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► **To cite this version:**

A. Demeyer, H. Benhelli-Mokrani, Benoît Chénais, P. Weigel, F. Fleury. Inhibiting homologous recombination by targeting RAD51 protein. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 2021, 1876 (2), pp.188597. 10.1016/j.bbcan.2021.188597 . hal-03347460

HAL Id: hal-03347460

<https://univ-lemans.hal.science/hal-03347460v1>

Submitted on 2 Aug 2023

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Inhibiting homologous recombination by targeting RAD51 protein

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Abstract

Homologous recombination (HR) is involved in repairing DNA double-strand breaks (DSB), the most harmful for the cell. Regulating HR is essential for maintaining genomic stability. In many forms of cancer, overactivation of HR increases tumor resistance to DNA-damaging treatments. RAD51, HR's core protein, is very often over-expressed in these cancers and plays a critical role in cancer cell development and survival. Targeting RAD51 directly to reduce its activity and its expression is therefore one strategy to sensitize and overcome resistance cancer cells to existing DNA-damaging therapies which remains the limiting factor for the success of targeted therapy.

This review describes the structure and biological roles of RAD51, summarizes the different targeted sites of RAD51 and its inhibitory compounds discovered and described in the last decade.

Introduction

In cells, chemical and physical aggression, and replicative stress can alter DNA. Unrepaired damage then causes genomic instability leading to cancer. DNA repair mechanisms have been put in place by cells to prevent these phenomena. HR is one of these mechanisms. It is an error-free process, the aim of which is to repair a double-strand break (DSB) using an intact DNA copy (sister chromatid), most often during the S and G2 phases. An increase in HR activity in cancer cells confers resistance to treatment [1]. Of the proteins involved in HR, RAD51 is considered key because of its recombinase

activity, which is essential for allowing HR to function correctly. Deregulating this protein increases the risk of developing cancer, and contributes to the resistance of tumor cells to treatment [2]. The chemo and radio resistance observed in tumor cells overexpressing RAD51 makes this protein a very interesting target for the treatment of resistant tumors.

HR and the involvement of RAD51 in DDR and reversion stalled forks

Different types of DNA damage can occur, depending on the nature of the attack. DSB is the most harmful of these lesions. HR is an efficient and faithful repair system for DSB as it uses a homologous template that makes it possible to restore the damaged sequence to its original state [3].

After a DSB, the DNA ends are resected and coated with RPA. The RPA is then replaced by RAD51, mediated by BRCA2, which allows nucleation of the RAD51 protomer on the ssDNA, to form the nucleoprotein filament. Once the filament has been formed, it associates by sequence homology with the sister chromatid forming a synaptic complex consisting of 3 strands of DNA, known as the D-loop. Finally, when the D-loop is in place, RAD51 is displaced to allow polymerases to synthesize the new DNA, forming Holliday junctions. These junctions are then processed by resolvases which, depending on the type of resolution, may or may not result in cross-over.

In addition, RAD51 plays three roles in the stalled replication fork process in response to replicative stress [4]. First, RAD51 promotes replication fork reversal when the fork meets an obstacle, thus preventing chromosome breakage [5]. The second role is to protect the newly synthesized ssDNA from degradation by the MRE11, EXO1 and DNA2 exonucleases. The filament of RAD51 prevents excessive resection. Finally, the last role is to allow collapsed replication forks to restart [6].

Regulating RAD51

RAD51 is finely regulated to reduce any aberrant chromosomal recombination. Its expression varies throughout the cell cycle, with a low level in the G1 phase which increases during the S phase and is highest in the G2 phase [7]. HR is preferentially performed in the G2 phase, when the sister chromatid is used as a repair model, although cases have been observed with other models such as a homologous chromosome or a sequence of a heterologous chromosome [8]. The deregulation found in a wide variety of cancers leads to very high levels of mRNA, protein, and promoter activity. This is linked to metastasis and increased disease aggressiveness [9,10].

There is regulation at the level of the promoter of RAD51. This promoter contains E2F1 and EGR1 transcription factor binding sites that will promote the expression of RAD51 [11,12]. For example, in colon cancer cells, E2F1 facilitates DNA repair and promotes the expression of HR factors [13]. On the

other hand, P53, very often mutated in cancers, acts on the promoter of RAD51 by reducing the promoter's activity [14].

Post-translational modifications (PTM) are also involved in the regulation of RAD51 activity (Figure S1). For example, the c-Abl tyrosine kinase phosphorylates RAD51 on residues Y54 and Y315, resulting in an increase in the protein's recombinase activity [15]. Another example is the phosphorylation of RAD51 on residue T309 by Chk1, which is necessary for HR repair after DNA damage induced by replicative stress [16]. More recently, several phosphorylations of RAD51 by MET tyrosine kinase were identified which lead to stabilize RAD51 nucleofilament [72]. Other PTMs, such as SUMOylation or ubiquitination, also modulate the activity of the protein [17].

