The interplay of DNA damage, epigenetics and tumour heterogeneity in driving cancer cell fitness

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Intra-tumor heterogeneity is a primary cause of therapeutic failure, driving tumor progression. Within tumors, diverse cell states coexist, maintained by a specific chromatin landscape that influences various cell functions, including cancer stemness. Among factors that induce chromatin changes affecting cell state fitness, DNA damage and its repair have emerged as significant contributors. This perspective examines recent advances that elucidate the interplay between DNA repair, epigenome, and cell plasticity. We discuss how epigenome affects DNA repair and, conversely, how DNA repair-induced chromatin changes influence cell plasticity. Finally, we discuss emerging concepts and highlight the therapeutic implications of these interconnected mechanisms.

Intratumor heterogeneity (ITH) is driven by (epi)genomic remodeling and microenvironmental changes, and present therapeutic resistance hurdles that must be overcome to optimize cancer treatments.

Over the past decade, technological advances have enabled the exploration of ITH at single-cell resolution, revealing a multitude of functional genetic and non-genetic cell states within the same tumor[1–3]. Certain cell states, such as those harboring stem cell traits (hereafter named cancer stem cells, CSCs), have been repeatedly associated with tumor progression and therapeutic failure[4–6]. Malignant cells can adapt to various stresses, including cancer treatment, by transitioning between states, a phenomenon known as cell plasticity. This transition between cell states is thought to be the major source of drug-resistance adaptation[7]. In this context, epigenetic remodeling, such as histone modifications, plays a significant role in tumor evolution processes[8–11]. For example, breast cancer cells can reach a drug-tolerant state by reducing H3K27me3 histone marks[9]. Conversely, inhibition of H3K27me3 demethylation in combination with chemotherapy prevents the transition to this drug-tolerant state. These studies emphasize the fact that the epigenetic heterogeneity and plasticity act as reservoir of cell states and therefore as a determinant of cell fate upon treatment exposure[12]. In this context epigenetic modifiers represent potential therapeutic targets to overcome therapy resistance.

From a fundamental perspective, each cell state may reflect a distinct configuration of gene regulatory networks (GRNs), which emerge from the complex interplay among chromatin structure, transcription factors, and gene expression[13]. The differential response of cancer cell states to treatment may be explained by variations in their chromatin architecture itself and the resulting activation of specific GRNs. While most chemotherapies used in cancer treatment act as DNA-damaging agents, several studies have demonstrated the role of chromatin features—such as chromatin folding, nucleosome remodeling, and histone modifications—in influencing DNA repair responses. Conversely, chromatin aberrations that confer either epigenetic restriction or increased plasticity can drive adaptive cell fate transitions[14]. More recently, studies have highlighted the crucial role of the DNA repair machinery in modulating chromatin marks, organization, and mobility[15,16]. This suggests that DNA double-strand breaks (DSBs) induced by treatment, along with their repair, can reshape chromatin organization, ultimately altering intracellular signaling pathways. These changes influence the cell's capacity for adaptation and contribute to the evolutionary dynamics of cancer.

In this perspective, we discuss recent evidence for the interplay between DNA repair, the epigenetic landscape, and cancer cell plasticity. We discuss how the epigenome can impact the DNA damage

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repair response and, conversely, how DNA repair induces chromatin mis-restoration with direct effects on ITH. Finally, we highlight the promising therapeutic implications that could result from elucidating this coordinated process.

Interplay between DNA damage repair and epigenetic landscapes

Accumulating evidences suggest that DNA damage and the subsequent activation of DNA repair machinery depends on the chromatin structure that specify cell identity. Here, we have summarized studies that relate the impact of cell identity on DNA damage mapping and the response to these damages.

Influence of cell identity on the spatial mapping of DNA damage

Cell identity is determined by specific (epi)genetic landscape, which governs the activity of a given gene regulatory network (GRN). As a result, the identity of a cell has a unique genomic DNA packaging that restricts the localization of DNA damage. Indeed, genome-wide

mapping of DSB, using BLESS or DSB Capture methodologies, demonstrates a relationship between genomic instability and nucleosome density. DSB are enriched in regions bearing epigenetic marks of transcriptionally active genes (H3K4me2/3), enhancer loci (H3K27ac, H3K9ac, and H3K4me1), regions rich in structural proteins (such as CCCTC-binding factor (CTCF)), or with motifs from several transcription factors (e.g. FOS, JUN, P53), and RNA polymerase II[17–20]. Thus, DSB do not appear randomly, but their localization is impacted by numerous cellular processes, DNA structures and sequences, histone modifications, and ultimately cell identity (Fig. 1A). The mapping of genomic breaks or "breakome" would therefore be influenced by cell identity[17,21–24]. Indeed, such heterogeneity in DNA damage mapping has been observed in tumors with glioblastoma cancer stem cells (CSCs) that presents a high expression activity of genes located at common fragile sites (CFS) compared to the glioblastoma cells composing the tumor bulk. This transcriptional activity promotes a transcription-replication conflict due to the specific DNA structure at CFS and leads to DSB formation[25]. Another source of genomic breaks that may be
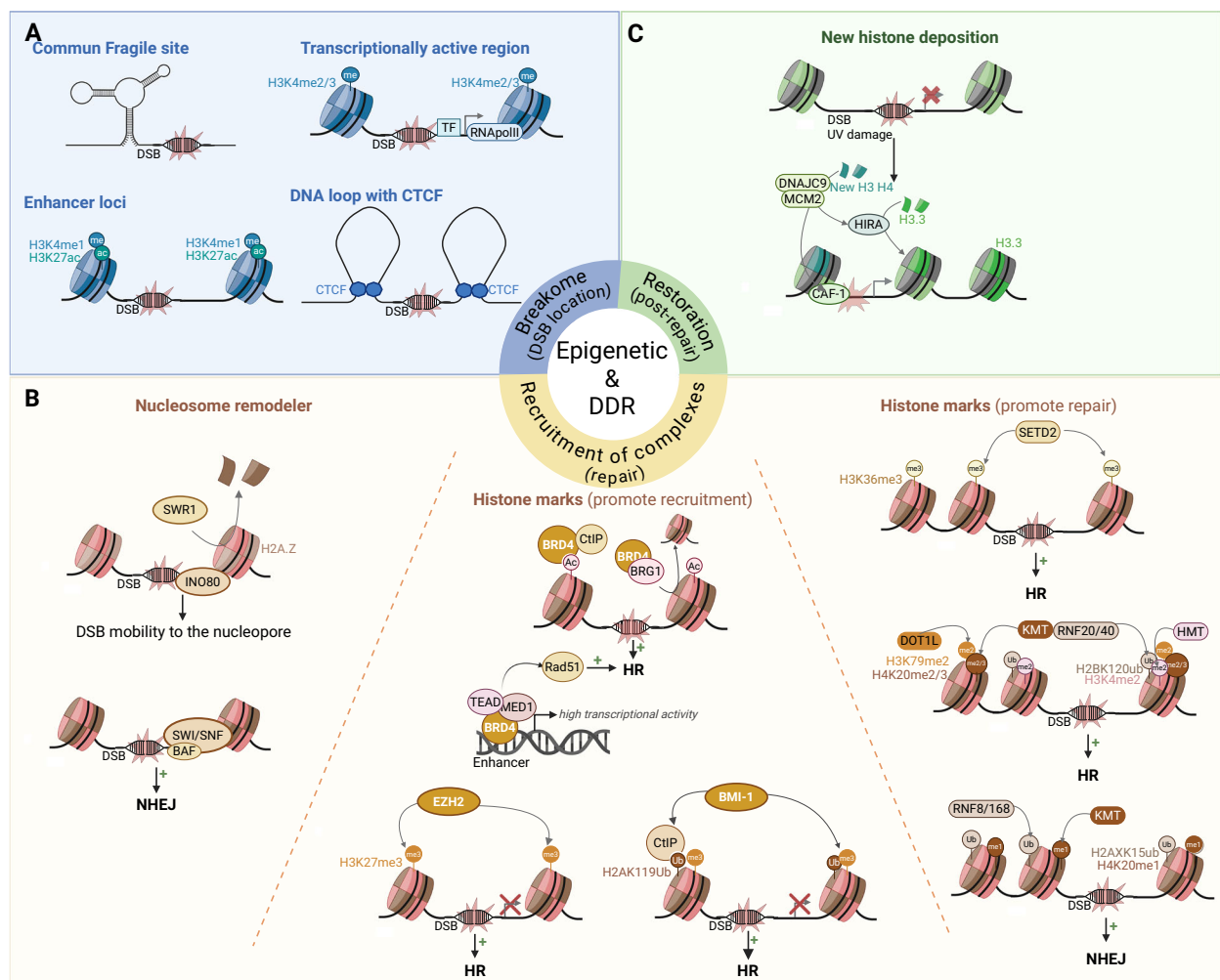

