homology to the repair partner and in this way strand integrity is restored. In human cells, the product of break resolution by HR will rarely be cross-over events, as this would result in loss of heterozygosity and could be mutagenic [73]. The molecular events of HR are summarised in Figure 2C.

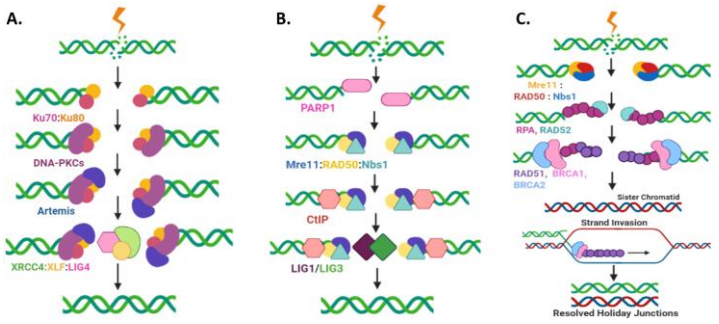

Figure 2: Mechanistic Pathways of DNA Double Stranded Break Repair
A. Classical NHEJ - a low-fidelity DSB repair mechanism available throughout the entirety of the cell cycle. In response to breaks the Ku70/80 heterodimer is recruited, followed by DNA-PKcs. The end-processing enzyme Artemis may be recruited if so required. The action of XRCC4, XLF and DNA Lig4 leads to joining of break ends and sealing of the lesion, completing the repair process.
B. Alternative NHEJ - acts in the absence of functional cNHEJ components. In response to damage PARP1 is recruited, followed by MRN and CtIP which act in concert to resect break ends. It's believed that the dual action of DNA Ligase 1/3 leads to sealing of the DSB.
C. Homologous Recombination - simplified schematic of high-fidelity repair. MRN is recruited to resect break ends, these are quickly bound by RPA and RAD52. RPA is displaced by RAD51 with the aid of BRAC1/2 and RAD51 paralogs. A sister chromatid is used as the template for strand invasion and DNA synthesis. Holiday junction resolution terminates the HR repair.

7. Regulation of Repair Pathway Choice

With two key mechanisms available to repair DSBs, how does the cell decide which path to take? Studies performed in hamster cells demonstrated that enzymatically induced breaks are directed towards HR for repair in 10-50% cases [77], whereas research in human glioblastoma cells has shown that *I-SceI* induced DSBs are repaired by HR just 10% of the time [70]. This would indicate that NHEJ is the primary repair pathway in mitotically replicating cells, which is indeed the opinion within the wider field [78]. However, as previously discussed, HR performs error-free repair which is crucial to prevent propagation of damaged genetic information. Therefore, it's believed that HR is the desired repair pathway during DNA replication[65]. Indeed, cells defective for BRCA1, a protein crucial to HR repair, are sensitive to IR-induced damage, indicating that HR is required to process complex lesions [79].

The choice between NHEJ and HR is also heavily regulated by the cell cycle [80]. Given that HR requires a homologous template for repair it is active at two specific stages, late S and G2, when replication has occurred, and a sister chromatid can be used [49]. However, NHEJ remains active throughout S and G2 indicating that there is competition for the DSB by both repair mechanisms.

The resection of DSB ends 5' to 3' can directly dictate the use of HR

over NHEJ as only HR can be employed once the break is resected and RPA binds [81]. Therefore, the resection of breaks must be tightly regulated to prevent erroneous attempts at repair by NHEJ. Resection is inhibited in G1 of the cell cycle by the binding of p53 binding protein 1 (53BP1) at chromatin surrounding the DSB, and this inhibition is later relieved in G2 by association of 53BP1 and BRCA1 [82]. In G2, the enlistment of BRCA1 to breaks leads to the phosphorylation and subsequent activation of CtIP, thus recruiting BRCA2 [83]. This requires the action of associated proteins PALB2, $CRL3^{KEAP1}$ and USP11 [81]. The activity of CDK1 is required to promote HR as it phosphorylates several key HR proteins, additionally, CDK1 activity is downregulated in G1, efficiently inhibiting the onset of HR [70,81].

An additional level of regulation occurs in S phase, allowing the distinction of DSBs in replicating from non-replicating DNA in the cell [81]. In the G1 phase of the cell cycle DNA has not yet been replicated and so the chromatin expresses a specific methylation mark on the histone H4 (H4K20me2), during S phase replication this methylation is lost (H4K20me0). TONSL, a protein involved in the repair or stalled/collapsed replication forks, is then capable of binding H4K20me0, it will complex with its binding partner MMS22L, and HR is instigated. When methylated, TONSL will not bind, but 53BP1 is recruited in its place, inhibiting HR [84].

Additional levels of regulation are provided by the upregulation of central HR proteins RAD51 and RAD52 during S and G2 phases of the cell cycle [85]. Additionally, a recent publication proposed that a protein called CYREN (cell cycle regulator of NHEJ) is responsible for promoting HR by binding to and inhibiting the Ku heterodimer [86]. Furthermore, NHEJ is downregulated during S phase demonstrated by the reduced phosphorylation of DNA-PKcs in cells which have been irradiated, therefore shunting the cells towards HR [87]. Therefore, there are a multitude of mechanisms the cell employs to control repair pathway choice which are heavily dependent on the cell cycle.

8. Sensors, Transducers and Effectors of the DNA Damage Response

8.1. Damage Sensors

Having discussed how DNA lesions are repaired by the cell, it's necessary to define how the cell is initially alerted to their presence. Mammalian cells possess an evolutionary conserved signalling mechanism which stimulates a coordinated response of DNA damage repair and cell cycle checkpoint activation. This network is proposed to function via the recognition of DNA breaks by damage sensors, this signal is then relayed to damage transducers, and these proteins will then signal through damage effectors to initiate either repair, transcription, cell cycle arrest or cell death [88, 89].

DNA damage sensors are poorly defined and there is much contest around the proposed sensor proteins. The role of the damage sensor is as the name suggests, to identify DNA lesions and kick-start the signalling network. In a 2003 publication, Petrini and colleagues outlined four criteria to be filled in order to classify a protein as a damage sensor [88]:

1. Upon recognition, the sensor protein should physically associate with damaged DNA.
2. The sensor protein should possess the innate ability to associate with damaged DNA without the need for an activation modification such as phosphorylation.
3. Mutations of the sensor protein, or conditions which impair its association with damaged DNA, should mar the activation of downstream cell cycle checkpoints.
4. It should thus be possible to identify so called 'up-mutants', i.e. mutated genes encoding sensor proteins will lead to constitutive signalling in the absence of damage.