Finally, the mediators of RAD51 are necessary for ensuring assembly of the nucleoprotein filament in a timely manner at the DSB [18]. BRCA2, PALB2 and the paralogues of RAD51 are known as mediators of RAD51. BRCA2 allows RAD51 to load on to ssDNA, initiating the formation of the nucleoprotein filament [19]. The RAD51 paralogues (RAD51B, RAD51C, RAD51D, XRCC2, XRCC3 and SWSAP1) form different complexes, such as the BCDX2 heterotetramer, the CX3 heterodimer, and the Shu complex. The exact functions of these complexes are still poorly known but they are believed to both play a role in the recruitment of RAD51 at the DSB, and promote RAD51-mediated activities [20,21].

In vivo, RAD51 is regulated at several levels, from transcription to post-translational modifications. Dysfunctions at any of these levels can therefore lead to irreversible effects in both the cell and the organism.

Structure of RAD51

Human RAD51 is a 37kDa protein consisting of an N-ter (1-84) and C-ter (89-339) domain linked by a linker (86-89). The N-ter part is capable of interacting with ssDNA and dsDNA through the HhH (helix-hairpin-helix) motif it contains, but also with the BRCA2 protein. [22-24]. The C-ter part is also capable of linking the DNA through its patterns Loop 1 (232-238) and Loop 2 (269-287) [25,26]. In addition to interacting with DNA, RAD51 can bind and hydrolyze ATP. If ATP binding is enough to form the nucleoprotein filament, hydrolysis of ATP is needed to disassemble it during the post-synaptic stage of HR [27]. RAD51 therefore has a Walker A (127-134) and Walker B (217-225) pattern that allows interaction with the ATP [28]. Nucleoprotein filament structures obtained by crystallization show that the Walker A motif of one protomer is in close proximity to the aa 315-323 sequence of the adjacent protomer. Fixing ATP would therefore strengthen the nucleoprotein filament structure. Finally, there are polymerization units that promote oligomerization of RAD51. The amino acids 190 and 192 and the pattern (86-FTTA-89) [19,29], in addition to playing a role in the interaction with BRCA2, facilitate the protein's self-association [30]. During polymerization, the FTTA region of one protomer

(containing the FXXA motif or motif 1 in figure 1) interacts with the region containing Ala190-Ala192 (motif 2 in Figure 1) of the second protomer to form the nucleofilament.

Mutations at these domains showed a decrease in the activities of RAD51 [30–33]. Targeting these domains with small compounds is promising in the fight against cancer.