Fig. 1 | From breaks to restoration: the of chromatin architecture in the DNA Damage Response. A, B During the DNA repair process the chromatin structure is playing an essential by influencing the location of DSB accumulation (Breakome) and the recruitment of the DDR machinery to the DSB locus (Repair). **A** DSBs preferentially accumulate in specific chromatin contexts, including common fragile sites (CFS), transcriptionally active regions, DNA loops enriched in structural proteins such as CCCTC-binding factor (CTCF), and active enhancers (**B**). The epigenetic landscape critically influences the recruitment of DNA repair complexes. Changes in nucleosome composition enhance DSB mobility toward the nuclear pores, which are areas of increased repair activity. Nucleosome remodelers, such as the SWI/SNF complex, facilitate the recruitment of NHEJ factors by remodeling chromatin flanking DSBs. Similarly, the choice between homologous recombination (HR) and NHEJ is influenced by the specific pattern of histone post-translational modifications (PTMs) around the DSB sites. **C** Restoration and maintenance of chromatin structure following DNA repair are essential for preserving cellular function. Among the emerging mechanisms, the deposition of new histones H3 and H4 by DNAJC9 and MCM2, as well as H3.3 incorporation by HIRA in collaboration with CAF-1, appear to play a central role in chromatin reassembly post-repair. Created in BioRender. Mitoyan, L. (2025) https://BioRender.com/5je9jd0.

directly linked to cell identity is the accumulation of single-strand breaks (SSBs) hotspots that tend to occur in the immediate vicinity around the TSSs of genes that are actively transcribed[26]. Thus, SSBs hotspots location will be dependent on the activity of a given GRN and inextricably linked to cell identity. If the mechanistic reason behind this phenomenon is still unknown, we can suppose that the repair efficiency of breaks around TSSs is less efficient due to the special chromatin environment around TSSs compared to elsewhere in the genome. Another explanation may reside in Transcription-Replication conflicts that occur when the two critical cellular machineries responsible for gene expression and genome duplication collide with each other on the same genomic location. A recent study reports that DNA loci of hyper-transcribed genes accumulate DNA damage due to the TOP1 cleavage complex trapped in the R-loop that interferes with the resolution of these supercoiling events, ultimately leading to DSBs[27].

Until now, the concept of breakome is mainly explained by transcription-induced DNA breaks but we cannot exclude that some DNA damage hotspots are not related to transcription initiation but still represent breaks consistently occurring in different cell states. It may be caused by exogenous factors that has a strong preference for specific chromatin structures. In line with this hypothesis, the use of clickable cisplatin derivatives revealed a unique genomic distribution of induced DNA-Pt lesions according to chromatin structure[28]. Indeed, the modification of chromatin folding by histone deacetylase inhibitor increases the number of induced DNA-Pt lesions. Thus, chromatin relaxation due to histone hyperacetylation reveals new genomic targets for cisplatin. This observation sustains an influence of chromatin structure on DSB mapping following exposition to exogenous DNA damaging agents.

Cell identity guide the DNA repair pathway choice

In addition to its influence on DNA damage mapping, cell identity also has a direct impact on the DNA repair response. The type of DNA repair pathway activated in response to DNA lesions has always been considered to be the result of the type of damage and the phase of the cell cycle in which the injured cell is located. However, over the past few years, several studies have highlighted the influence of cell state on the DNA damage response and repair capacity.

First, several regulators that maintain cell identity present a dual role with the capacity to activate specific DDR pathways. The anticlastogenic function of these cell-fate regulators directly linked cell identity to a singular DNA damage response. Second, the chromatin structure itself appears to have an impact on DNA repair pathway activation. These both elements will strongly influence the ability of each cancer cell state to maintain genome integrity, respond to genotoxic agents, and will impact tumor adaptability to treatment.

Dual role of cell identity safe guarders. Among the various regulators of cell identity and the DNA damage response, the Polycomb group complexes (PRC1 and PRC2) play essential roles. Their synergistic activity leads to the formation of transcriptionally repressive Polycomb domains, characterized by compacted chromatin enriched in the histone modifications H2AK119ub1 (catalyzed by PRC1) and H3K27me3 (catalyzed by PRC2). Initial models proposed a hierarchical recruitment mechanism, where PRC2 is first recruited to target loci to deposit H3K27me3. This mark is then recognized by canonical PRC1 complexes via chromodomain-containing subunits, facilitating H2AK119ub1 deposition and further chromatin compaction[29–31]. Importantly, components of both PRC1 and PRC2 have been implicated in stem cell identity regulation as well as in the detection and response to DNA lesions, suggesting their dual role in maintaining genome integrity and cellular plasticity. Among these components, BMI1 (PRC1) is the most prominent. BMI1 is associated with self-renewal capacity of various adult stem cells[32,33]. The preponderant role

of BMI1 in maintaining stemness in malignant cells has been demonstrated in different cancers such as breast, colon, head and neck, or lung[34–37]. The inhibition of BMI1 is sufficient to decrease the proportion of CSC and to limit tumor progression in colon or prostate cancers[35,38]. Besides its role as an epigenetic regulator of cell identity, BMI1 appears to contribute to the DNA damage response by depositing H2AK119ub mark at the DNA lesions. It allows the recruitment of CtIP and DNA end resection to promote DNA repair via HR[30] (Fig. 2, line1). This central regulatory node connecting cell identity and DDR activation may explain the higher capacity of CSC compared to the tumor bulk to resist to genotoxic agents as demonstrated in glioblastoma or breast tumors[39,40]. Similar to PRC1, components of PRC2 are also involved into the maintenance of cell identity and the activation of DNA damage response[41–43] (Fig. 2, line2). Enhancer of zeste homolog 2 (EZH2), the PRC2 catalytic component, is able to increase H3K27me3 mark during chemotherapeutic treatment to regulate the expression of the DNA/RNA helicase SLFN11, inhibit transcriptional activity at DNA damage site and promote DNA repair[44,45]. Because EZH2 is frequently overexpressed in CSCs[46], we can assume that EZH2 capacity to control gene expression during treatment will contribute to CSC resistance. Actually, it was demonstrated that the MELK-FOXM1-EZH2 signaling axis is essential for GSC radioresistance[47].

More recently, it was demonstrated that other epigenetic regulators such as the BET protein BRD4 known to bind to active enhancers and control cell identity gene induction[48] may also play a role in regulating HR during DSB repair (Fig. 2, **line3**). Actually, the interaction between BRD4, BRG1, and CtIP appears to be required to achieve homology-directed repair of DSBs[49]. Moreover, the regulatory machinery at super-enhancers involving BRD4, MED1, and TEAD appears to recruit RAD51 to repair DSBs generated by the high transcriptional activity of these loci[20].

This dual role of PRC1/2 complex or BRD4 in conferring efficient DNA damage response to specific cancer cell states is not restricted to epigenetic effectors. The Epithelial-to-Mesenchymal transition (EMT) transcription factor ZEB1 is a critical regulator of cancer cell plasticity[50] but also strongly contributes to DNA damage response and repair (Fig. 2, **line4**). ZEB1 is required for DNA repair and the clearance of DNA breaks by controlling the expression of ATM, and its phosphorylation enhance ZEB1 interaction with USP7 and CHK1 to promote HR pathway activity[51]. ZEB1 can also inhibit polθ expression leading to a lower error-prone Alt-EJ pathway activity and consequently increasing genome stability of EMT-like cells[52]. As a consequence, ZEB1 inhibition is sufficient to impair DNA damage repair in CSCs and sensitize tumors to radiotherapy[51].