Very few proteins meet some or all these criteria. The proteins which have been suggested as sensors include the MRN complex [90] and PCNA-like proteins [89]. The MRN complex is involved in the early steps of both NHEJ and HR repair of DNA damage [39, 52], but in its capacity as a break sensor it is responsible for binding the damaged chromatin and recruiting Ataxia-Telangiectasia-Mutated (ATM) which can then initiate downstream signalling [88-92]. The second group of damage sensors are the PCNA-like proteins RAD1, RAD9 and Hus1, which are collectively termed 911 [89, 91, 93]. As previously discussed, ssDNA forms during resection of DSBs and is quickly bound by RPA [60], this ssDNA: RPA complex is required to recruit the protein ATM- and Rad3-Related (ATR) and its regulatory subunit ATRIP, as well as RAD17 [91]. Once at the site of the break, ATR-ATRIP and RAD17 initiate the loading of 911, this leads to the phosphorylation of RAD17, 911 and TopBP1, which binds 911, by ATR kinase. This in turn will promote ATR's kinase activity [89, 91, 92,94].

8.2. Damage Transducers

Once the cell is alerted to the presence of damage, it will signal through its master transducers, ATM and ATR, as well as DNA-PKcs, although this complex is primarily required to initiate NHEJ [94]. These proteins are members of the phosphatidylinositol-3-kinase-like kinase (PI3K) family which are responsible for activating a vast array of downstream proteins at Serine/Threonine residues via phosphorylation [94, 95]. ATM and ATR share structural similarity and are both activated by autophosphorylation in response to DNA damage, however they differ in that ATM is primarily activated by DSBs, whereas ATR responds to a diverse range of damage including DSBs [95]. ATM is mutated in a disease known as AT (ataxia telangiectasia) which is characterised by chromosomal instability [96]. In contrast no diseases have been linked to ATR

mutations, although studies of mammalian cells which express a dominant-negative ATR show sensitivity to DNA damage and impaired checkpoint function [97].

Both ATM and ATR share downstream substrates including p53 [98, 99] which is phosphorylated at the same residues by both proteins, but they also have independent targets [100]. One objective of activated ATM and ATR is to initiate DNA damage repair via NHEJ and HR. ATM is responsible for controlling the response to DSBs induced by IR whilst ATR acts to trigger repair of damage induced by a wide variety of sources e.g. UV and Hydroxyurea (HU) [89, 101]. To trigger HR repair, the MRN complex first localises to damaged DNA, acting in a damage sensor capacity, this is followed by ATM recruitment and activation which in turn leads to phosphorylation of MRN by ATM as Nbs1 directly binds the kinase [94, 102]. Subsequently, the repair cascade is activated.

Although ATR can act to repair DSBs, its recruitment and activation are mediated in a different way to ATM. It appears that its recruitment is dependent upon its interaction with ATRIP, and furthermore it requires ssDNA: RPA complexes to localise [103]. Furthermore, ssDNA produced by resection not only activates ATR, but it causes the progressive exchange of ATM for ATR activation at DSB sites [94]. This switch allows for the coordinated activity of ATM and ATR, eliciting activation of their individual downstream targets in a sequential manner [104]. In addition to triggering DSB repair, ATM and ATR have many substrates, or damage transducers, which are responsible for activating the cell cycle checkpoint mechanisms, and this will be addressed later in the text. The DNA damage response is a vast and integrated signalling network and its structure is summarised in Figure 3.

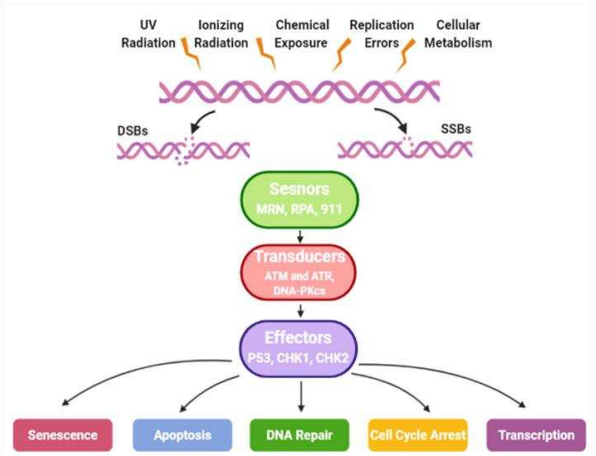

Figure 3: The DNA Damage Response Network
The DDR can be summarised as a vast signalling network comprised of damage sensors, which then signal to transducers, followed by signalling to effector proteins which trigger many cellular responses.

9. The Cell Cycle and its Checkpoints

9.1. Organisation of the Cell Cycle

The accurate replication of the genetic code is governed by a highly regulated sequence of events which are evolutionary conserved, this is collectively termed 'The Cell Cycle' (Figure 4) [105]. The canonical model of the cell cycle is comprised of four individual phases, G1, S, G2 and M, which each represent a different stage of the DNA replication process [106]. G1, S and G2 collectively form interphase, in which G1 and G2 are 'gap phases' where the cell prepares for DNA replication and prepares for division respectively. S phase, or synthesis phase, is when the entirety of a cell's genetic information is duplicated in preparation for division into two daughter cells. If a cell decides that it's not in a ready position to proceed with replication it can enter a phase known as G0 before it commits to G1, this is a 'resting phase' and is where many non-proliferative cells reside. M phase, or mitotic phase, can be further subdivided into prophase, metaphase, anaphase and telophase, in which the cell accurately segregates its duplicated information into two daughter cells [107, 108].

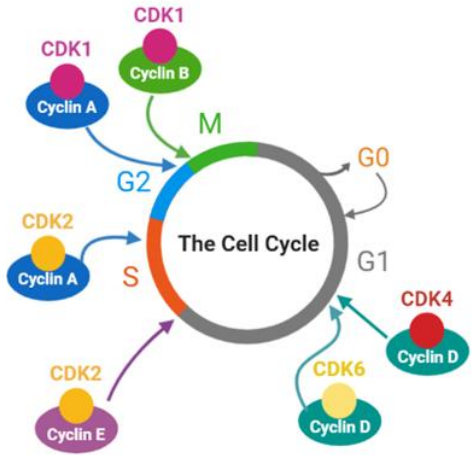

Figure 4: The Mammalian Cell Cycle
The cell cycle is composed of four sequential phases G1, S, G2 and M which culminate in the division of the parent cell into two daughter cells. The progression through interphase and mitosis is regulated by complexes of CDKs and cyclins.

9.2. Regulation of Cell Cycle Progression

The progression of a cell through the various cycle stages is under the control of CDK proteins. CDKs are large serine/threonine kinases which are responsible for phosphorylating downstream proteins to initiate further cellular functions [107]. There are four key CDKs (CDK1, CDK2, CDK4 and CDK6) which are activated at specific points in the cell cycle by activator proteins called cyclins. Certain cyclins will bind to and activate certain kinases, and this is summarised in (Table 1) [107, 109].