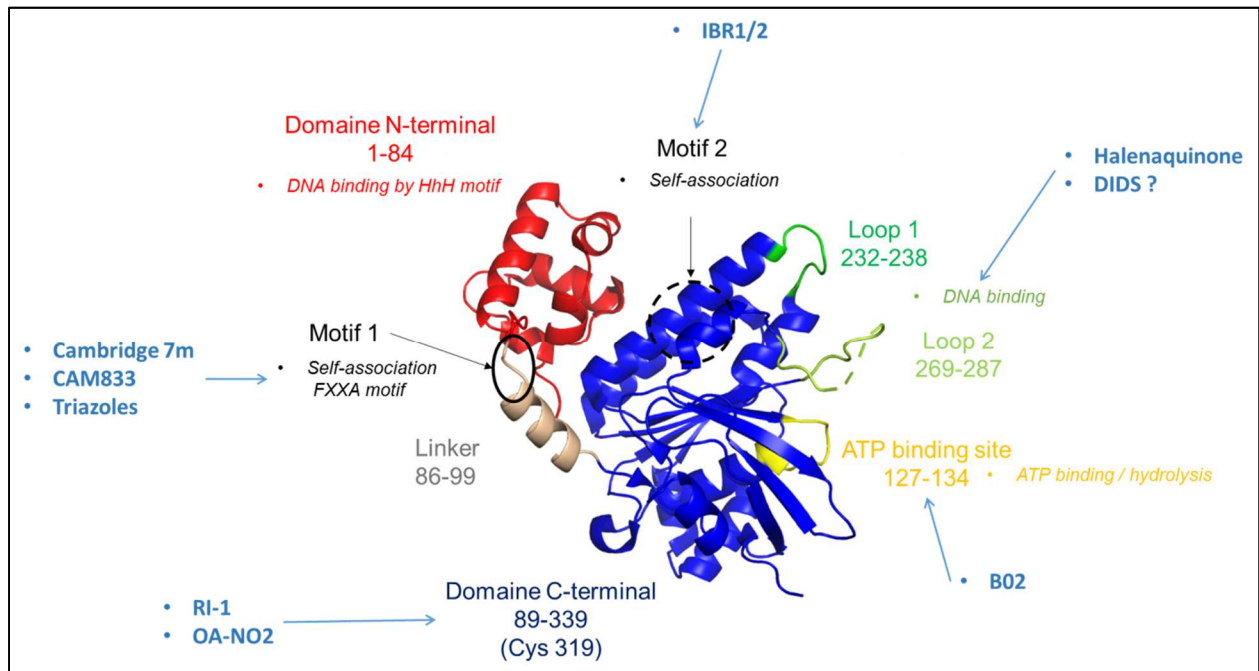


Figure 1: Structural domain and region of RAD51 essential for its activity and binding sites of RAD51 to small compound inhibitors (PDB: 5NW1). Motifs 1 and 2 correspond to amino acid sequence involved in the subunit-subunit interaction of RAD51. Both loop 1 and loop 2 (green) are involved in the recognition and interaction of DNA single- and double-strand respectively. ATP binding site is composed by both Walker A and B sites (yellow). Binding sites of each inhibitor are indicated with a blue narrow.

HR deficient cancer / PARP inhibitor resistance

A subset of cancers shows a deficiency in HR repair because of genetic and epigenetic alterations in the genes in the HR repair pathway. The best characterized HR-deficient cancers, such as certain ovarian cancers or breast cancers, have mutations in the BRCA1 and BRCA2 genes³⁴. These cancers therefore use alternative repair pathways that depend on poly (ADP-ribose) polymerase (PARP), protein particularly involved in BER [41].

PARP inhibitors have been developed to provide an effective therapy for HR-deficient cancers by inducing synthetic lethality [35]. Indeed, the principle of synthetic lethality is based on the fact that the concomitant failure of two complementary repair pathways leads to cell death, while the failure

of only one of these ways is not lethal. This concept has been particularly studied in the case of mutations of the genes of HR, BRCA1 and BRCA2, with the inhibition of PARP. Currently three PARP1 inhibitors have been approved by the FDA: olaparib, rucaparib and niraparib.

However, resistance to PARP inhibitors has been reported in patients treated over a long period of time. This resistance can be acquired in different ways and in particular by restoring HR in cells. Secondary mutations in the BRCA1 and BRCA2 genes can restore protein function and promote HR [36]. PARPi resistance was also observed with secondary mutations in the RAD51C and RAD51D genes [37]. A loss of function in the p53 binding protein 1 (53BP1) promotes the resection of DSBs in BRCA1-deficient cells and restores HR [38].

Because RAD51 is also implicated in the resistance of cancer cells to PARP inhibitors, the modulation of RAD51 is a great interest in the search of new anticancer therapies. Some molecules have been discovered for synthetic lethality with PARP inhibitors. These molecules specifically target the RAD51-BRCA2 interaction to mimic a BRCA-deficient effect and will be presented in the next section.

In addition to BRCA1 mutations that restore BRCA1-RAD51 interactions and facilitate the loading of RAD51 on ssDNA [39], there is a mitotic regulator involved in RAD51 degradation, EMI1, which induces an increase in the protein level of RAD51 in these cells [40]. To overcome PARPi resistance, the combination of PARPi and RAD51 inhibitors appears promising.

Inhibiting RAD51 activity

During HR, from resection to resolution, RAD51 is the main target in the development of new drugs. Several strategies have led to the discovery of inhibitors. Nucleotide inhibitors are DNA aptamers with a 3D conformation making specific interaction possible with RAD51. In 2010, three aptamers showed strong inhibition of the strand exchange activity of RAD51, with IC50s below μM [42]. Peptide inhibitors have also been developed to prevent protein-protein interactions with RAD51. BRC4 is a peptide that mimics the BRC4 motif of BRCA2. This peptide inhibits the formation of the RAD51-ssDNA complex [43]. A final strategy is to develop small, low molecular weight chemical compounds that specifically interact with RAD51. Since 2009, at least fifteen RAD51 inhibitors were described and studied (Table 1).

Table1: Small compound inhibitors of RAD51 described since 2009

Molecule	Structure	Target	In vitro IC50/Binding affinity	Clinical trials	References
DIDS		ssDNA binding site	D-loop assay: 1 to 10µM	/	46
Halenaquinone		dsDNA binding site	/	/	49
B02		Around ATP binding site	D-loop assay: 27,4µM	/	51
RI-2/RI-2		Cys319	D-loop assay: 5-30µM	/	54
IBR1/IBR2		Ala 190 / Ala192	Cellular assay : GI50: 11-16µM depending on cancer cell lines	/	56
Cambridge 7m		FXXA binding site	(ITC) K _D : 1,3µM	/	59
CAM833		FXXA binding site	(ITC) K _D : 366nM	/	60
CSB		Unknown	Strand exchange assay: 0,4µM	/	57
OA-NO2		Cys 319	/	/	63
Compound 26 (Triazoles)		FXXA binding site	HRR decreases (40%) Synergistic effect with olaparib in BxPC3 cells	/	61
CYT01A/CYT01B /CYT0851	/	/	Synergistic effect with PARPi in breast cancer cells	Phase I/II NCT03997968	67-68-69
Amuvatinib		Global translation diminution	/	Stopped in Phase II	70
Resveratrol		Decreased RAD51 expression	Increased apoptosis in etoposide resistant cancer cells	Phase II for multiple myeloma	73-74
Artesunate		Decreased RAD51 expression	Increased cisplatin sensitivity in cancer cells	/	76
SAHA		Decreased RAD51 amount Increased RAD51 degradation	Synergistic effect with PARPi in colorectal cancer cells	/	79-80
T007090		Decreased RAD51 amount	Increased radiosensitivity in cancer cells	/	81