Cell state-dependent chromatin structure and DNA repair. Beyond the dual role of certain cell identity safe-guarders to lineage-restrict DNA damage response and repair, they also guarantee a unique chromatin folding. This cell state-dependent chromatin structure will also constrain DNA repair. Indeed, it has been demonstrated that repair proteins spread according to chromatin topological features (Fig. 1B), using detailed analysis of DSB repair factor localization in single cells[19]. Several studies reported a preponderant role of the chromatin structure in governing DNA repair pathway choice, principally between homologous recombination (HR) and non-homologous end joining (NHEJ) pathways. We can identify HR restrictive domain as lamina-associated domain (LAD) that preferentially mobilized the error-prone repair pathway (NHEJ, Alt-EJ) to repair DSB[53,54]. Thus, the re-localization of DSB in HR permissive domain (euchromatin) is essential for genome stability and cell state maintenance[55]. Diverse chromatin remodeling factor are involved in increasing chromatin accessibility as BRG1 or INO80[56]. These chromatin remodelers promote the break relocation at the nuclear periphery by the incorporation of H2A.Z that increases the interaction of DSB with nuclear pore[57]. The SWI/SNF complex (with BAF sub-unit), is also required for efficient
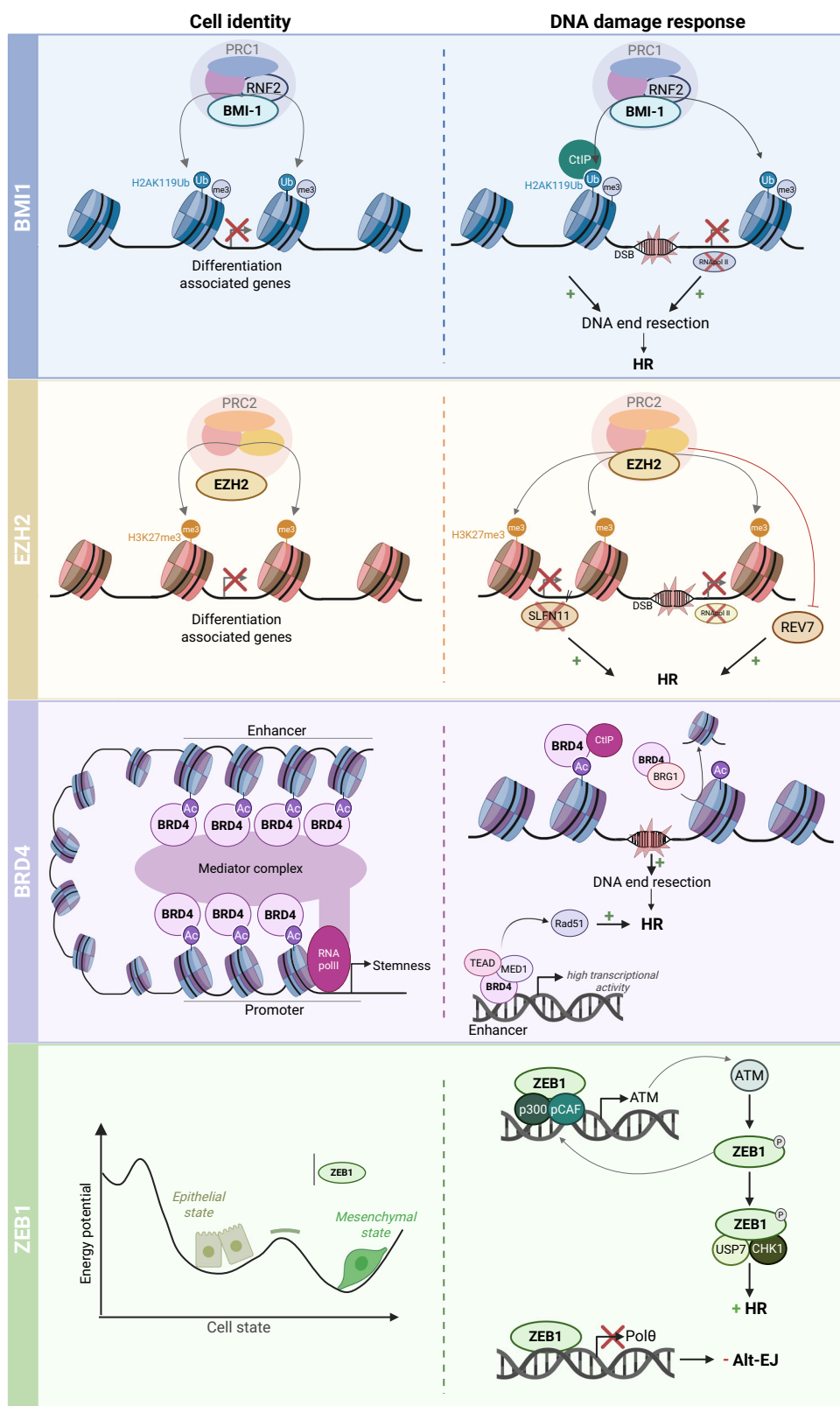
DNA repair pathways activation including NHEJ, by re-organizing the chromatin flanking the DNA lesion to promote the DNA repair[58–61]. If the chromatin mobility during DNA repair is essential, histone modifications are also preponderant to reshape chromatin landscape at DSBs. Using combined repair proteins (RAD51, XRCC4, 53BP1) and histone marks ChIP-seq with well-annotated DSB map, the group of Gaelle Legube offered a comprehensive picture of the DNA repair pathway choice according to the chromatin landscape[62]. HR and NHEJ appears to conceivably require very different chromatin settings. Concordant to previous studies, HR-competent chromatin contained elevated levels of H3K36me3. This histone mark, associated with transcription elongation machinery, is deposited by the trimethyl transferase SETD2 (SET domain containing 2)[63] and the NSD family members[64]. The H3K36me3 is bound by LEDGF (lens

Fig. 2 | Dual role of cell identity safe-guarders. (line 1) To control cell identity, BMI1 (a polycomb repressor complex 1 (PRC1) protein) increase mono-ubiquitinylation of H2AK119 to inhibit the transcription of differentiation-associated genes. To activate DNA repair, BMI1 ubiquitylate histones next to DSB. It leads to local transcription inhibition and recruitment of (C-terminal binding protein) interacting protein (CtIP) to promote DNA end resection and homologous recombination (HR). (line2) To control cell identity, EZH2 (a PRC2 protein) increase H3K27me3 marks to inhibit the transcription of differentiation-associated genes. To activate DNA repair EZH2 promote HR though the downregulation of Schlafen11 gene expression (SLFN11, an DNA/RNA helicase), and increase H3K27me3 marks on histones neighboring the DSB to induce local transcription silencing. Moreover, EZH2 is also known to inhibit REV7, hence favoring the HR repair pathway choice. (line 3) To control cell identity, BRD4 (Bromodomain-containing protein 4) regulates the transcription of genes-related to stemness by promoting enhancer-promoter interaction. To activate DNA repair, BRD4 interact with BRG1 to increase histone eviction and bind to histones next to DSB favoring CtIP recruitment and HR repair activity. In addition, BARD4 binds super-enhancers with MED1 and TEAD, to promote the recruitment of Rad51 and DNA repair on high transcript loci (line 4) To control cell identity, ZEB1 (Zinc finger E-box binding homeobox 1) play as a major regulator of the epithelial-to-mesenchymal transition (EMT) program. To activate DNA repair, ZEB1 promote HR and inhibit Alt-EJ by regulating the gene expression of ATM and polθ. Moreover, ATM phosphorylation enhance ZEB1 interaction with USP7 and CHK1 to promote HR pathway activity. Created in BioRender. Mitoyan, L. (2025) https://BioRender.com/5je9jd0.

epithelium-derived growth factor) that promotes recruitment of RAD51 and Ct-IP to facilitate DNA damage repair by HR[63,65]. Mechanistically, K354 deacetylation by SIRT1 (a HDAC protein) promotes SAMHD1 (Sterile alpha motif and HD domain-containing protein 1) recruitment to DSB and binding to ssDNA at DSB, which in turn facilitates Ct-IP ssDNA binding allowing genome integrity through the promotion of HR[66].HR-competent chromatin is also associated with H3K79me2, H4K20me2/3, H2BK120ub, H3K4me2 near the DSB and low level of H2AZ. Conversely, DSB repair by NHEJ exhibits high levels of H4K20me1 and H2AXK15ub[62]. These experimental approaches represent the first step in understanding how chromatin structure guides the choice of DNA repair pathway. Because the 3D chromatin architecture undergoes considerable remodeling during cell state transition[67], we can suspect that DNA damage response is constantly adjusted during cell plasticity. For example, the loss of H2A.Z near the TSS or the increase of H3K36me3 (implicated in HR pathway) modulate EMT by promoting expression of mesenchymal genes involved in first step of development[68]. The regulation of chromatin plasticity is essential to maintain cell identity in physiological and pathological conditions[14]. As a direct consequence, in tumors comprising various cell states (i.e. various epigenome), we can suspect a heterogeneity in terms of DNA damage response that may explain dissociated tolerance to DNA-damaging agents. Indeed, cancer cells undergoing EMT acquire stem cell traits[69], accompanied by a massive chromatin reprogramming. Although DNA methylation remains unchanged during EMT, cells undergo global chromatin remodeling including an increase in the transcriptional mark H3K36me3 known to be enriched in HR-competent chromatin[70]. Of note, our current understanding of H3K36me1/2/3 writers and erasers remains limited compared to other well-characterized marks (e.g., H3K9me), and further studies will be required to clarify their contribution to cell plasticity. During treatment, chemoresistant breast cancer cell activates persister transcriptional program, due to the loss of bivalent chromatin (H3K27me3) in favor of active chromatin mark (H3K4me3)[9]. As the histone mark H3K4me3 is known to promote recruitment of the Xeroderma Pigmentosum Complex (XPC) and nucleotide excision repair (NER) machinery to repair DNA damage, it could explain the drug-tolerant capabilities of these persisters cells[71]. In addition, several studies report a strong HR activity in CSC compared to their differentiated counterpart[40,72]. As a result, glioblastomas stem cells represent the radio-resistant sub-population in GBM tumor bulk and breast CSCs tackle more efficiently DNA lesions and replicative stress generated by genotoxic treatment than non-bCSCs.

Overall, these studies highlight how elucidating the molecular bases of DNA repair in the context of chromatin and cell identity can help unravel the non-genetic mechanisms of therapeutic resistance in cancer.

