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REVIEW

Targeting RAD51-Mediated Homologous Recombination as a Treatment for Advanced Solid and Hematologic Malignancies: Opportunities and Challenges Ahead

Erica S Tsang, Pamela N Munster

Division of Hematology and Oncology, University of California San Francisco, San Francisco, CA, 94158, USA

Correspondence: Pamela N Munster, Division of Hematology and Oncology, University of California San Francisco, Box 1711, 1450 Third Street, San Francisco, CA, 94158, USA, Tel +1-415-502-3598, Email Pamela.Munster@ucsf.edu

Abstract: RAD51 is integral in homologous recombination DNA damage repair and has garnered much interest as both a biomarker and potential therapeutic target in oncology. Multiple in vitro and in vivo studies have demonstrated its role as a predictive marker, particularly in the context of platinum-based therapies and poly ADP-ribose polymerase (PARP) inhibitors. In this review, we highlight the development of RAD51 inhibitors, with a focus on novel molecules and ongoing clinical trials. Despite many efforts to develop effective and tolerable direct RAD51 inhibitors, identification of these agents remains challenging. Clinically, however, there may be a role of pharmacological indirect RAD51 inhibition.

Keywords: homologous recombination deficiency, RAD51, DNA damage repair, HRD

Introduction

RAD51 plays an integral role in the repair of DNA damage by homologous recombination and represents a potential therapeutic target in oncology. In normal cells, exogenous and endogenous DNA damage is an unavoidable and continuous biological process. DNA damage may be triggered by multiple factors including oxidized radicals, ionizing radiation, ultraviolet light, and various chemicals. Impaired DNA repair after DNA insult may result in DNA single (SSB) and double strand breaks (DSB), and efficient and precise DNA damage repair is crucial to maintain genomic instability, protect cell survival and prevent the development of cancer.[1–4] DNA DSB are considered the most lethal, and homologous recombination (HR) serves as one of the most efficient and error-free repair strategies (Figure 1).[5] In the event of a DSB, the DNA ends are resected and coated with Replication Protein A (RPA).[6] RAD51 then replaces RPA, allowing the formation of a nucleoprotein filament around the single-stranded (SS) DNA with the help of BRCA2.[7] This filament invades the sister chromatid to search for the matching homology sequence, and then creates the displacement loop (D-loop). RAD51 is subsequently displaced for polymerases to synthesize new DNA for replication of the homologous template.[8]

In addition to its role in homologous recombination, RAD51 is highly involved in the response to replication stress, specifically at stalled replication forks where ssDNA is vulnerable to progress to a DSB without immediate and adequate repair and reversal mechanisms in place. Promotion of replication fork reversal by RAD51 at these replication blocks facilitates the continuation of replication and preserves chromosomal integrity.[9,10] The RAD51 associated regulation of exonucleases at stalled DNA replication forks also protects newly formed ssDNA from degradation.[11,12] And lastly, RAD51 is tasked to restart collapsed replication forks.[13]

In terms of regulation of RAD51, p53 reduces RAD51 mRNA and protein expression.[14] Post-translational modification of RAD51 via phosphorylation has also been reported. One example is c-MET tyrosine kinase, which