List of RAD51 inhibitors described from 2009, including Name, structure, target site, in vitro IC50 and binding affinity, clinical trials and corresponding references.

- ***DIDS (4, 4'-diisothiocyanostilbene-2, 2'-disulfonic acid) and derivatives***

DIDS is used as an inhibitor of anion transporters and chloride channels [44]. In an aqueous medium, DIDS hydrolyzes and polymerizes to form di-, tri-, and pentamers. These polymers block Cl⁻-K⁺ chloride channels [45]. This small compound (370g/mol) is composed of a substituted stilbene ring with four functions, two sulfonate functions (SO₃⁻) in positions 2 and 2' and two isothiocyanate functions (NCS) in positions 4 and 4'.

In 2009, by screening a library of 185 compounds, DIDS was shown to inhibit the strand exchange activity of RAD51 with an IC₅₀ between 1 and 10 μM. DIDS has also been shown to stimulate hydrolysis of ATP by RAD51 and to inhibit the binding of RAD51 to DNA [46]. DIDS may compete with ssDNA binding, by binding at or near RAD51's ssDNA binding site.

This compound also has an anti-leukemic effect by targeting RAD51. DIDS treatment inhibits repairs to activation-induced cytidine deaminase (AID) initiated DNA breaks, induces apoptosis, and promotes cytotoxicity preferentially in AID-expressing human chronic lymphocytic leukemia [47].

From DIDS, we have recently identified a new RAD51 inhibitor which is more stable and less toxic than DIDS (patent application n°WO2020104634A1). The treatment with Cisplatin on prostate cancer cells has shown that the level of RAD51 foci is significantly decreased when it is associated with this stilbene derivative (about 20%), that confirms the intracellular RAD51 targeting of this new inhibitor. This result shows that DIDS derivatives are promising molecules for inhibiting HR and thus their potential to sensitize resistant cells to anticancer treatments (publications in progress).

- ***Halenaquinone/xestoquinone***

Halenaquinone and xestoquinone, discovered in 1983, are natural compounds synthesized in *Xestospongia exigua*. These compounds are similar, except that xestoquinone does not contain a C-3 carbonyl group, while halenaquinone does. These compounds have been shown to inhibit topoisomerase I and cytotoxicity in leukemic lines P388, Molt-4 and L1210 [48]. From 160 crude extracts of marine sponges screened using the D-loop assay, halenaquinone was shown to inhibit the strand exchange activity of RAD51 [49]. By means of SPR, both compounds were shown to bind directly to RAD51, however halenaquinone appears to have better affinity than xestoquinone. *In vitro*, halenaquinone inhibits the binding of RAD51 to double-stranded DNA, but not to single-stranded DNA. At the cellular level, this compound dose-dependently decreases the formation of RAD51 foci in the GM0637 line after 12 Gy IR treatment without affecting protein expression.

- **B02 and its derivatives**

These compounds were discovered as inhibitors of RAD51 by high-throughput screening of more than 200,000 compounds from the NIH Small Molecule Repository [50]. The activities of the compounds were measured using a FRET-based DNA strand exchange assay. The most active compounds were tested in the D-loop and the compound B02 (3-(Phenylmethyl)-2-[(1E)-2-(3-pyridinyl)ethenyl]-4(3H)-quinazolinone) binds specifically to RAD51. It has shown strong inhibition of D-loop formation (IC50: 27.4 μ M with 1 μ M RAD51) and is not active on the prokaryotic homologue RecA (IC50>250 μ M). A structure-activity analysis (SAR) determined that the benzyl group (-R2) could be modified without affecting the activity of the compound, leading to the compounds B02-3a (ethyl in R2) and B02-3b (m-methyl phenyl in R2) with IC50s of 15.3 and 27.3 μ M, respectively [51].

B02 prevents nucleoprotein filament formation and dsDNA-RAD51 interaction. B02 reduces the recruitment of RAD51 (foci formation) at DNA break sites during cisplatin treatment of MDA-MB-231. The authors showed, in viability testing, that this compound sensitizes cells to cisplatin treatment. This compound was then tested on breast cancer lines and xenografts in mice and showed a decrease in tumor growth of xenografts (MDA-MB-231 cells) in mice [52].