Chromatin maintenance after DNA repair

While the chromatin landscape defines cell states with different susceptibility to DNA damage accumulation and with specific DNA repair pathways activation, the DNA repair process itself is not neutral on chromatin structure. Instead, it induces chromatin remodeling. Similarly, to replication, DNA repair processes provoke substantial epigenetic modification, due to chromatin disassembly needed to increase access to repair protein complex to the DNA lesion[73,74]. In fact, nucleosome is partially or totally disassembled around DSB in nucleolin-dependent manner, to allow the recruitment of repair protein such as RPA[75]. After DNA repair, the epigenetic landscape must be restored, following the access-repair-restore model[73], to maintain the transcriptional activity, and the subsequent cellular identity (Fig. 1C). Despite several decades of research, how transcription restarts after DNA damage repair in a chromatin context is not fully elucidated. Most studies described transcription coupled repair (TCR) pathway in the context of NER of UV damage[76]. The histone chaperone chromatin assembly factor-1 (CAF-1) is recruited at the UV damage locus to facilitate new histone deposition. Then, the histone chaperone HIRA (histone regulator A) recruit new histone 3.3 at UV and DSB damage chromatin to act as a chromatin bookmarking process to facilitate recovery of transcription activity[77–80]. The histone variant H3.3 and its dedicated chaperone (CAF-1, HIRA, DAXX) play an essential role in the regulation of promoter and enhancer activity, whereas the variants H3.1 and H3.2 are usually present in transcriptionally repressed region during S phase[81]. Thus, the restoration of H3.3 is crucial for the maintenance of transcription activity immediately after DNA damage repair. Recently, new players in this process, the DNAJC9 histone chaperone and MCM2, has been demonstrated to provide new H3-H4 histones to CAF-1 and HIRA for its deposition into chromatin, and also stimulates old H3-H4 histone recovery[82]. Thus, in addition of parental histone recovery[74] the integration of new histone on DNA damage locus is essential to preserve the epigenetic memory and cell identity after DNA repair.

Nevertheless, in a malignant context with substantial stalled replication fork associated with replicative stress, mis-restoration of epigenetic marks after DNA replication and repair might not be a rare event, but a common failure. The loss of initial chromatin architecture could lead to cell plasticity and participate to shape the non-genetic tumor heterogeneity.

Epigenetic damage scar challenge cell identity

While DNA repair pathways typically restore the DNA sequence to its original state before damage occurred, the accuracy of chromatin restoration remains unclear. In several context, including cancer or aging, restoration of the epigenetic landscape after DNA damage is not always allowed leading to the generation of "epigenetic damage scar"[15,83]. It was first demonstrated that during DSB repair, silencing proteins (e.g. SIRT1, EZH2, DNMT1, and DNMT3B) are recruited to the damage site with enrichment of their corresponding histone marks (hypoacetyl H4K16, H3K9me2, H3K9me3, and H3K27me3) and are maintain after repair[84–88]. Although promoter's activity in the immediate vicinity of DSB is mainly preserved, in some case promoter regions harbored increased DNA methylation in the CpG island or on promoter close to the recombination site, leading to heritable silencing[86,89]. In addition to histone mark and DNA methylation, the

chromatin condensation must be restored after DNA repair in part by the reestablishment of nucleosome following the reincorporation of new histone variant, such as H3.3[76,77]. Importantly, this H3.3 distribution and relative abundance profoundly impact cellular identity and plasticity by epigenetically regulating gene expression[90]. These new incorporated histones present diverse posttranslational modifications (PTMs) that differ from the parental ones[91]. As an example, new H3.3 present accumulation of K9/K14ac2 and K9me2 that could participate to the establishment of an epigenetic damage scar[91].

A direct consequence of these epigenetic scars linked to DNA lesions is an impact on the transcriptional activity of neighboring genes. It was first demonstrated in cancer cells using the reporter construct DRGFP (Direct-Repeat GFP) to monitor epigenetic modifications following DSB repair by HR in the GFP locus. Even though the damaged GFP locus was properly repaired, epigenetic scars, including DNA methylation and H3K9me2/3 modifications, appeared and generate cells with different but heritable GFP expression levels[88]. More recently, using a mouse model (named ICE, ERT2-HA-I-*Ppo*I-IRES-GFP) that induce non-mutagenic DSB repair following the induction of the endonuclease I-*Ppo*I, it was observed that ICE cells had relatively less chromatin-bound H3K27ac and H3K56ac (2% and 5%, respectively)[92]. This epigenetic erosion was sufficient to weaken insulation and disordered promoter-enhancer (P-E) communication accelerating the epigenetic clock and age-related changes to chromatin, gene expression, and cellular identity. These epigenetic damage scars can be assimilated to post-repair chromatin fatigue which can affect numerous genes expression within the topologically defines chromatin neighborhood that recovered from a single DNA breakage[93]. This notion of aging–driven epigenetic changes could causally contribute to tumor initiation. Indeed, transient depletion of PRC1 cellular subunits is associated with irreversible activation of genes that promote cell growth, proliferation, migration, cell polarity, promoting neoplastic transformation[94]. This work introduces the concept of epigenetically initiated cancers (EICs) with epigenetic dysregulation that can lead to inheritance of altered cell fates sufficient to initiate tumor. In this context several reports suggest that loss of epigenetic regulation mitigates tissue homeostasis causing a susceptible state, in which cells are more prone to be transformed[95,96].

Mechanistically this post-repair chromatin fatigue may be explain by the three-dimensional chromatin structure that is established by hierarchical folding at multiple scales starting from small functional loops, followed by larger topologically associated domains (TADs)[97]. The chromatin loop that contains DSBs presents a local DNA replication attenuation[98]. The persistence of DSB in this context could change the replication timing, and at the end, the transcriptome. DSBs directed to specific locations within an entire TAD have a lasting impact on transcription even if the lesion has been generated (and subsequently properly repaired) at megabase distances from the gene itself[93]. In addition, a recent study demonstrated that, the formation of DSB in heterochromatin rapidly moved outside the polycomb body (compact nuclear condensation) associated with a reduction of H3K27me3[99]. This could persist and influence the transcriptional program. Importantly, these 3D epigenetic damage scars are inherited to the next generations of daughter cells and inevitably lead to a derail cell identity.

The inheritance of these epigenetic damage scars is surprising knowing the complex mechanisms that drive post-mitotic chromatin reconfiguration to maintain chromatin integrity and eliminate chromatin alterations to prevent the spread to the progeny[100]. One mechanistic hypothesis could reside in the process named mitotic bookmarking which assure the transmission of cell identity. It consists in the persistence of transcription factor DNA binding during mitosis allowing the rapid transcriptional activation upon mitotic exit[101–103]. Mitotic bookmarking is dependent on SWI/SNF complex that is required for appropriate reactivation of bound genes after mitosis[104].

Interestingly, it was demonstrated that SWI/SNF complex is enriched in DSB-flanking chromatin[60]. Thus, we can hypothesize that accumulated SWI/SNF complex on the epigenetic damage scar loci will allow the inheritance of transcriptional damage memory to the progeny and could challenge cell fate. How epigenetic scar could promote tumor recurrences by favoring the emergence of treatment-resistant cell states is a fascinating area that deserved to be explored.

Therapeutic opportunities

The intricate relationship between DNA damage repair and cell identity offers novel therapeutic opportunities to tackle adaptive mechanisms fueling the dynamism integral to tumor heterogeneity. Several studies indicate that CSCs have developed a robust replicative stress response (RSR) to reduce and tolerate replicative stress observed in neoplasia[105]. CSCs benefit from a super-active HR system including RAD51 upregulation. This cellular state of super-active homologous recombination appears to resolve replication stress by promoting efficient stressed replication fork stabilization, reversal and restart. This stress tolerance, which benefits from the DDR, is in fact a sign of targeted vulnerability in the CSCs. The use of different RSR inhibitors appears to be highly effective to eradicate the CSC-state and limit tumor progression. The combined inhibition of ATR and PARP provided GSC-specific cytotoxicity and complete abrogation of GSC radiation resistance[25]. In colorectal cancers, the combined treatment of MRE11 and RAD51 inhibitors (respectively mirin and B02) eradicate CSC by inducing mitotic catastrophe[106]. Similarly, in breast cancers, RAD51 inhibition increase replicative stress in CSC and sensitize these tumor-initiating cells to cisplatin, thus reducing tumors' ability to relapse[40]. Recently, it was demonstrated that nifuroxazide treatment, a prodrug that is specifically bioactivated in breast CSC, induces a chemical HRDness in breast CSCs that (re)sensitizes breast cancers with innate or acquired resistance to PARP inhibitor (PARPi)[107]. Preclinical and clinical development of antitumor agents targeting the RSR machinery is extending with the new generation of ATMi, ATRi, WEE1i, CHK1i, DNA-PKi, RAD51i, or POLθi[108] (Table 1).