The levels of CDK proteins remain stable throughout the cell cycle, but the cyclin levels fluctuate, thereby controlling which CDKs are active at any one time. CDK activity is inactivated in a number of ways: cyclins are targeted for degradation by ubiquitination at the end of their cycle phase, CDK1 is inactivated via inhibitory phosphorylation by Wee1 and Myt1 kinases, and finally, binding of CDK inhibitory proteins to the CDKs or CDK: cyclin complexes controls their activity [107, 109]. The CDK7: Cyclin H complex,

also known as CAK, does not behave as the others do, it is active throughout the entirety of the cell cycle and is required for the complete activation of CDK1 [107].

Table 1: CDKs, their Cyclin Partners and their Activity Throughout the Cell Cycle
CDKs are bound by cyclin proteins which activate them and allow the progression of cells through the various cell cycle phases.

Cyclin-Dependent Kinase (CDK)	Cyclin	Cell Cycle Phase Activity
CDK4	Cyclin D1, D2 and D3	G1 Entry
CDK6	Cyclin D1, D2 and D3	G1 Entry
CDK2	Cyclin E	G1 to S Transition
CDK2	Cyclin A	S Phase
CDK1	Cyclin A	S to G2/M Transition
CDK1	Cyclin B	Mitosis
CDK7	Cyclin H	All Phases

9.3. Cell Cycle Checkpoints

To ensure that the cell cycle is completed, and DNA is replicated and passed on without error the cell has evolved a highly conserved surveillance system known as cell cycle checkpoints [110]. The significance of these checkpoint mechanisms is highlighted by the fact that many proteins involved in checkpoint activation are tumour suppressors, whose loss leads to cancer [111]. It is the responsibility of the checkpoint network to identify DNA damage and structural abnormalities, render these stimuli into signals and transduce the signals to downstream effector proteins which can coordinate a repair response. Furthermore, the checkpoint proteins must orchestrate the repair response in harmony with cell cycle progression to prevent the transmission of faulty genetic information to daughter cells [112]. The typical effects of cell cycle checkpoint activation include the slowing, or halting of cell cycle progression to allow time for DNA damage repair to occur, prior to mitosis [107, 112]. The checkpoints activated in response to DNA lesions lie at the G1/S boundary before DNA is replicated, during S (intra-S), and at the G2/M boundary before cell division [107].

As described previously in the text, the DDR is a complex signalling network composed of damage sensors, transducers and effectors. The key transducer proteins are ATM and ATR kinases which activate a myriad of damage effector proteins via phosphorylation modifications89. Therefore, ATM and ATR are responsible for initiating cell cycle checkpoints and do so through activation of two key proteins, Checkpoint Kinase 2 (CHK2) and Checkpoint Kinase 1 (CHK1) respectively [111-113]. The axis chosen depends on the nature of the DNA damage, the ATM-CHK2 branch will primarily respond to IR-induced DNA damage, whilst the ATR-CHK1 is called upon mainly for UV-induced damage and DNA replication stress [111, 113, 114].

An important and well characterised substrate of ATM and ATR is the tumour suppressor p53, which is phosphorylated at several residues in response to DNA damage [115]. MDM2, an oncogene, is a key regulator of p53 in vivo, binding to and targeting it for degradation at the proteasome via ubiquitination [116]. The transcription of MDM2 is also initiated by p53, hence establishing a negative feedback loop [117]. In response to damage p53 is stabilised by the dissociation of MDM2, owing to the phosphorylation of p53 at distinct residues [116, 117]. The successful activation of the G1/S checkpoint is dependent upon p53 [118], and the molecular mechanisms of this pathway will be examined below.

9.4. The G1/S Checkpoint

At the G1/S checkpoint cells are examined to ensure that they meet the necessary requirements to commit to progression through DNA synthesis [113]. The presence of DNA damage or replication stress activate the checkpoint if required. p53 mediates G1/S activation, and its absence results in complete abrogation of the checkpoint [119]. In response to IR-induced damage, p53 is stabilised by MDM2 removal and rapidly accumulates in the cell [116]. p53 is phosphorylated by the ATM kinase at the serine 15 residue located within its amino-terminal transactivating domain [99]. In the absence of ATM, ATR serves as a back-up kinase and can phosphorylate p53 at ser15, furthermore it has been shown ATR may be required to maintain a sustained phosphorylation of p53 [120]. One key protein which is transcriptionally activated by p53 is the CDK inhibitor p21. Upregulation of p21 leads to inhibition of the CDK2: cyclin E/A activity, thereby preventing the G1 to S phase transition [111, 112, 115]. In addition to phosphorylating p53, ATM phosphorylates the CHK2 kinase at the threonine 68 residue [121, 122], and subsequently CHK2 phosphorylates p53 at the ser20 residue123. This phosphorylation event further enhances the accumulation of p53 by directly preventing MDM2 and p53 association [112]. Activated CHK2 is also responsible for phosphorylation of the CDC25A protein on its ser123 residue [124]. Under normal circumstances CDC25A is responsible for activation of CDK2 which is instrumental in S phase progression, therefore degradation of CDC25A by CHK2-mediated phosphorylation leads to CDK2 inhibition and G1/S checkpoint activation [107, 124]. Interestingly, the ATM-CHK2-CDC25A G1/S activation occurs faster than p53-mediated activation, which requires the build-up of newly transcribed proteins. In this way, CDC25A degradation leads to a quick but shorter activation of the G1/S checkpoint, and if required the p53 branch can promote its sustained activation [111]. For cells which have sustained irreparable DNA damage, they can be targeted for death via the transcriptional activation of apoptotic proteins by p53 [117].

9.5. The Intra-S Checkpoint

The intra-S phase checkpoint is critical for slowing the rate of DNA synthesis to allow time for repair of DNA damage, primarily through HR86, [111]. There are three distinct mechanisms of

intra-S activation which culminate in the inhibition of new origin firing and prevention of strand elongation at active replication origins [111, 112, 125]. Firstly, as previously discussed, inhibitory phosphorylation of CDC25A targets it for ubiquitin-mediated proteasomal degradation, thereby preventing CDK2: cyclin A activity, and inhibiting S phase progression [107, 124]. Secondly, during DNA replication the cell division control protein 45 (CDC45) is loaded onto DNA in a CHK2-dependent manner and it recruits DNA polα to pre-replication complexes [126, 127]. Therefore, the inhibition of CHK2 allows transiently slows DNA synthesis by the suppression of new origin firing. Finally, another route of S-phase checkpoint activation is via the NBS1 protein, a component of MRN. In response to IR ATM is recruited to the site of DSBs in an NBS1 and BRCA1 dependent manner, both are phosphorylated by ATM and this promotes the phosphorylation of SMC1 at ser957 and ser966. The phosphorylation of Cohesin component SMC1 is critical for the activation of the intra-S checkpoint, evidenced by the abrogation of the checkpoint in SMC1 knockout cells [128, 129].