In 2017, another derivative, compound 17 (R2-parachlorobenzyl), was tested on a panel of triple negative breast cancers. This compound is 15 times more effective than B02 at inhibiting the growth of SUM159T cells, and inhibits the appearance of RAD51 foci twice as much after 6 Gy of irradiation in MDA-MB-231 [53].

- **RI-1 and RI-2**

Detected by screening of 10,000 compounds (Chembridge DIVERSet), RI-1 (3-chloro-1-(3,4-dichlorophenyl)-4-(4-morpholinyl)-1H-pyrrole-2,5-dione), binds directly to RAD51 at Cys319. The chromaleimide group reacts with the sulfurhydryl group of cysteine through the Michael reaction, thus forming a covalent bond between the compound and the protein. By interacting with Cys319, the compound RI-1 destabilizes the protein-protein interface, which is essential for formation of the nucleoprotein filament. *In vitro* results suggest that the IC50 of RI-1 is between 5 and 30 μ M. Cellular experiments show that RI-1 is capable of specifically inhibiting HR and sensitizes cancer cells (HeLa, MCF-7 and U2OS) to mitomycin C (MMC) treatment [54]. RI-2 (1-(3,4-dichlorophenyl)-3-(4-methoxyphenyl)-4-morpholino-1H-pyrrole-2,5-dione) has been developed to both reduce the chances of off-targeting and increase the stability of the compound in living organisms [55]. RI-2 reversibly binds to RAD51 in the same pocket as RI-1. This compound inhibits HR *in cellulo* and sensitizes the HEK293 line to MMC treatment.

- ***IBR1/IBR2***

IBR1 and IBR2 were discovered from a bank of 24,000 chemical compounds. IBR2 is more effective in inhibiting the growth of cancer cells. IBR2 binds directly and specifically to RAD51 at alanines 190 and 192, preventing the multimerization of RAD51 and formation of the nucleoprotein filament [56]. This is the same interaction site as the phenylalanines of the BRC4 peptide. Like BRC4, IBR2 inhibits RAD51 by mimicking the BRC repeat sequence. In particular, this sequence allows BRCA2 to bind to RAD51. At the cellular level, IBR2 used at 5µM is capable of reducing the rate of homologous recombination within cells and inhibits the formation of RAD51 foci in MCF-7 (breast cancer) cells after IR treatment. The compound does not appear to decrease the amount of RAD51 transcriptionally but does seem to encourage degradation of the protein via the ubiquitination-proteasome pathway. The compound was also tested on a panel of cancer cells K562 (GI50: 12.5 µM), HeLa (GI50: 14.5 µM), MDA-MB-231 (GI50: 14.2 µM), MDA-MB-435 (GI50: 11.5 µM), MDA-MB-468 (GI50: 13.2 µM), MCF7 (GI50: 12.3 µM), T47D (GI50: 12.1 µM) and HBL100 (GI50: 16.0 µM), suggesting potential for a broad-spectrum anti-cancer treatment. *In vivo*, IBR2 inhibits the growth of breast cancer xenografts in mice. In addition, IBR2 appears to be of interest in difficult-to-treat cancer cells, such as imatinib-resistant CML (chronic myeloid leukemia). The compound has been used on imatinib-resistant T315I mutant mouse cells and shows 50% inhibition of cell growth (GI50) as early as 12µM.

- ***Chicago Sky Blue (CSB)***

CSB is a dye used for coloring cottons or as a pigment for inks. It is a powerful inhibitor of RAD51 with an IC50 of 0.4µM determined using the strand exchange technique. Identified by screening more than 1000 compounds, CSB appears to act by disrupting the association of RAD51 with single-stranded DNA and inhibiting nucleoprotein filament formation [57]. In addition to inhibiting the activity of RAD51, this compound has recently demonstrated some efficacy in mice in cardiac repair after infarction by inhibiting both the CaMKII pathway and activation of macrophages and neutrophils [58]. CSB does not appear to be a good candidate for anti-cancer treatment because of its activity on the cardiac system.

- ***Cambridge 7m (C7m)***

This compound was developed from screening molecular fragments. The fragments are screened at high concentrations to identify effective ligands. The structural information of the fragment-protein complex is then used to optimize the fragment for additional interactions and the improved affinity of the compound. Cambridge 7m was developed to target the protein-protein

interaction between RAD51 and BRCA2, as well as the polymerization domain of RAD51. The authors carried out screening by calorimetry (ITC), and C7m was shown to be the most inhibitory compound, with a K_D of $1.3\mu\text{M}$ [59]. The binding of the molecule may be located on RAD51 at the FXXA binding site of RAD51.