In addition to super-active HR system, other targetable DNA repair mechanisms may be selectively active in cancer stem cells (CSCs), such as templated-sequence insertions (TSIs)–a form of DNA double-strand break (DSB) repair that depends on hTERT activity[109,110]. Notably, hTERT expression is tightly restricted to stem and progenitor cells. Inhibition of hTERT using imetelstat has been shown to sensitize leukemic stem cells to genotoxic agents by suppressing this telomerase-mediated DNA repair pathway[109,111,112]. Under these premises, it becomes a priority to enhance our understanding of the mechanisms driving cell state specific DNA repair. This knowledge is crucial for identifying targets for intervention to limit tumor adaptation and evolution. Another way of exploiting the association between cell identity and the choice of DNA repair mechanisms is to corrupt the GRN of the cancer cell-state in order to reprogram cells into states that respond to treatment[113]. This last decade, the genetic concept of synthetic lethality has been exploited with success to target tumors HR-deficient with PARP inhibitors[114,115]. In tumors without any germline (or even somatic) mutation in HR-related genes, we can propose reprogramming cells in a state of low HR-activity by promoting a loss of epigenetic heterogeneity. A few years ago, following an epidrug screen, it was demonstrated that bromodomain (BRD) and extraterminal domain inhibitors (BETis) were able to sensitize HR-proficient breast cancer cells to PARPi. BETi-treated cells presented repression of HR-related genes expression leading to the induction of chemical HRDness[116]. One possible explanation of this synthetic lethality could lie in a phenotypic change induced by BETi, leading to a homogenized tumor in a cell state with limited HR activity. Indeed, BETi treatment is able to reduce the CSC pool by inducing a cell state transition to a non-CSC state, known to be less HR-active[117]. Similarly, the use of HDAC inhibitor has been repeatedly observed to be synthetic lethal with

Table 1 | Registered clinical trials of drugs targeting replicative stress response machinery in cancers

Target	Drug	Clinical trial ID (refs)	Stage	Cancer	Combination
ATR	M6620 (VX-970)	NCT03641547/NCT02157792/NCT02723864	Phase I	Solid Tumors	with convential chemotherapies or with PARPi and cisplatin
		NCT02487095	Phase I/II	Small Cell Lung Cancers	with Topotecan
		NCT03896503/NCT02595892	Phase II	Small Cell Lung Cancers/ Ovarian or Fallopian Cancers	with Topotecan or Gemcitabine
	VX-970	NCT02723864	Phase I	Refractory Solid Tumors	with PARPi
	M4344	NCT04655183	Phase I/II	Breast Cancers (with DDR mutations)	with PARPi
	M1774	NCT05986071	Phase I/II	Breast Cancers (HR+ and HER2-)	with Fulverstrant
	AZD6738(Ceralasctib)	NCT02264678/NCT03022409/NCT02223923/ NCT03328273	Phase I	Solid Tumors and Chronic Lymphocytic Leukemia	with Acalabrutinib or PARPi or PARPI with Carboplatin
		NCT03682289/NCT0342342/NCT0378680/ NCT04065269/ NCT03330847	Phase II	Solid Tumors	with PARPi or WEEIi and PARPi
	BAY1895344 (Elimusertib)	NCT04535401/NCT04491942/NCT03188965/ NCT04576091/ NCT04616534/ NCT04267939	Phase I	Solid Tumors	with convential chemotherapy or pembrolizumab (anti-PD-1) or PARPi
	RP-3500 (Camonsertib)	NCT04972110/NCT04497116	Phase I/II	Solid Tumors	with PARPi
	M1774 (tuvusertib)	NCT05396833/NCT06337630	Phase I	Solid tumors	with ATMi (Lartesertib) or Avelumab (anti-PD-L1) or Topo1i (PLXO38)
		NCT05882734	Phase I/II	Non-Small Cell Lung Cancers	with Cemiplimab (anti-PD-1)
		NCT06424717/NCT05947500/NCT06518564	Phase II	Urothelial Cancers/Merkel Cell Skin Cancers/ Endometrial Cancers (ARID1A mutated)	with Avelumab (anti-PD-L1)
		NCT06433219	Phase II	Ovarian Cancers	with ATMi (M4076, artesertib) and PARPi
	berzosertib	NCT05246111	Phase I	Solid Tumors	with Topotecan
		NCT04802174	Phase I/II	Small Cell Lung Cancers/ Neuroendocrine Cancers	with Lurbinectedin
	ATG-018	NCT05338346	PhaseI	Solid Tumors and Hematological Malignancies	
	M4344	NCT04149145	PhaseI	Ovarian cancers	with PARPi
	ARTO380	NCT04657068	Phase I/II	Solid Tumors	with Gemcitabine and Irinotecan
	IMP9064	NCT05269316	Phase I/II	Solid Tumors	with PARPi
ATM	AZD0156	NCT02588105 [131,132]	Phase I	Solid Tumors	with conventional chemotherapies or with PARPi
	WSD0628	NCT05917145 [133]	Phase I	Brain Tumors	
	AZD1390	NCT05182905	Phase 0/Ib	Glioma	
		NCT04550104/NCT05678010/NCT05116254/ NCT03423628/ NCT06894979	Phase I	Solid Tumors	with Radiotherapy or Radiotherapy and Durvlaumab
	M3541	NCT03225105 [134]	Phase I	Solid Tumors	with Radiotherapy
	M4076	NCT04882917 [135]	Phase I	Solid Tumors	
	KU-60019	NCT03571438	Phase I	Renal Tumors	with CK2i
	AZD0156	NCT02588105	Phase I	Solid Tumors	with PARPi or Irinotecan or Fluoruoracil or Folinic acid

Table 1 (continued) | Registered clinical trials of drugs targeting replicative stress response machinery in cancers

Target	Drug	Clinical trial ID (refs)	Stage	Cancer	Combination
CHK1	SCH900776	NCT01870596	Phase II	Acute Myeloid Leukemia	with Cytarabine
	LY2880070	NCT05275426	Phase II	Ewing sarcoma/Desmoplastic Small Round Cell Tumors	with Gemcitamine
	LY2606368 (Prexasertib)	NCT04023669/NCT02808650/NCT03495323/NCT03735446/NCT03057145	Phase I	Solid Tumors/Acute Myeloid Leukemia	with conventional chemotherapies or PARPi
		NCT02203513/NCT02873975	Phase II	Solid Tumors	
	PEPO7	NCT05983523/NCT05659732	Phase I	Solid Tumors	
	SRA737	NCT02797964/NCT02797977	Phase I/II	Solid Tumors	with conventional chemotherapies
Target	**Drug**	**Clinical trial ID (refs)**	**Stage**	**Cancer**	**Combination**
PolQ	ART4245	NCT04991480	Phase I/II	Solid Tumors	with PARPi
	GSK4524101	NCT06077877	Phase I/II	Solid Tumors	with PARPi
	ART6043	NCT05898399	Phase I/II	Solid Tumors	with PARPi
	Novobiocin	NCT05687110	Phase I	Solid Tumors (with DDR mutations)	
	ART4215	NCT04991480	Phase I/II	Breast cancers (BRCA-deficient)	with PARPi
Rad51	CYT-0851	NCT03997968[136]	Phase I/II	B-cell malignancies and Solid Tumors	with conventional chemotherapies or Rituximab and bendamustine
Wee1	ZN-c3 (Azenosertib)	NCT05368506	Phase I	Breast Cancers (TNBCs) and Ovarian Cancers	
		NCT05198804/NCT05568217O	Phase I/II	Ovarian cancers (Platinum resistant)/AML	with PARPi or BCL-2i
		NCT06015659/NCT04814108/NCT05128825	Phase II	Pancreatic Cancers/Uterine serous carcinoma/Ovarian cancers (Platinum resistant), Fallopian Tube or Primary Peritoneal Cancers	with Gemcitabine
	AZD1775 (MK-1775, Adavosertib)	NCT02617277/NCT01748825/NCT00648648/ NCT02508246/ NCT02791919/NCT03028766/ NCT02381548/NCT03345784	Phase I	Solid Tumors and Acute Myeloid Leukemia	with conventional chemotherapies or Belinostat
		NCT02194829	Phase I/II	Pancreatic Cancers	with Paclitaxel or Gemcitabine
		NCT02037230	Phase I/II	Pancreatic Cancers	with Gemcitabine and Radiotherapy
		NCT02906059	Phase Ib	Cololorectal Cancers	with Irinotecan
		NCT01164995/NCT02272790/NCT02101775/ NCT01357161/ NCT02448329/NCT02196168/ NCT02666950/NCT03253679/ NCT03012477/ NCT03668340/NCT03718143[37,138]	Phase II	Solid Tumors and Acute Myeloid Leukemia	with conventional chemotherapies
	SY-4835	NCT05291182	Phase I	Solid Tumors	
DNA-PK	AZD7648	NCT03907969[139]	Phase I/IIa	Solid Tumors	with doxorubicin
	CC-115	NCT01353625[140]	Phase I	Solid Tumors and Hematological Malignancies	with mTORi
	BY101298	NCT06524804	Phase Ib/II	Solid Tumors	with Radiotherapy
	M3814 (peposertib)	NCT02316197/NCT05711615/NCT05687136	Phase I	Solid Tumors and Chronic Lymphocytic Leukemia	with doxorubicin or ATRi
		NCT02516813	Phase Ia/Ib	Solid Tumors	with Radiotherapy
		NCT04172532/NCT04071236	Phase I/II	Pancreatic cancers/Prostate Cancers	with Radiotherapy

PARPi in tumors initially HR-competent[118–120]. In this case again, we observe a reduction of HR-related genes such as RAD51 or FANCD2 in HDACi-treated cells and HDACi have been identified as one of the first differentiation therapies[121] capable to induce CSC differentiation[122]. EZH2 inhibitors (EZH2i) have been also consistently reported as a potent therapeutic strategy for targeting CSCs in both hematologic and solid tumors[123]. Although the precise mechanism of action remains unclear, EZH2's role in regulating HR activity may partly explain this effect, as several studies have shown that EZH2i sensitize cancer cells to PARP inhibitors (PARPi)[124,125]. A similar outcome may also be achieved by inhibiting other histone methyltransferases, such as G9a/GLP, which appear to regulate cancer stemness and potentially synergize with PARPi treatment[126,127] or by inhibiting DNA methyltransferase using cytosine analogs 5-azacitidine (5-AZA) that impairs leukemia or breast cancer stem cells function[128,129] and induces an HRDness phenotype that sensitizes cancer cells to PARPi[130].