9.6. The G2/M Checkpoint

The G2/M checkpoint monitors cells for the presence of DNA damage before they divide to produce daughter cells, and therefore it's crucial to prevent erroneous genetic code being passed on [112]. For a cell to qualify for entry to M phase the CDK1: cyclin B complex must be active which requires reversal of the inhibitory phosphorylation at threonine residues 14 and 15, imparted by the Wee1 and Myt1 kinases [107, 109]. These modifications are removed from CDK1 through the action of CDC25C phosphatase, thereby promoting entry into mitosis [113, 130].

In response to DNA damage induced during S phase the G2/M checkpoint is activated via the phosphorylation of CHK1 and CHK2 by ATR and ATM respectively. Unlike the G1 checkpoint, G2/M is dependent upon the action of ATR for activation, whereas ATM is dispensable, apart from when the checkpoint is triggered by damage that occurs during the G2 phase itself [112]. This highlights the critical crosstalk between ATM and ATR in the initiation of the DDR and checkpoint mechanisms. The G2/M checkpoint is activated by the dual action of CHK1 and CHK2 which phosphorylate CDC25C on it ser216 residue [131]. Phosphorylation of CDC25C creates a binding site for 14-3-3 proteins, the transcription and accumulation of which is p53 dependent, this leads to the sequestration of CDC25C in the cytoplasm, preventing the activation of CDK1 thereby blocking mitosis [130, 131]. Furthermore, p53 is responsible for the accumulation of p21 protein which has also been shown to promote arrest of damaged cells in G2/M, providing a further means of checkpoint regulation [132].

Cell cycle checkpoints are critical to allow for DNA repair in a manner coordinated intimately with the progression of the cell cycle, this is summarised in Figure 5.

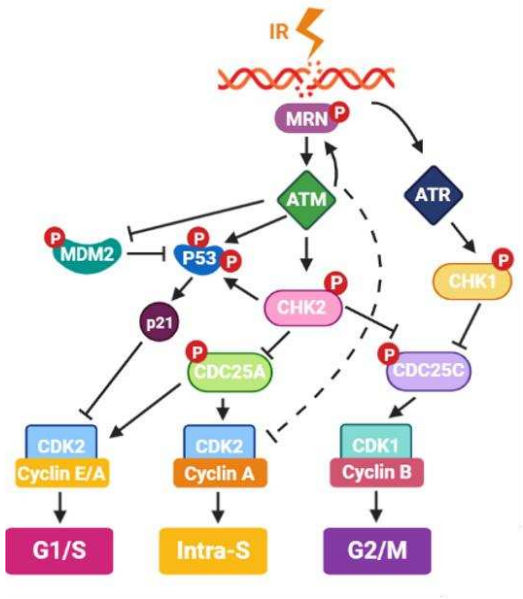

Figure 5: The Cell Cycle Checkpoint Response
In response to DNA damage the cell activates the DDR, a component of which is the cell cycle checkpoint response. ATM is the primary coordinator of the response, phosphorylating CHK2 which can then relay the signal further through its downstream targets to activate the G1/S and intra-S phase checkpoints. G1/S activation is P53 dependent. G2/M checkpoint activation, unlike G1/S and intra-S is mainly reliant on the ATR-CHK1 axis, although ATM can also play a role when damage is incurred during G2 itself [112].

10. Exploiting DNA Repair Deficiency in Cancer Therapy

As previously discussed, functional DDR pathways are crucial for maintaining genome stability. Defective repair results in the accumulation of genetic mutations as well as chromosomal aberrations and this can lead to cancer development. Furthermore, defects in DDR are the cause of a number of human diseases including Ataxia-Telangiectasia (A-T), Fanconi Anaemia, and Li-Fraumeni syndrome caused by an ATM mutation, inefficient repair of DNA crosslinks and a p53 mutation respectively [133–135]. Traditionally, cancer treatment has involved the use of DNA-damaging chemotherapeutic drugs, as well as damage-inducing radiotherapy. These strategies take advantage of the inherent genomic instability of cancer cells by overwhelming them with damage that they will struggle to repair, hence targeting them for apoptotic cell death. In recent years, the concept of exploiting synthetic lethality to treat cancer patients with mutations in DNA repair proteins has gained traction. This review will focus on the use of PARP inhibitors (PARPi) in cancer therapy, although many inhibitors of other DNA repair proteins are now available.

Synthetic lethality is a term used to describe a state where a mutation in one of two given genes permits cell viability, but mutation of both genes results in cell lethality. Perhaps the most pertinent demonstration of this phenomenon relates to the synthetic lethal interaction observed between HR proteins BRCA1 and BRCA2, and PARP1 proteins. Poly (ADP-ribose) polymerase 1 (PARP1) is a nuclear protein that functions in both the repair of SSBs and DSBs by catalysing the addition of a poly (ADP-ribose) chain to target

proteins, known as PARylation, which ultimately leads to the recruitment of other DNA repair factors. PARP1 eventually imparts the PARylation post-translational modification upon itself, leading to its dissociation from repaired breaks [136-138]. The mechanism of action of PARPi's is summarised in Figure 6.

Bryant et al. and Farmer et al. both published data in 2005 illustrating that tumour cells with a BRCA2 mutation showed significantly enhanced cell death compared to controls after treatment with PARPi drugs. Both groups proposed that PARPi's mediate their function by impeding the repair of SSBs, which upon collision with replication machinery lead to the collapse of replication forks. This will result in the activation of DSB repair by HR, however this pathway is defective in BRCA2 mutant cancers, hence collapsed forks will not be repaired and the cell will be targeted for death [139, 140]. More recent publications however have suggested that PARPi's effect death by preventing the self-PARylation of the enzyme, causing it to be 'trapped' on the DNA, preventing DNA replication and repair, and causing cytotoxicity [141].

The use of PARPi's as single agent therapies for BRCA1 and BRCA2 mutant cancers has shown survival benefit for breast and ovarian patients in the clinic [142-145]. Current US FDA approved PARPi's include 1st generation inhibitors Olaparib, Rucaparib, Niraparib, and 2nd generation Talazoparib, which are all employed in the treatment of ovarian cancer, although Olaparib is also approved for breast cancer [146]. Furthermore, efforts are focused on testing the efficacy of PARPi's in a range of other cancers which possess mutations in DNA repair proteins [147, 148]. Another consideration is the potential for acquired and de novo resistance to PARPi treatment amongst patients receiving the drugs at early stages of disease and those on long-term treatment, necessitating the need for development of novel combination therapies [149].