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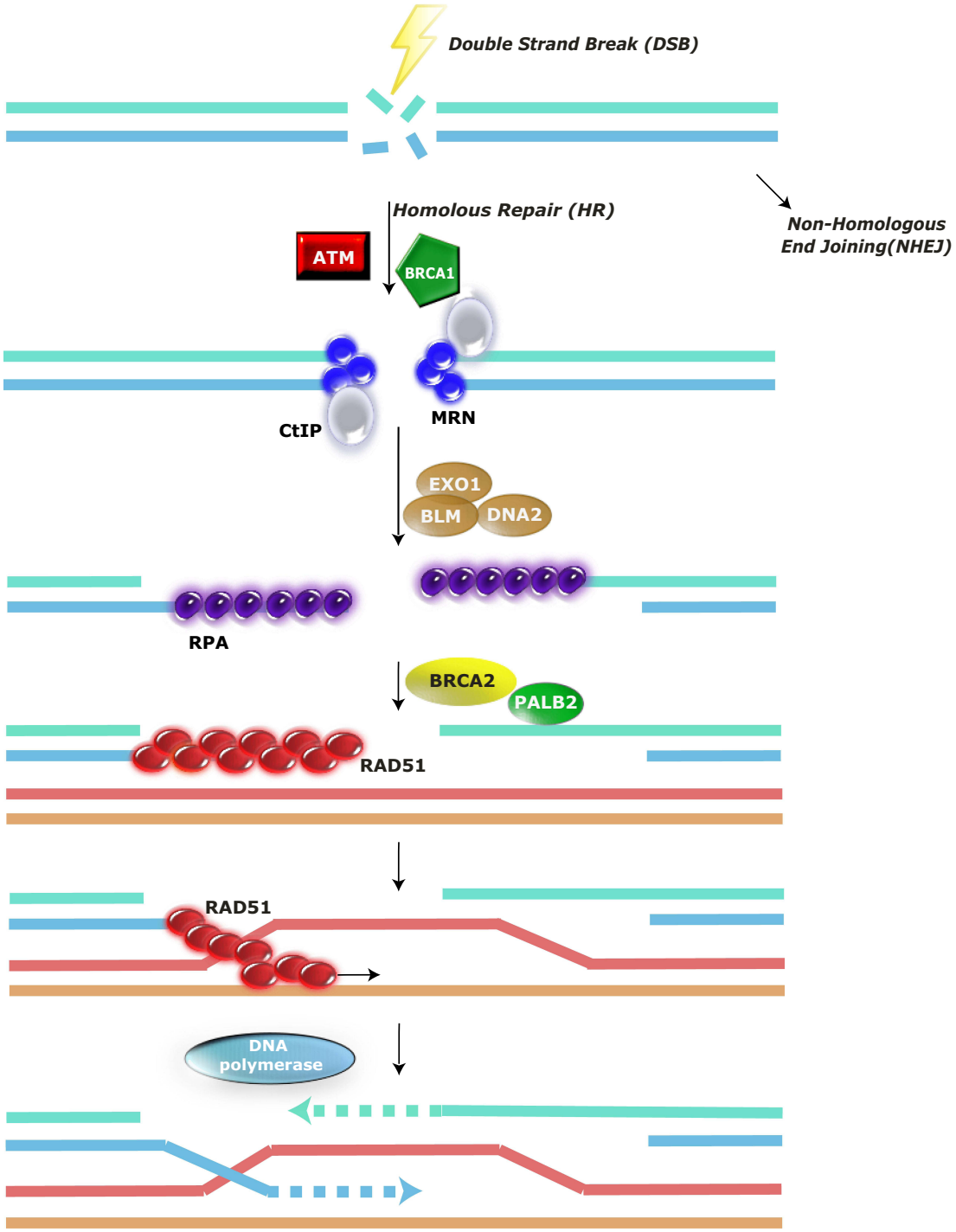

Figure 1 Schematic diagram of homologous recombination repair mediated by RAD51. In the setting of a double strand break (DSB), DNA resection occurs. The resected 3′ ends are coated with Replication Protein A (RPA). RAD51 replaces RPA, facilitating the formation of a nucleoprotein filament around the single-stranded (ss)DNA. This is mediated by BRCA2 and PALB2. The RAD51 nucleoprotein filament invades the sister chromatid to find the matching homology sequence. RAD51 is displaced to allow DNA polymerases to synthesize new DNA for replication of the homologous template.

phosphorylates RAD51 and subsequently stabilizes the nucleofilament.[15] Another example involves Polo-like kinase 1 (Plk1) phosphorylating RAD51 during cell damage, which leads to RAD51 binding to the Nijmegen breakage syndrome gene product (NBS1), strengthening resistance to genotoxic stress.[16]

RAD51 as a Biomarker and Therapeutic Target

Homologous recombination relies heavily on intact RAD51 function to preserve genomic integrity, and RAD51 dysregulation and mutations have been associated with malignancy.[17–20] Perhaps most well recognized are patients with Fanconi anemia (FA) and FA-like syndrome who demonstrate alterations in *RAD51* and its paralogs, with manifestations including an increased disposition to malignancies such as squamous cell carcinoma and acute myeloid leukemia.[21] *RAD51* mutations or alterations in important regulatory genes including *BRCA2, PALB2*, and *RAD51* paralogs can affect RAD51 activity.[22] Unlike *BRCA2* and *PALB2* mutations which are clearly linked to multiple defined cancers, germline *RAD51* mutations have been predominantly associated with an increased risk of ovarian cancers, whereas the link to other malignancies including breast cancer is less clear.[23,24] For example, 0.35% and 0.41% of individuals with ovarian cancers were found to have germline mutations in known RAD51 regulatory genes *RAD51C* and *RAD51D*, respectively.[25]