In this context, we can propose that differentiation therapy is a new opportunity to induce a chemical HRDness, due to the shift from a HR-competent state to a state presenting poor HR functionality.

Beyond the various hurdles that must be overcome to introduce DNA damage response inhibitors (DDRi) into clinical practice—such as the identification of predictive biomarkers of response and the frequent emergence of acquired resistance due to DDR restoration—one promising strategy to enhance the efficacy of antitumor DDR therapies is to leverage the interplay between epigenetics and DDR, tailoring treatment to the specific cell state.

References

1. Tirosh, I. & Suvà, M. L. Deciphering human tumor biology by single-cell expression profiling. *Annu. Rev. Cancer Biol.* **3**, 151–166 (2019).
2. Gonzalez Castro, L. N., Tirosh, I. & Suvà, M. L. Decoding cancer biology one cell at a time. *Cancer Discov.* **11**, 960–970 (2021).
3. Tirosh, I. Stochastic transitions as a major source of cancer heterogeneity. *Nat. Rev. Genet* **23**, 582–583 (2022).
4. Ginestier, C. et al. ALDH1 Is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* **1**, 555–567 (2007).
5. Eppert, K. et al. Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat. Med.* **17**, 1086–1093 (2011).
6. Merlos-Suárez, A. et al. The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. *Cell Stem Cell* **8**, 511–524 (2011).
7. Gargiulo, G., Serresi, M. & Marine, J.-C. Cell states in cancer: drivers, passengers, and trailers. *Cancer Discov.* **14**, 610–614 (2024).
8. Risom, T. et al. Differentiation-state plasticity is a targetable resistance mechanism in basal-like breast cancer. *Nat. Commun.* **9**, 3815 (2018).
9. Marsolier, J. et al. H3K27me3 conditions chemotolerance in triple-negative breast cancer. *Nat. Genet* **54**, 459–468 (2022).
10. Mani, N., Daiya, A., Chowdhury, R., Mukherjee, S. & Chowdhury, S. Chapter seven - epigenetic adaptations in drug-tolerant tumor cells. In *Advances in Cancer Research* (eds. Landry, J. W., Das, S. K. & Fisher, P. B.) 293–335 (Academic Press, 2023).
11. Moore, P. C., Henderson, K. W. & Classon, M. Chapter One - The epigenome and the many facets of cancer drug tolerance. In *Advances in Cancer Research* (eds. Landry, J. W., Das, S. K. & Fisher, P. B.) 1–39 (Academic Press, 2023).
12. Laisné, M., Lupien, M. & Vallot, C. Epigenomic heterogeneity as a source of tumour evolution. *Nat. Rev. Cancer* **25**, 7–26 (2025).
13. Huang, S. Genetic and non-genetic instability in tumor progression: link between the fitness landscape and the epigenetic landscape of cancer cells. *Cancer Metastasis Rev.* **32**, 423–448 (2013).
14. Flavahan, W. A., Gaskell, E. & Bernstein, B. E. Epigenetic plasticity and the hallmarks of cancer. *Science* **357**, eaal2380 (2017).
15. Dabin, J., Fortuny, A. & Polo, S. E. Epigenome maintenance in response to DNA damage. *Mol. Cell* **62**, 712–727 (2016).
16. Dabin, J., Giacomini, G., Petit, E. & Polo, S. E. New facets in the chromatin-based regulation of genome maintenance. *DNA Repair (Amst.)* **140**, 103702 (2024).
17. Lensing, S. V. et al. DSBCapture: in situ capture and sequencing of DNA breaks. *Nat. Methods* **13**, 855–857 (2016).
18. Mourad, R., Ginalski, K., Legube, G. & Cuvier, O. Predicting double-strand DNA breaks using epigenome marks or DNA at kilobase resolution. *Genome Biol.* **19**, 34 (2018).
19. de Luca, K. L. et al. Genome-wide profiling of DNA repair proteins in single cells. *Nat. Commun.* **15**, 9918 (2024).
20. Hazan, I., Monin, J., Bouwman, B. A. M., Crosetto, N. & Aqeilan, R. I. Activation of oncogenic super-enhancers is coupled with DNA repair by RAD51. *Cell Rep.* **29**, 560–572.e4 (2019).
21. Hoffman, E. A., McCulley, A., Haarer, B., Arnak, R. & Feng, W. Break-seq reveals hydroxyurea-induced chromosome fragility as a result of unscheduled conflict between DNA replication and transcription. *Genome Res* **25**, 402–412 (2015).
22. Canela, A. et al. Genome organization drives chromosome fragility. *Cell* **170**, 507–521.e18 (2017).
23. Yan, W. X. et al. BLISS is a versatile and quantitative method for genome-wide profiling of DNA double-strand breaks. *Nat. Commun.* **8**, 15058 (2017).
24. Biernacka, A. et al. i-BLESS is an ultra-sensitive method for detection of DNA double-strand breaks. *Commun. Biol.* **1**, 181 (2018).
25. Carruthers, R. D. et al. Replication stress drives constitutive activation of the DNA damage response and radioresistance in glioblastoma stem-like cells. *Cancer Res.* **78**, 5060–5071 (2018).
26. Cao, H. et al. Hotspots of single-strand DNA 'breakome' are enriched at transcriptional start sites of genes. *Front Mol. Biosci.* **9**, 895795 (2022).
27. Hidmi, O. et al. TOP1 and R-loops facilitate transcriptional DSBs at hypertranscribed cancer driver genes. *iScience.* **27**, 109082 (2024).
28. Zacharioudakis, E. et al. Chromatin regulates genome targeting with cisplatin. *Angew. Chem. Int Ed. Engl.* **56**, 6483–6487 (2017).
29. Plotnik, J. P. Fighting PRC1 via the RING. *Nat. Chem. Biol.* **17**, 753–754 (2021).
30. Fitieh, A. et al. BMI-1 regulates DNA end resection and homologous recombination repair. *Cell Rep.* **38**, 110536 (2022).
31. Yu, J.-R., Lee, C.-H., Oksuz, O., Stafford, J. M. & Reinberg, D. PRC2 is high maintenance. *Genes Dev.* **33**, 903–935 (2019).
32. Park, I. et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* **423**, 302–305 (2003).
33. Molofsky, A. V. et al. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* **425**, 962–967 (2003).
34. Paranjape, A. N. et al. Bmi1 regulates self-renewal and epithelial to mesenchymal transition in breast cancer cells through Nanog. *BMC Cancer* **14**, 785 (2014).
35. Kreso, A. et al. Self-renewal as a therapeutic target in human colorectal cancer. *Nat. Med.* **20**, 29–36 (2014).
36. Lin, E.-H. et al. Targeting cancer stemness mediated by BMI1 and MCL1 for non-small cell lung cancer treatment. *J. Cell. Mol. Med.* **26**, 4305–4321 (2022).
37. Herzog, A. E., Somayaji, R. & Nör, J. E. Bmi-1: A master regulator of head and neck cancer stemness. *Front Oral. Health* **4**, 1080255 (2023).
38. Yoo, Y. A. et al. The role of castration-resistant Bmi1+Sox2+ cells in driving recurrence in prostate cancer. *J. Natl Cancer Inst.* **111**, 311–321 (2018).
39. Facchino, S., Abdouh, M., Chatoo, W. & Bernier, G. BMI1 confers radioresistance to normal and cancerous neural stem cells

through recruitment of the dna damage response machinery. *J. Neurosci.* **30**, 10096–10111 (2010).

40. Azzoni, V. et al. BMI1 nuclear location is critical for RAD51-dependent response to replication stress and drives chemoresistance in breast cancer stem cells. *Cell Death Dis.* **13**, 96 (2022).

41. Margueron, R. & Reinberg, D. The polycomb complex PRC2 and its mark in life. *Nature* **469**, 343–349 (2011).

42. Comet, I., Riising, E. M., Leblanc, B. & Helin, K. Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. *Nat. Rev. Cancer* **16**, 803–810 (2016).

43. Campbell, S., Ismail, I. H., Young, L. C., Poirier, G. G. & Hendzel, M. J. Polycomb repressive complex 2 contributes to DNA double-strand break repair. *Cell Cycle* **12**, 2675–2683 (2013).

44. Gardner, E. E. et al. Chemosensitive relapse in small cell lung cancer proceeds through an EZH2-SLFN11 Axis. *Cancer Cell* **31**, 286–299 (2017).

45. Caron, P., van der Linden, J. & van Attikum, H. Bon voyage: a transcriptional journey around DNA breaks. *DNA Repair* **82**, 102686 (2019).

46. Wen, Y., Cai, J., Hou, Y., Huang, Z. & Wang, Z. Role of EZH2 in cancer stem cells: from biological insight to a therapeutic target. *Oncotarget* **8**, 37974–37990 (2017).