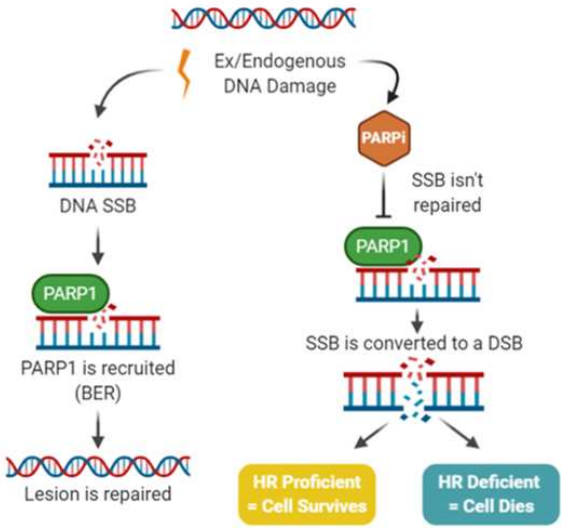

Figure 6: PARP Inhibitor Therapy
PARP inhibitors (PARPi) exploit the synthetic lethal interaction between BER protein PARP, and DSB repair proteins such as BRCA1/2. By inhibiting PARP, the repair of simple DNA lesions is inhibited, causing them to potentiate to DSBs. In cells defective for HR the cell will be unable to repair these breaks and the cell will apoptose, thereby enhancing cancer cell death. Normal cells with functional HR pathways will be unaffected.

11. Conclusion

DNA damage elicits the activation of the highly complex DNA damage response. Depending on the DNA lesion induced the cell can activate one of the five core repair mechanisms discussed above, each of which should resolve the damage and maintain genome stability. In addition, the cell skilfully activates its DDR network in coordination with cell cycle checkpoint activation to allow time for the complete resolution of damage. The system does possess a degree of functional redundancy, with two high-fidelity repair pathways (NHEJ and HR) which can compensate for the loss of one another to a degree. However, mutations of DNA repair proteins are a common feature of cancer cells. PARPi therapies discussed in this review are currently used to treat a number of cancers carrying mutations in HR proteins BRCA1/2, and they represent an advance in the field of targeted treatments. Future research in this arena will most likely highlight novel synthetic lethal partners, akin to BRCA and PARP1, which could be targets for therapeutic exploitation with PARPi's.

References

1. Kanaar R, Hoeijmakers JHJ, Van Gent DC. Molecular mechanisms of DNA double-strand break repair. Trends Cell Biol. 1998; 8: 483-9.
2. Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S. Molecular Mechanisms of Mammalian DNA Repair and the DNA Damage Checkpoints. Annu. Rev. Biochem. 2004; 73: 39-85.
3. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009; 461: 1071-8.
4. Tubbs A, Nussenzweig A. Endogenous DNA Damage as a Source of Genomic Instability in Cancer. Cell. 2017; 168: 644-56.
5. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions. 2006; 160: 1-40.
6. Kawanishi S, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. Biol. Chem. 2006; 387: 365-72.
7. Ward JF. DNA Damage Produced by Ionizing Radiation in Mammalian Cells: Identities, Mechanisms of Formation, and Reparability. Prog. Nucleic Acid Res. Mol. Biol. 1988; 35: 95-125.
8. Bauer NC, Corbett AH, Doetsch PW. The current state of eukaryotic DNA base damage and repair. Nucleic Acids Res. 2015; 43: 10083-101.
9. Wood RD. DNA Repair in Eukaryotes. Annu. Rev. Biochem. 1996; 65: 135-67.
10. Sancar A, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S. Molecular Mechanisms of Mammalian DNA Repair and the DNA Damage Checkpoints. Annu. Rev. Biochem. 2004; 73: 39-85.
11. Jiricny J. The multifaceted mismatch-repair system. Nat. Rev. Mol.

Cell Biol. 2006; 7: 335-46.

12. Hang B. Base excision repair. DNA Repair, Genet. Instab. Cancer. 23–64 (2007) doi:10.1142/9789812706782_0002.
13. Dianov G, Lindahl T. Reconstitution of the DNA base excision-repair pathway. Curr. Biol. 1994; 4: 1069-76.
14. Curtin NJ. DNA repair dysregulation from cancer driver to therapeutic target. Nature Reviews Cancer vol. 2012; 12: 801-17.
15. David SS, O'Shea VL, Kundu S. Base-excision repair of oxidative DNA damage. Nature. 2007; 447: 941-50.
16. Hoeijmakers JHJ. The consequences of DNA injury Genome maintenance mechanisms for preventing cancer. Nature. 2001; 411: 366-74.
17. Wood RD. Nucleotide excision repair in mammalian cells. Journal of Biological Chemistry vol. 1997; 272: 23465-8.
18. Rich T, Allen RL, Wyllie A. Defying death after DNA damage. Nature. 2000; 407: 777-83.
19. Sonoda E, Hochegger H, Saberi A, Taniguchi Y, Takeda S. Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. DNA Repair (Amst). 2006; 5: 1021-9.
20. Gupta A, Yang Q, PanditaRK, Hunt CR, Xiang T, Misri S et al. Cell cycle checkpoint defects contribute to genomic instability in PTEN deficient cells independent of DNA DSB repair. Cell Cycle. 2009; 8: 2198-210.
21. Liang F, Han M, Romanienko PJ, Jasin M. Homology-directed repair is a major double-strand break repair pathway in mammalian cells. Proc. Natl. Acad. Sci. U. S. A. 1998; 95; 5172-7.
22. Rulten SL, Grundy GJ. Non-homologous end joining: Common interaction sites and exchange of multiple factors in the DNA repair process. BioEssays. 2017; 39.
23. Gu Y, Jin S, Gao Y, Weaver DT, Alt FW. Ku70-deficient embryonic stem cells have increased ionizing radiosensitivity, defective DNA end-binding activity, and inability to support V(D)J recombination. Proc. Natl. Acad. Sci. U. S. A. 1997; 94: 8076-81.
24. Nussenzweig A, Sokol K, Burgman P, Li L, Li GC. Hypersensitivity of Ku80-deficient cell lines and mice to DNA damage: The effects of ionizing radiation on growth, survival, and development. Proc. Natl. Acad. Sci. U. S. A. 1997; 94; 13588-93.
25. Gao Y, Sun Y, Frank KM, Dikkes P, Fujiwara Y, Seidl K et al. A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. Cell. 1998; 95; 891-902.
26. Frank KM, Sharpless NE, Gao Y, Sekiguchi JM, Ferguson DO, Zhu C et al. DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the p53 pathway. Mol. Cell. 2000; 5: 993-1002.
27. Barnes DE, Stamp G, Rosewell I, Denzel A, Lindahl T. Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. Curr. Biol. 1998; 8; 1395-8.
28. Burma S, Chen BPC, Chen DJ. Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. DNA Repair (Amst). 2006; 5: 1042-8.
29. Walker JR, Corpina RA, Goldberg J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. Nature. 2001; 412: 607-14.
30. Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochemical Journal vol. 2009; 417; 639-50.
31. Meek K, Dang V, Lees-Miller SP. DNA-PK: the means to justify the ends? Adv. Immunol. 2008; 99: 33-58.
32. Lieber MR. The mechanism of human nonhomologous DNA End joining. J. Biol. Chem. 2008; 283: 1-5.
33. Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. Cell Res. 2008; 18; 134-47.
34. Ciccia A, Elledge SJ. Review The DNA Damage Response : Making It Safe to Play with Knives. Mol. Cell. 2010; 40: 179-204.
35. Jiafeng Gu, Haihui Lu, Albert G. Tsai, Klaus Schwarz MRL. Single-stranded DNA ligation and XLF-stimulated incompatible DNA end ligation by the XRCC4-DNA ligase IV complex: influence of terminal DNA sequence. Nucleic Acids Res. 2007; 35: 5755-62.
36. Calsou P, Delteil C, Frit P, Drouet J, Salles B. Coordinated assembly of Ku and p460 subunits of the DNA-dependent protein kinase on DNA ends is necessary for XRCC4-ligase IV recruitment. J. Mol. Biol. 2003; 326: 93-103.
37. Ma Y, Pannicke U, Schwarz K, Michael RL. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell. 2002; 108: 781-94.
38. Bennardo N, Cheng A, Huang N, Stark JM. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. PLoS Genet. 2008; 4.
39. Deriano L, Roth DB. Modernizing the Nonhomologous End-Joining Repertoire: Alternative and Classical NHEJ Share the Stage. Annu. Rev. Genet. 203; 47: 433-55.
40. Corneo B, Wendland RL, Deriano L, Cui X, Klein IA, Wong SY et al. Rag mutations reveal robust alternative end joining. Nature. 2007; 449: 483-6.
41. McVey M, Lee SE. MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. Trends in Genetics vol. 2008; 24: 529-38 (2008).
42. Sharma S, Javadekar SM, Pandey M, Srivastava M, Kumari R, Raghavan SC. Homology and enzymatic requirements of microhomology-dependent alternative end joining. Cell Death Dis. 2015; 6: e1697.
43. Kabotyanski EB, Gomelsky L, Han JO, Stamato TD, Roth DB. Dou-