- ***CAM 833***

CAM 833 was discovered using a structure-led fragment based approach by targeting the FXXA binding site of RAD51 in order to inhibit the BRCA2-RAD51 protein-protein interaction. CAM 833 has a high affinity for RAD51 with a K_d of 366 nM (ITC). Crystal structure shows CAM833 binds RAD51 at the same site as the BRCA2 FxxA motif. In addition to preventing oligomerization of RAD51, CAM 833 is able to reduce in a dose-dependent manner (IC_{50} $6\mu\text{M}$) the foci of RAD51 after exposure to IR (3Gy) and alters DNA repair by homologous recombination. This molecule also appears to induce apoptotic cell death and cell cycle arrest and to sensitize different cancer lines to IR and to the PARPi AZD2461 [60].

- ***Triazoles derivatives (Compound 26)***

By virtual screening of Asinex and Life chemicals databases, Roberti et al. have targeted the FxxA pocket of RAD51, which is involved in the interaction with BRCA2 protein.

Following cell-based assays, the authors have identified a triazole hit compound (compound 26) able to inhibit the BRCA2-RAD51 interaction. The objective of this study was to achieve synthetic lethality by combining a PARP1 inhibitor, olaparib, with a disruptor of the BRCA2-RAD51 interaction [61].

This compound 26 is able to reduce the rate of homologous recombination in BxPC3 cells (Pancreatic cancer cells) with 40% inhibition at $20\mu\text{M}$. Moreover, the use on BRCA2 deficient cells (Capan-1 cells) does not impact the HR which reinforces the hypothesis of mechanism of action.

Combination treatments with olaparib showed an increase in DNA damage of nearly 70% (compound 26 a single treatment did not show an increasing DNA damage). Cell viability measurements suggested a potential synergistic effect between olaparib and compound 26. The co-treatment showed an increased decrease in cell viability (BxPC3) compared to the single treatment molecules.

However, this compound does not completely achieve the synthetic lethality phenomenon because the inhibition of HR is only reduced by 40%. It remains to improve these triazole compounds in order to observe a greater effect of co-treatments with olaparib.

- ***Fatty acid nitroalkene 10-nitro-octadec-9-enoic acid (OA-NO2)***

Fatty acid nitroalkenes are products of nitric oxide and nitrite-dependent metabolic and inflammatory reactions with unsaturated fatty acids. The electrophile nature of fatty acid nitroalkenes mediates post-translational modifications of hyper-reactive nucleophilic cysteines in proteins modulating protein structure and function [62]. Asan *et al.* showed from xenograft and immunostaining experiments that OA-NO₂ inhibits triple negative breast cancer (TNBC) cell growth, RAD51 foci formation, and sensitivity to ionizing radiation [63]. In addition, OA-NO₂ specifically targets HR, but not NHEJ, efficiency. O₂-NO₂ interacts directly with RAD51 via Cys-319. Computational analysis revealed that OA-NO₂ alkylation of RAD51 Cys319 is stabilized by hydrophobic interactions with the Pro-318 of RAD51 and hydrogen bonding with Glu-322.

Other ways of modulating HR: decrease RAD51 expression with small compounds

Decreasing the expression of RAD51 is another strategy for modulating HR activity within cells. Studying the mechanism of action of these compounds is generally more complex, but their efficacy on sensitizing cancer cells remains of interest.

- ***CYT01A, CYT01B et CYT-0851***

Over activation of cytidine deaminase (AID) is found in some cancers [64,65]. Some solid tumors show ectopic expression of AID and targeting synthetic lethal interaction between RAD51 and AID can be a solution for treating certain tumors. AID activity induces mutations that can destabilize DNA replication and lead to double-strand breaks that will be managed by RAD51 in order to maintain cell survival. In this context, inhibiting RAD51 by CYT01A (EC₅₀= 150nM) makes it possible to increase the number of double-strand breaks with the aim of inducing the death of this type of cell [66]. Without describing the mechanism of action of the compound or its structure, the authors of the study showed a reduced amount of RAD51 in the nucleus and a decrease in RAD51 repair foci, which was correlated with a decrease in homologous recombination activity.

CYT01B showed the same effect as CYT01A. This compound also appears to sensitize some cancer cells to treatment with PARP inhibitors [67].

Finally, CYT-0851 was tested in breast cancer cell models and showed anticancer activity and synergy with PARP inhibitor treatment. Compared to the effect of monotreated compounds, the co-treatment showed an increase in γ -H2AX foci with immunofluorescence, as well as an increase in DNA damage observed using the Comet assay [68]. This compound is in clinical phase 1

(NCT03997968) since 2019 in the treatment of patients with advanced lymphoma or solid tumors. A patent related to this work has been filed by Cytair Therapeutics^[69].

- ***Amuvatinib***

Amuvatinib is a synthetic carbothioamide targeting several proteins with tyrosine kinase activity. In particular, it inhibits c-Met, c-RET and mutant forms of c-KIT, PDGFR and FLT3. It has been used in Phase II clinical trials for the treatment of non-platinum-resistant small cell lung cancer ^[70]. Amuvatinib also suppresses the induction of RAD51 to potentiate the activities of DNA-damage-inducing agents. This decrease in expression is thought to be associated with reduced phosphorylation of the S6 ribosome protein and inhibition of global translation ^[71]. In addition, C-met has recently been shown to phosphorylate and stimulate the activity of RAD51 ^[72]. This link between c-Met and RAD51 would therefore further increase the impact of amuvatinib on RAD51.