47. Kim, S.-H. et al. EZH2 protects glioma stem cells from radiation-induced cell death in a MELK/FOXM1-dependent manner. *Stem Cell Rep.* **4**, 226–238 (2015).

48. Jahangiri, L. et al. Core regulatory circuitries in defining cancer cell identity across the malignant spectrum. *Open Biol.* **10**, 200121 (2020).

49. Barrows, J. K. et al. BRD4 promotes resection and homology-directed repair of DNA double-strand breaks. *Nat. Commun.* **13**, 3016 (2022).

50. Chaffer, C. L. et al. Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. *Cell* **154**, 61–74 (2013).

51. Zhang, P. et al. ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. *Nat. Cell Biol.* **16**, 864–875 (2014).

52. Prodhomme, M. K. et al. EMT transcription factor ZEB1 represses the mutagenic POLθ-mediated end-joining pathway in breast cancers. *Cancer Res.* **81**, 1595–1606 (2021).

53. Lemaître, C. et al. Nuclear position dictates DNA repair pathway choice. *Genes Dev.* **28**, 2450–2463 (2014).

54. Schep, R. et al. Impact of chromatin context on Cas9-induced DNA double-strand break repair pathway balance. *Mol. Cell* **81**, 2216–2230.e10 (2021).

55. Burgess, R. C., Burman, B., Kruhlak, M. J. & Misteli, T. Activation of DNA damage response signaling by condensed chromatin. *Cell Rep.* **9**, 1703–1717 (2014).

56. Jiang, Y. et al. INO80 chromatin remodeling complex promotes the removal of UV lesions by the nucleotide excision repair pathway. *Proc. Natl Acad. Sci. USA.* **107**, 17274–17279 (2010).

57. Horigome, C. et al. SWR1 and INO80 chromatin remodelers contribute to dna double-strand break perinuclear anchorage site choice. *Mol. Cell* **55**, 626–639 (2014).

58. Ogiwara, H. et al. Histone acetylation by CBP and p300 at double-strand break sites facilitates SWI/SNF chromatin remodeling and the recruitment of non-homologous end joining factors. *Oncogene* **30**, 2135–2146 (2011).

59. Watanabe, R. et al. SWI/SNF factors required for cellular resistance to DNA damage include ARID1A and ARID1B and show interdependent protein stability. *Cancer Res.* **74**, 2465–2475 (2014).

60. Harrod, A., Lane, K. A. & Downs, J. A. The role of the SWI/SNF chromatin remodelling complex in the response to DNA double strand breaks. *DNA Repair.* **93**, 102919 (2020).

61. Hao, F., Zhang, Y., Hou, J. & Zhao, B. Chromatin remodeling and cancer: the critical influence of the SWI/SNF complex. *Epigenetics Chromatin* **18**, 22 (2025).

62. Clouaire, T. et al. Comprehensive mapping of histone modifications at DNA double-strand breaks deciphers repair pathway chromatin signatures. *Mol. Cell* **72**, 250–262.e6 (2018).

63. Pfister, S. X. et al. SETD2-dependent histone H3K36 trimethylation Is required for homologous recombination repair and genome stability. *Cell Rep.* **7**, 2006–2018 (2014).

64. Barral, A. et al. SETDB1/NSD-dependent H3K9me3/H3K36me3 dual heterochromatin maintains gene expression profiles by bookmarking poised enhancers. *Mol. Cell* **82**, 816–832.e12 (2022).

65. Aymard, F. et al. Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat. Struct. Mol. Biol.* **21**, 366–374 (2014).

66. Kapoor-Vazirani, P. et al. SAMHD1 deacetylation by SIRT1 promotes DNA end resection by facilitating DNA binding at double-strand breaks. *Nat. Commun.* **13**, 6707 (2022).

67. Yadav, T., Quivy, J.-P. & Almouzni, G. Chromatin plasticity: a versatile landscape that underlies cell fate and identity. *Science* **361**, 1332–1336 (2018).

68. Domaschenz, R., Kurscheid, S., Nekrasov, M., Han, S. & Tremethick, D. J. The histone variant H2A.Z is a master regulator of the epithelial-mesenchymal transition. *Cell Rep.* **21**, 943–952 (2017).

69. Thiery, J. P., Acloque, H., Huang, R. Y. J. & Nieto, M. A. Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871–890 (2009).

70. McDonald, O. G., Wu, H., Timp, W., Doi, A. & Feinberg, A. P. Genome-scale epigenetic reprogramming during epithelial-to-mesenchymal transition. *Nat. Struct. Mol. Biol.* **18**, 867–874 (2011).

71. Maritz, C. et al. ASH1L-MRG15 methyltransferase deposits H3K4me3 and FACT for damage verification in nucleotide excision repair. *Nat. Commun.* **14**, 3892 (2023).

72. Obara, E. A. A. et al. SPT6-driven error-free DNA repair safeguards genomic stability of glioblastoma cancer stem-like cells. *Nat. Commun.* **11**, 4709 (2020).

73. Polo, S. E. & Almouzni, G. Chromatin dynamics after DNA damage: the legacy of the access–repair–restore model. *DNA Repair* **36**, 114–121 (2015).

74. Adam, S. et al. Real-time tracking of parental histones reveals their contribution to chromatin integrity following DNA damage. *Mol. Cell* **64**, 65–78 (2016).

75. Goldstein, M., Derheimer, F. A., Tait-Mulder, J. & Kastan, M. B. Nucleolin mediates nucleosome disruption critical for DNA double-strand break repair. *Proc. Natl Acad. Sci.* **110**, 16874–16879 (2013).

76. Polo, S. E., Roche, D. & Almouzni, G. New histone incorporation marks sites of UV repair in human cells. *Cell* **127**, 481–493 (2006).

77. Adam, S., Polo, S. E. & Almouzni, G. Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. *Cell* **155**, 94–106 (2013).

78. Bouvier, D. et al. Dissecting regulatory pathways for transcription recovery following DNA damage reveals a non-canonical function of the histone chaperone HIRA. *Nat. Commun.* **12**, 3835 (2021).

79. Li, X. & Tyler, J. K. Nucleosome disassembly during human non-homologous end joining followed by concerted HIRA- and CAF-1-dependent reassembly. *eLife* **5**, e15129 (2016).

80. Zhang, H. et al. RPA interacts with HIRA and regulates H3.3 deposition at gene regulatory elements in mammalian cells. *Mol. Cell* **65**, 272–284 (2017).

81. Martire, S. & Banaszynski, L. A. The roles of histone variants in fine-tuning chromatin organization and function. *Nat. Rev. Mol. Cell Biol.* **21**, 522–541 (2020).

82. Plessier, A. et al. Proteomic profiling of UV damage repair patches uncovers histone chaperones with central functions in chromatin repair. *bioRxiv* https://doi.org/10.1101/2024.08.23.609352 (2024).

83. Ferrand, J., Plessier, A. & Polo, S. E. Control of the chromatin response to DNA damage: Histone proteins pull the strings. *Semin. Cell Dev. Biol.* **113**, 75–87 (2021).

84. Mortusewicz, O., Schermelleh, L., Walter, J., Cardoso, M. C. & Leonhardt, H. Recruitment of DNA methyltransferase I to DNA repair sites. *Proc. Natl Acad. Sci. USA* **102**, 8905–8909 (2005).

85. Tamburini, B. A. & Tyler, J. K. Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand dna repair. *Mol. Cell. Biol.* **25**, 4903–4913 (2005).

86. O'Hagan, H. M., Mohammad, H. P. & Baylin, S. B. Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. *PLoS Genet* **4**, e1000155 (2008).

87. O'Hagan, H. M. et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer Cell* **20**, 606–619 (2011).

88. Russo, G. et al. DNA damage and repair modify DNA methylation and chromatin domain of the targeted locus: mechanism of allele methylation polymorphism. *Sci. Rep.* **6**, 33222 (2016).

89. Morano, A. et al. Targeted DNA methylation by homology-directed repair in mammalian cells. Transcription reshapes methylation on the repaired gene. *Nucleic Acids Res.* **42**, 804–821 (2014).

90. Choi, J., Kim, T. & Cho, E.-J. HIRA vs. DAXX: the two axes shaping the histone H3.3 landscape. *Exp. Mol. Med,* **56**, 251–263 (2024).

91. Loyola, A., Bonaldi, T., Roche, D., Imhof, A. & Almouzni, G. PTMs on H3 variants before chromatin assembly potentiate their final epigenetic state. *Mol. Cell* **24**, 309–316 (2006).

92. Yang, J.-H. et al. Loss of epigenetic information as a cause of mammalian aging. *Cell* **186**, 305–326.e27 (2023).

93. Bantele, S. et al. Repair of DNA double-strand breaks leaves heritable impairment to genome function. *biorxiv* https://doi.org/10.1101/2023.08.29.555258 (2023).

94. Parreno, V. et al. Transient loss of polycomb components induces an epigenetic cancer fate. *Nature* **629**, 688–696 (2024).

95. Richart, L. et al. XIST loss impairs mammary stem cell differentiation and increases tumorigenicity through mediator hyperactivation. *Cell* **185**, 2164–2183.e25 (2022).