ble-strand break repair in Ku86- and XRCC4-deficient cells. Nucleic Acids Res. 1998; 26: 5333-42.

44. Paul K, Wang M, Mladenov E, Bencsik-Theilen A, Bednar T,Wu W et al. DNA Ligases I and III Cooperate in Alternative Non-Homologous End-Joining in Vertebrates. PLoS One. 2013; 8: e59505.
45. Iliakis G, Wu W, Wang M, Terzoudi GI, Pantelias GE. Backup Pathways of Nonhomologous End Joining May Have a Dominant Role in the Formation of Chromosome Aberrations. in Chromosomal Alterations. 2007; 67-85.
46. Wael Y, Mansour, Tim Rhein JDD. The alternative end-joining pathway for repair of DNA double-strand breaks requires PARP1 but is not dependent upon microhomologies. Nucleic Acids Res. 2010; 38: 6065-77.
47. Wang M, Wu W, Wu W, Rosidi B, Zhang L, Wang H et al. PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. Nucleic Acids Res. 2006; 34: 6170-82.
48. Mateos-Gomez PA, Gong F, Nair N, Miller KM, Lazzerini-Denchi E, Sfeir A. Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. Nature. 2015; 518; 254-7.
49. Dudas A, Chovanec M. DNA double-strand break repair by homologous recombination. Mutation Research - Reviews in Mutation Research vol. 2004; 566: 131-67.
50. Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. Cell Res. 2008; 18: 99-113.
51. Helleday T. Pathways for mitotic homologous recombination in mammalian cells. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis vol. 2003; 532: 103-15.
52. Paull TT, Gellert M. Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. Genes Dev. 1999; 13: 1276-88.
53. Desai-Mehta A, Cerosaletti KM, Concannon P. Distinct Functional Domains of Nibrin Mediate Mre11 Binding, Focus Formation, and Nuclear Localization. Mol. Cell. Biol. 2001; 21: 2184-91.
54. Powell SN, Kachnic LA. Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. Oncogene vol. 2003; 22: 5784-91.
55. Chen L, Nievera CJ, Lee AYL, Wu X. Cell cycle-dependent complex formation of BRCA1·CtIP·MRN is important for DNA double-strand break repair. J. Biol. Chem. 2008; 283: 7713-20.
56. MS W. Replication protein A: heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annu. Rev. Biochem. 1997; 66: 61-92.
57. Serrano MA, et al. DNA-PK, ATM and ATR collaboratively regulate p53-RPA interaction to facilitate homologous recombination DNA repair. Oncogene. 2013; 32: 2452-62.
58. Gasior SL, Wong AK, Kora Y, Shinohara A, Bishop DK. Rad52 associates with RPA and functions with Rad55 and Rad57 to assemble meiotic recombination complexes. Genes Dev. 1998; 12: 2208-21.
59. Parsons CA, Baumann P, Van Dyck E, West SC. Precise binding of single-stranded DNA termini by human RAD52 protein. EMBO J. 2000; 19: 4175-81.
60. Sugiyama T, Zaitseva EM, Kowalczykowski SC. A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the Saccharomyces cerevisiae Rad51 protein. J. Biol. Chem. 1997; 272: 7940-5.
61. San Filippo J, Sung P, Klein H. Mechanism of Eukaryotic Homologous Recombination. Annu. Rev. Biochem. 2008; 77: 229-57.
62. Taylor MRG, et al. Rad51 Paralogs Remodel Pre-synaptic Rad51 Filaments to Stimulate Homologous Recombination. Cell. 2015; 162: 271-86.
63. Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. Mol. Cell. 2001; 7: 263-72.
64. Moynahan ME, Jasin M. Loss of heterozygosity induced by a chromosomal double-strand break. Proc. Natl. Acad. Sci. U. S. A. 1997; 94: 8988-93.
65. Haber JE. Partners and pathways - Repairing a double-strand break. Trends in Genetics. 2000; 16: 259-64.
66. Wright WD, Heyer WD. Rad54 Functions as a Heteroduplex DNA Pump Modulated by Its DNA Substrates and Rad51 during D Loop Formation. Mol. Cell. 2014; 53: 420-32.
67. Helleday T, Lo J, van Gent DC, Engelward BP. DNA double-strand break repair: From mechanistic understanding to cancer treatment. DNA Repair (Amst). 2007; 6: 923-35.
68. Nimonkar AV, Sica RA, Kowalczykowski SC. Rad52 promotes second-end DNA capture in double-stranded break repair to form complement-stabilized joint molecules. Proc. Natl. Acad. Sci. U. S. A. 2009; 106: 3077-82.
69. Holliday R. A mechanism for gene conversion in fungi. Genet. Res. 1964; 5: 282-304.
70. Valerie K, Povirk LF. Regulation and mechanisms of mammalian double-strand break repair. Oncogene. 2003; 22: 5792-812.
71. Punatar RS, Martin MJ, Wyatt HDM, Chan YW, West SC. Resolution of single and double Holliday junction recombination intermediates by GEN 1. Proc. Natl. Acad. Sci. USA. 2017; 114: 443-50.
72. Wechsler T, Newman S, West SC. Aberrant chromosome morphology in human cells defective for Holliday junction resolution. Nature. 2011; 471: 642-6.
73. Matos J, West SC. Holliday junction resolution: Regulation in space and time. DNA Repair (Amst). 2014; 19: 176-81.
74. Constantinou A, Davies AA, West SC. Branch migration and Hol-