- ***Resveratrol***

The structure of resveratrol is stilbene-based and is composed of two phenolic rings connected by a double bond to produce 3, 4', 5 - trihydroxystilbene. Resveratrol is a polyphenol naturally present in certain fruits, such as grapes or blackberries, when they are subjected to environmental stress. Resveratrol is known to have many therapeutic effects, including anti-inflammatory and anti-oxidative effects, and affects the onset and progression of many diseases ^[73]. Clinical studies have been conducted on patients with cancer, neurological disorders, cardiovascular disease, diabetes and non-alcoholic liver steatosis ^[74]. At the biochemical level, resveratrol is capable of inhibiting the expression of RAD51 in certain cancers. One study used resveratrol as an inhibitor of RAD51 in cancer stem cells resistant to etoposide. In this study, the use of resveratrol in combination with etoposide was shown to reduce cell viability and increase apoptosis in cancer stem cells ^[75].

- ***Artesunate***

Artesunate is a sesquiterpenic peroxide derived from artemisinin extracted from *Artemisia annua*, originally developed for the treatment of malaria. In cancer cells, the toxic effects of artesunate are caused by oxidative stress and the induction of double-strand breaks. A study of ovarian cancer cell lines showed that artesunate was capable of reducing the protein levels of RAD51 in several cell lines after 24 hours of treatment (0-25µg/mL) ^[76]. At the same time, the number of cisplatin-induced RAD51 foci was significantly reduced, leading to increased cell sensitivity to cisplatin. In addition, in two types of non-malignant cells (normal human fibroblasts and immortalized epithelial cells) the quantity of RAD51 was not modified by artesunate ^[76].

Another study synthesized a prodrug with artesunate conjugated to cisplatin which may be able to increase cytotoxicity in BRCA-proficient ovarian and breast cancer cells [77].

- ***Suberoylanilide hydroxamic acid (SAHA) and other HDIs***

SAHA is a histone deacetylase inhibitor (HDI). Histone deacetylases (HDACs) and histone acetyltransferases (HATs) modulate chromatin conformation by adding or removing the acetyl moiety of histones and non-histone proteins. They also play an important role in many biological processes, including intracellular signaling. HDIs are a class of therapeutic agents for human malignant diseases [78]. Studies in multiple myeloma (MM) show that cells exposed to SAHA appear to have both reduced RAD51 and decreased formation of RAD51 foci in their nuclei after irradiation. It was also found that SAHA may increase the degradation of RAD51 through the proteasome in irradiated MM cells. [79].

In addition to SAHA, other HDi appear to have an impact on HR and RAD51 in response to IR treatment. The compound PCI-24781 reduces the transcription of HR proteins, including RAD51, after 24 hours of treatment to 0.2 μ M, and the combination with PARP inhibitors shows a synergistic effect on apoptosis. *In vivo*, RAD51 protein levels were reduced in the tumors of treated mice, and a 69% decrease was observed when the total duration of drug exposure was more than 24 hours [80].

- ***T007090 (T007)***

T007 is a PPAR γ inhibitor. PPAR γ (peroxisome proliferator-activated receptor gamma) is a nuclear receptor that regulates the transcription of numerous genes involved in the differentiation, proliferation, and apoptosis of various cell types. T007 increases radiosensitivity in some cancer lines and appears to inhibit IR-induced repair of DSB. In cervical cancer cells, T007 significantly reduces levels of the RAD51 protein, leading to the persistence of IR-induced DNA damage [81]. Studies on ME-180 and SiHA cell lines treated with T007 (0-50 μ M/12-24h) showed a decrease in the protein content of DNAPKcs and RAD51. In the same cell lines, a combination with irradiation resulted in a decrease in RAD51 foci in the cell nuclei [81].

Conclusions and future perspectives for RAD51 inhibitors

Developing inhibitors and modulators of DNA repair proteins is currently the subject of much research. RAD51 is a protein that is of particular interest in the HR mechanism and its deregulation contributes to genomic instabilities that can lead to cancer, particularly ovarian and breast cancer. In certain cancers, RAD51 is over-expressed and induces resistance to cancer treatments. In recent

years, inhibitors have been developed to limit the impact of RAD51 in these resistances and to improve current therapies.

In this review, we described several compounds able to modulate the activity or the expression of RAD51. We focused on the main inhibitors interacting directly with RAD51 and we indicated the interaction site localization of each molecule on the RAD51 protein in order to highlight the interfaces targeted. Indeed several pockets can be targeted, such as the ATP-binding pocket, DNA interaction sites or self-oligomerization motifs of RAD51.