96. Lin, S., Margueron, R., Charafe-Jauffret, E. & Ginestier, C. Disruption of lineage integrity as a precursor to breast tumor initiation. *Trends Cell Biol.* **33**, 887–897 (2023).

97. Jerkovic, I. & Cavalli, G. Understanding 3D genome organization by multidisciplinary methods. *Nat. Rev. Mol. Cell Biol.* **22**, 511–528 (2021).

98. Fedkenheuer, M. et al. A dual role of Cohesin in DNA DSB repair. *Nat. Commun.* **16**, 843 (2025).

99. Wensveen, M.R. et al. Double-strand breaks in facultative heterochromatin require specific movements and chromatin changes for efficient repair. *Nat. Commun.* **15**, 8984 (2024).

100. Zhang, H. et al. Chromatin structure dynamics during the mitosis-to-G1 phase transition. *Nature* **576**, 158–162 (2019).

101. Palozola, K. C., Lerner, J. & Zaret, K. S. A changing paradigm of transcriptional memory propagation through mitosis. *Nat. Rev. Mol. Cell Biol.* **20**, 55–64 (2019).

102. Bellec, M. et al. The control of transcriptional memory by stable mitotic bookmarking. *Nat. Commun.* **13**, 1176 (2022).

103. Yu, Q. et al. Dynamics and regulation of mitotic chromatin accessibility bookmarking at single-cell resolution. *Sci. Adv.* **9**, eadd2175 (2023).

104. Zhu, Z. et al. Mitotic bookmarking by SWI/SNF subunits. *Nature* **618**, 180–187 (2023).

105. Manic, G. et al. Replication stress response in cancer stem cells as a target for chemotherapy. *Semin. Cancer Biol.* **53**, 31–41 (2018).

106. Manic, G. et al. Control of replication stress and mitosis in colorectal cancer stem cells through the interplay of PARP1, MRE11 and RAD51. *Cell Death Differ.* **28**, 2060–2082 (2021).

107. Rouault, C. D. et al. Inhibition of the STAT3/fanconi anemia axis is synthetic lethal with PARP inhibition in breast cancer. *Nat. Commun.* **16**, 2159 (2025).

108. Pilié, P. G., Tang, C., Mills, G. B. & Yap, T. A. State-of-the-art strategies for targeting the DNA damage response in cancer. *Nat. Rev. Clin. Oncol.* **16**, 81–104 (2019).

109. Onozawa, M. et al. Repair of DNA double-strand breaks by templated nucleotide sequence insertions derived from distant regions of the genome. *Proc. Natl Acad. Sci. USA* **111**, 7729–7734 (2014).

110. Hidaka, D. et al. Short-term treatment with imetelstat sensitizes hematopoietic malignant cells to a genotoxic agent via suppression of the telomerase-mediated DNA repair process. *Leuk. Lymphoma* **61**, 2722–2732 (2020).

111. Bruedigam, C. et al. Telomerase inhibition effectively targets mouse and human AML stem cells and delays relapse following chemotherapy. *Cell Stem Cell* **15**, 775–790 (2014).

112. Barwe, S. P., Huang, F., Kolb, E. A. & Gopalakrishnapillai, A. Imetelstat induces leukemia stem cell death in pediatric acute myeloid leukemia patient-derived xenografts. *J. Clin. Med.* **11**, 1923 (2022).

113. Tammela, T. & Sage, J. Investigating tumor heterogeneity in mouse models. *Annu. Rev. Cancer Biol.* **4**, 99–119 (2020).

114. Murai, J. et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer Res.* **72**, 5588–5599 (2012).

115. Tutt, A. N. J. et al. Adjuvant olaparib for patients with BRCA1- or BRCA2-mutated breast cancer. *N. Engl. J. Med.* **384**, 2394–2405 (2021).

116. Yang, L. et al. Repression of BET activity sensitizes homologous recombination-proficient cancers to PARP inhibition. *Sci. Transl. Med.* **9**, eaal1645 (2017).

117. Arfaoui, A. et al. A genome-wide RNAi screen reveals essential therapeutic targets of breast cancer stem cells. *EMBO Mol. Med.* **11**, e9930 (2019).

118. Zhu, Q. et al. Novel dual inhibitors of PARP and HDAC induce intratumoral STING-mediated antitumor immunity in triple-negative breast cancer. *Cell Death Dis.* **15**, 1–12 (2024).

119. Drzewiecka, M. et al. Class I HDAC inhibition leads to a down-regulation of FANCD2 and RAD51, and the eradication of glioblastoma eclls. *J. Pers. Med* **13**, 1315 (2023).

120. Rasmussen, R. D., Gajjar, M. K., Jensen, K. E. & Hamerlik, P. Enhanced efficacy of combined HDAC and PARP targeting in glioblastoma. *Mol. Oncol.* **10**, 751–763 (2016).

121. Minucci, S. & Pelicci, P. G. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat. Rev. Cancer* **6**, 38–51 (2006).

122. Salvador, M. A. et al. The histone deacetylase inhibitor abexinostat induces cancer stem cells differentiation in breast cancer with low Xist expression. *Clin. Cancer Res.* **19**, 6520–6531 (2013).

123. Scott, M. T. et al. Epigenetic reprogramming sensitizes CML stem cells to combined EZH2 and tyrosine kinase inhibition. *Cancer Discov.* **6**, 1248–1257 (2016).

124. Karakashev, S. et al. EZH2 inhibition sensitizes CARM1-high, homologous recombination proficient ovarian cancers to PARP inhibition. *Cancer Cell* **37**, 157–167.e6 (2020).

125. Yamaguchi, H. et al. EZH2 contributes to the response to PARP inhibitors through its PARP-mediated poly-ADP ribosylation in breast cancer. *Oncogene* **37**, 208–217 (2018).

126. Haebe, J. R., Bergin, C. J., Sandouka, T. & Benoit, Y. D. Emerging role of G9a in cancer stemness and promises as a therapeutic target. *Oncogenesis* **10**, 76 (2021).

127. Watson, Z. L. et al. Histone methyltransferases EHMT1 and EHMT2 (GLP/G9A) maintain PARP inhibitor resistance in high-grade serous ovarian carcinoma. *Clin. Epigenetics* **11**, 165 (2019).

128. Trowbridge, J. J. et al. Haploinsufficiency of Dnmt1 impairs leukemia stem cell function through derepression of bivalent chromatin domains. *Genes Dev.* **26**, 344–349 (2012).

129. Pathania, R. et al. DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. *Nat. Commun.* **6**, 6910 (2015).

130. Abbotts, R. et al. DNA methyltransferase inhibitors induce a BRCAness phenotype that sensitizes NSCLC to PARP inhibitor and ionizing radiation. *Proc. Natl Acad. Sci.* **116**, 22609–22618 (2019).

131. Kostaras, E. et al. A systematic molecular and pharmacologic evaluation of AKT inhibitors reveals new insight into their biological activity. *Br. J. Cancer* **123**, 542–555 (2020).

132. Chen, C.-W., Buj, R., Dahl, E. S., Leon, K. E. & Aird, K. M. ATM inhibition synergizes with fenofibrate in high grade serous ovarian cancer cells. *Heliyon* **6**, e05097 (2020).

133. Xue, Z. et al. The novel brain penetrant ataxia-telangiectasia mutated inhibitor WSD0628 provides robust radiosensitization of brain tumor patient-derived xenografts. *Neuro Oncol.* **15**, noaf102 (2025).

134. Waqar, S. N. et al. Phase I trial of ATM inhibitor M3541 in combination with palliative radiotherapy in patients with solid tumors. *Invest N. Drugs* **40**, 596–605 (2022).

135. Siu, L. L. et al. Abstract CT171: a first-in-human phase I study of the ATM inhibitor M4076 in patients with advanced solid tumors (DDRiver solid tumors 410): part 1A results. *Cancer Res.* **83**, CT171 (2023).

136. Lynch, R. C. et al. First-in-human phase I/II study of CYT-0851, a first-in-class inhibitor of RAD51-mediated homologous recombination in patients with advanced solid and hematologic cancers. *JCO* **39**, 3006–3006 (2021).

137. Leijen, S. et al. Phase I study evaluating WEE1 inhibitor AZD1775 as monotherapy and in combination with gemcitabine, cisplatin, or carboplatin in patients with advanced solid tumors. *J. Clin. Oncol.* **34**, 4371–4380 (2016).

138. Oza, A. M. et al. A biomarker-enriched, randomized phase ii trial of adavosertib (AZD1775) plus paclitaxel and carboplatin for women with platinum-sensitive TP53-mutant ovarian cancer. *Clin. Cancer Res* **26**, 4767–4776 (2020).

139. Yap, T. A. et al. The DNA-PK inhibitor AZD7648 alone or combined with pegylated liposomal doxorubicin in patients with advanced cancer: results of a first-in-human phase I/IIa study. *Br. J. Cancer* **133**, 168–177 (2025).

140. Munster, P. et al. First-in-human phase I study of a dual mTOR kinase and DNA-PK inhibitor (CC-115) in advanced malignancy. *Cancer Manag Res* **11**, 10463–10476 (2019).

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