liday junction resolution catalyzed by activities from mammalian cells. Cell. 2001; 104: 259-68.

75. Heyer WD. Recombination: Holliday Junction Resolution and Crossover Formation. Current Biology vol. 2004; 14: R56-8.
76. Bizard AH, Hickson ID. The dissolution of double Holliday junctions. Cold Spring Harb. Perspect. Biol. 2014; 6: a016477.
77. Sargent RG, Brenneman MA, Wilson JH. Repair of site-specific double-strand breaks in a mammalian chromosome by homologous and illegitimate recombination. Mol. Cell. Biol. 1997; 17: 267-77.
78. Jackson SP, Jeggo PA. DNA double-strand break repair and V(D)J recombination: involvement of DNA-PK. Trends in Biochemical Sciences vol. 1995; 20: 412-5.
79. Scully R, et al. Genetic analysis of BRCA1 function in a defined tumor cell line. Mol Cell. 1999; 4: 1093-9.
80. Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. Cell Res. 2008; 18: 134-47.
81. Murray JM, Carr AM. Integrating DNA damage repair with the cell cycle. Curr. Opin. Cell Biol. 2018; 52: 120-5.
82. Ceccaldi R, Rondinelli BDA. Repair Pathway Choices and Consequences at the Double-Strand Break. Trends Cell Biol. 2016; 26: 52-64.
83. Escribano-Díaz C, et al. A Cell Cycle-Dependent Regulatory Circuit Composed of 53BP1-RIF1 and BRCA1-CtIP Controls DNA Repair Pathway Choice. Mol. Cell. 2013; 49: 872-83.
84. Saredi G, Huang H, Hammond CM, Alabert C, Bekker-Jensen S, Forne I, et al. H4K20me0 marks post-replicative chromatin and recruits the TONSL-MMS22L DNA repair complex. Nature. 2016; 534: 714-8.
85. Chen FQ, Nastasi A, Shen ZY, et al. Cell cycle-dependent protein expression of mammalian homologs of yeast DNA double- strand break repair genes RAD51 and RAD52. Mutat. Res. - Fundam. Mol. Mech. Mutagen. 1997; 384: 205-11.
86. Arnoult N, et al. Regulation of DNA repair pathway choice in S and G2 phases by the NHEJ inhibitor CYREN. Nature. 2017; 549: 548-52.
87. Chen BP, Chan DW, Kobayashi J, et al. Cell cycle dependence of DNA-dependent protein kinase phosphorylation in response to DNA double strand breaks. J. Biol. Chem. 2005; 280: 14709-15.
88. Petrini JHJ, Stracker TH. The cellular response to DNA double-strand breaks: Defining the sensors and mediators. Trends Cell Biol. 2003; 13: 458-62.
89. Zhou BBS, Elledge SJ. The DNA damage response: Putting checkpoints in perspective. Nature vol. 2000; 408: 433-9.
90. Lee JH, Paull TT. Direct Activation of the ATM Protein Kinase by the Mre11/Rad50/Nbs1 Complex. Science (80). 2004; 304: 93-6.
91. Harper JW, Elledge SJ. The DNA Damage Response: Ten Years After. Molecular Cell vol. 2007; 28: 739-45.
92. Lowndes NF, Murguia JR. Sensing and responding to DNA damage. Current Opinion in Genetics and Development vol. 2000; 10: 17-25.
93. Volkmer E, Karnitz LM. Human homologs of Schizosaccharomyces pombe Rad1, Hus1, and Rad9 form a DNA damage-responsive protein complex. J. Biol. Chem. 1999; 274: 567-70.
94. Maréchal A, Zou L. DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb. Perspect. Biol. 2013; 5: 1-17.
95. Lempiäinen H, Halazonetis TD. Emerging common themes in regulation of PIKKs and PI3Ks. EMBO J. 2009; 28: 3067-73.
96. Lavin MF, Shiloh Y. The Genetic Defect in Ataxia-Telangiectasia. Annu. Rev. Immunol. 1997; 15: 177-202.
97. Cliby WA, et al. Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA- damaging agents and defects in cell cycle checkpoints. EMBO J. 1998; 17: 159-69.
98. Tibbetts R, Kathryn S, Brumbaugh M, Josie M, Williams, Jann N, et al. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev. 1999; 13: 152-7.
99. Banin S, Moyal L, Shieh SY, Taya Y, Anderson CW, Chessa L, et al. Enhanced phosphorylation of p53 by ATM in response to DNA damage Science. Science (80). 1998; 281: 1674-7.
100. Kim ST, Lim DS, Canman CE, Kastan MB. Substrate specificities and identification of putative substrates of ATM kinase family members. J. Biol. Chem. 1999; 37538-43.
101. Shiloh Y. ATM and ATR: Networking cellular responses to DNA damage. Current Opinion in Genetics and Development vol. 2001; 11: 71-7.
102. Dupré A, Boyer-Chatenet L, Gautier, J. Two-step activation of ATM by DNA and the Mre11-Rad50-Nbs1 complex. Nat. Struct. Mol. Biol. 2006; 13: 451-7.
103. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science (80-.). 2003; 300: 1542-8.
104. Shiotani B, Zou L. Single-Stranded DNA Orchestrates an ATM-to-ATR Switch at DNA Breaks. Mol. Cell. 2009; 33: 547-58.
105. Nurse P, Masui Y, Hartwell L. Understanding the cell cycle. Nature Medicine vol. 1998; 4: 1103-6.
106. Chao HX, et al. Evidence that the human cell cycle is a series of uncoupled, memoryless phases. Mol. Syst. Biol. 2019; 15.
107. Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif. 2003; 36: 131-49.
108. Cooper GM. The Cell: A Molecular Approach. in The Cell: A Molecular Approach (Sinauer Associates, 2000).
109. Grana X, Reddy EP. Cell cycle control in mammlian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin dependent kinase inhibitors (CKIs). Oncogene. 1995; 11:

211-9.