In the context of synthetic lethality with PARP, the interaction surfaces between BRCA2 and RAD51 have been targeted and several molecules (triazole derivatives, CAM833) have shown to act with PARPi in a synergic manner. Other molecules, such as IBR1/IBR2, directly target RAD51 and seem to have an antineoplastic activity as a single agent. Targeting RAD51 alone is not sufficient to strongly decrease the viability of cancer cells because other DNA repair mechanisms can be involved and partially compensate the inhibition of RAD51. Hence the interest in targeting different DNA repair protein as PARP and RAD51 to sensitize resistant cancer cells to radio or chemotherapies. The current research dynamic is increasingly oriented towards precision medicine, and it is necessary to deepen our knowledge of DNA repair mechanisms in order to optimize molecules and limit off-target effects.

However, developing these compounds could be of use well beyond the context of cancer therapy. For example, in order to fight the emergence of antibiotic resistance, it might be interesting to target RecA (RAD51 homologue) [82]. Uses in the field of virology are also possible. RAD51 is involved in the HIV-1 integration mechanism in infected cells by inhibiting viral integrase [83]. Inhibiting integration could be explained by dissociating the integrase-DNA complex from RAD51, and stimulating RAD51 with the RS-1 compound [84] revealed a decrease in integration efficiency [85]. The development of RAD51 modulators remains an interesting and relevant approach for targeting cancer, antibiotic resistance, and HIV.

Acknowledgments

This work was supported by the French Regional Council of Pays-de-la-Loire in the PIRAMID program.

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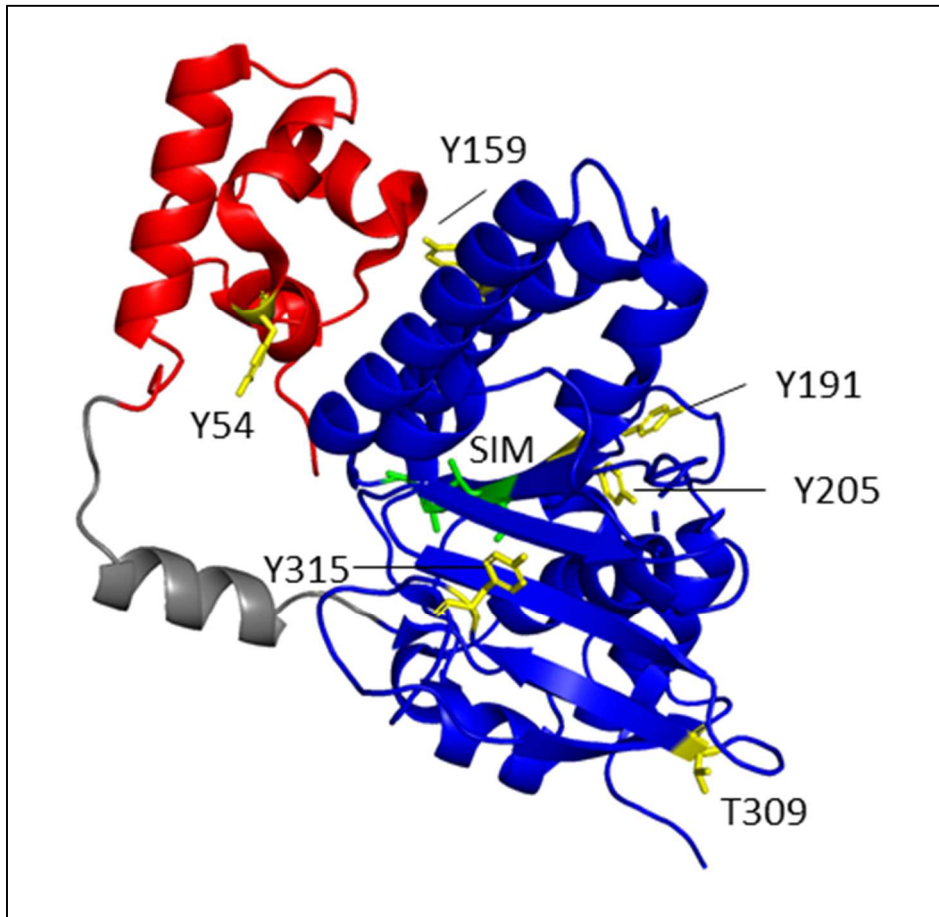


Figure 1S. Main sites of post-translational modifications of RAD51(PDB: 5NW1) .

Tyrosines Y54 and Y315 are the target of c-Abl kinase and its phosphorylations modulate the recruitment of RAD51 on DNA damage sites and its activity of DNA strand exchange. T309 is phosphorylated by Chk1 that promotes the foci formation of RAD51 on DNA damage. Tyrosines Y159, Y191, Y205 and Y315 are phosphorylated *in vitro* by c-Met kinase that modulates the stability of nucleoprotein of RAD51 and the HR pathway. SIM corresponds to sumoylation interaction Motif.