110. Hartwell LH, Weinert TA. Checkpoints: Controls that ensure the order of cell cycle events. Science (80-.). 1989; 246: 629-34.

111. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. Nature vol. 2004; 432: 316-23.

112. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes and Development vol. 2001; 15: 2177-96.

113. Samuel T, Weber HO, Funk JO. Linking DNA damage to cell cycle checkpoints. Cell cycle (Georgetown, Tex.) vol. 2002; 1: 161-7.

114. Zhao H, Piwnica-Worms H. ATR-Mediated Checkpoint Pathways Regulate Phosphorylation and Activation of Human Chk1. Mol. Cell. Biol. 2001; 21: 4129-39.

115. Siliciano JD, Canman CE, Taya Y, Sakaguchi K, Appella E, KM. DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev. 1997; 11: 3471-81.

116. Haupt Y, Maya R, Kazaz A, OM. Mdm2 promotes the rapid degradation of p53. Nature. 1997; 387: 296-9.

117. Agarwal ML, Taylor WR, Chernov MV, Chernova OB, SG. The p53 network. J. Biol. Chem. 1998; 273: 1.

118. Walworth NC. Cell-cycle checkpoint kinases: Checking in on the cell cycle. Current Opinion in Cell Biology vol. 2000; 12: 697-704.

119. Chen X, Ko LJ, Jayaraman L, Prives C. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. Genes Dev. 1996; 10: 2438-51.

120. Hall-Jackson CA, Cross DA, Morrice E, Smythe C. ATR is a caffeine-sensitive, DNA-activated protein kinase with a substrate specificity distinct from DNA-PK. Oncogene. 1999; 18: 6707-13.

121. Matsuoka S, Huang M, E S. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science (80-.). 1998; 282: 1893-7.

122. Ahn JY, Schwarz JK, Piwnica-Worms H, Canman CE. Threonine 68 Phosphorylation by Ataxia Telangiectasia Mutated Is Required for Efficient Activation of Chk2 in Response to Ionizing Radiation. Cancer Res. 2000; 60: 5934LP-6.

123. Chehab NH, Malikzay A, Appel M, Halazonetis TD. Chk2/hCds1 functions as a DNA damage checkpoint in G1 by stabilizing p53. Genes Dev. 2000; 14: 278-88.

124. Falck J, Mailand N, Syljuåsen RG, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature. 2001; 410; 842-7.

125. Painter RB, Young BR. Radiosensitivity in ataxia-telangiectasia: A new explanation. Proc. Natl. Acad. Sci. USA. 1980; 77: 7315-317.

126. Bartek J, Lukas C, Lukas J. Checking on DNA damage in S phase. Nature Reviews Molecular Cell Biology vol. 2004; 5: 792-804.

127. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell vol. 2003; 3: 421-9.

128. Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes Dev. 2004; 18: 1423-38.

129. Yazdi PT, et al. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. Genes Dev. 2002; 16: 571-82.

130. Takizawa CG, Morgan DO. Control of mitosis by changes in the subcellular location of cyclin-B1-Cdk1 and Cdc25C. Current Opinion in Cell Biology vol. 2000; 12: 658-65.

131. Sanchez Y, et al. Conservation of the Chk1 checkpoint pathway in mammals: Linkage of DNA damage to Cdk regulation through Cdc25. Science (80-.). 1997; 277: 1497-501.

132. Bunz F, et al. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science (80-.). 1998; 282: 1497-501.

133. Lavin MF, Shiloh Y. The Genetic Defect In Ataxia-Telangiectasia. Annu. Rev. Immunol. 1997; 15: 177-202.

134. Joenje H, Patel KJ. The emerging genetic and molecular basis of Fanconi anaemia. Nature Reviews Genetics vol. 2001; 2: 446-57.

135. Frebourg T, et al. Germ-line p53 mutations in 15 families with Li-Fraumeni syndrome. Am. J. Hum. Genet. 1995; 56: 608-15.

136. Ray Chaudhuri A, Nussenzweig A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat. Rev. Mol. Cell Biol. 2017.

137. Woodhouse BC, Dianova II, Parsons JL Dianov GL. Poly(ADP-ribose) polymerase-1 modulates DNA repair capacity and prevents formation of DNA double strand breaks. DNA Repair (Amst). 2008; 7: 932-40.

138. Wei H, Yu X. Functions of PARylation in DNA Damage Repair Pathways. Genomics, Proteomics and Bioinformatics vol. 2016; 14: 131-9.

139. Farmer H, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005; 434: 917-21.

140. Bryant HE, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005; 434: 913-7.

141. Murai J, et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res. 2012; 72: 5588-99.

142. Rodler ET, et al. Phase I study of veliparib (ABT-888) combined with cisplatin and vinorelbine in advanced triple-negative breast cancer and/or BRCA mutation-associated breast cancer. Clin. Cancer Res. 2016; 22: 2855-64.

143. Gray HJ, et al. Phase I combination study of the PARP inhibitor veliparib plus carboplatin and gemcitabine in patients with advanced ovarian cancer and other solid malignancies. Gynecol. Oncol. 2018; 148: 507-14.

144. Del Campo JM, et al. The successful phase 3 niraparib EN- GOT-OV16/NOVA trial included a substantial number of patients with platinum resistant ovarian cancer (OC). J. Clin. Oncol. 2017;

35: 5560.

145. Cerrato A, Morra F, Celetti A. Use of poly ADP-ribose polymerase [PARP] inhibitors in cancer cells bearing DDR defects: The rationale for their inclusion in the clinic. Journal of Experimental and Clinical Cancer Research vol. 2016; 35: 1-13.

146. Yi M, et al. Advances and perspectives of PARP inhibitors. Experimental Hematology and Oncology vol. 2019; 8: 1-12.

147. Pilié PG, Gay CM, Byers LA, O'Connor MJ, Yap TA. PARP inhibitors: Extending benefit beyond BRCA-mutant cancers. Clinical Cancer Research vol. 2019; 25: 3759-71.

148. Veneris JT, Matulonis UA, Liu JF, Konstantinopoulos PA. Choosing wisely: Selecting PARP inhibitor combinations to promote anti-tumor immune responses beyond BRCA mutations. Gynecologic Oncology vol. 2020; 156: 488-97.

149. Dréan A, Lord CJ, Ashworth A. PARP inhibitor combination therapy. Critical Reviews in Oncology/Hematology vol. 2016; 108: 73-85.