Emerging evidence over recent years have increasingly pointed to the relevance of homologous recombination deficiency (HRD) in its predictive role in the response to specific therapies. Traditionally, more emphasis has been placed on the role of *BRCA1* and *BRCA2*, as well as *PALB2* mutations in the response to DNA damaging agents. However, RAD51 has now been recognized as an independent as well as an interlinked predictive marker in response to DNA damage and its repair, and assays more specific for HRD and genomic instability are being developed.[26–29] RAD51-specific testing has also been proposed, highlighting its importance as an emerging biomarker. A RAD51 immunohistochemistry (IHC) assay examining RAD51 foci showed a high degree of accuracy in predicting poly ADP-ribose polymerase (PARP) inhibitor and platinum-based therapy response using in vivo models.[30] In a retrospective analysis from the GeparSixto trial of neoadjuvant treatment for patients with triple-negative breast cancer, this same assay was evaluated as a response predictor of response to neoadjuvant carboplatin.[31] Comparing to the commercially available genomic HRD score (Myriad myChoice) this RAD51 assay was useful in identifying RAD51-low tumors, which further correlated with pathological complete response. An easy, readily available histology-based assay could provide a less expensive test option which also requires less tissue per sample.[27] RAD51 immunostaining performed on tumor biopsies before and after olaparib in the Phase II PETREMAC trial, also predicted the response to PARP inhibition in patients with early triple-negative breast cancer. This trial allowed patients to receive olaparib prior to chemotherapy for up to 10 weeks.[32]

While low RAD51 expression changes have been linked to therapy response, RAD51 mutations may convey resistance to therapy, particularly to PARP inhibitors. PARP inhibitors are effective in many patients with HR-deficient cancers by exploiting their defective DNA repair functions.[33,34] However, even in the presence of effective PARP inhibition, primary or acquired resistance occurs frequently and often develops rapidly. One of the putative mechanisms of resistance is believed to stem from secondary mutations in *RAD51C* and *RAD51D* genes.[35] However as a clear role of RAD51 as a responsive predictor and genomic culprit in carcinogenesis is emerging in multiple in vitro, in vivo and clinical trials, its role as a therapeutic target is just emerging. There is much interest in the development of RAD51 inhibitors in advanced solid and hematologic malignancies, with many challenges and opportunities. This review focuses on the current understanding of RAD51 as a therapeutic target, highlighting novel molecules targeting RAD51 and ongoing clinical trials (Table 1).

Discovering Small Molecule Approaches

Amuvatinib

Like many other compounds, imatinib, a tyrosine kinase inhibitor, was initially found to modulate RAD51 expression indirectly via the c-ABL, c-KIT, and PDGF pathways. Compelled by the relevance of RAD51, these findings prompted further testing of other tyrosine kinase inhibitors, including amuvatinib.[36] In glioblastoma multiforme cell culture studies, amuvatinib, known to target c-KIT, PDGFRα, and FLT3, was found to potentiate radiation therapy and was linked to decreased RAD51 expression and increased DBSs.[37] These findings led to a Phase I study of amuvatinib in 22 patients with advanced solid tumors. Amuvatinib was well tolerated, and in the absence of dose-limited toxicities, the maximum tolerated dose was not reached in this trial.[38] RAD51 protein expression was measured in serial skin punch biopsies in eight patients

Table 1 Drugs in Development That Target RAD51 Activity

Compound	Target	Clinical Trial	Reference
Small molecules			
Amuvatinib	Indirect inhibition of RAD51 expression by targeting c-KIT, PDGFRα, and FLT3 pathways	NCT01357395 – Negative phase II trial of amuvatinib with platinum/etoposide in patients with platinum-refractory small cell lung cancer	[40]
DIDS	Inhibit RAD51 binding to ssDNA	–	[41]
B02	ATP binding site	–	[47]
Fatty acid nitroalkene OA-NO_2	Alkylation of cysteine 319	No clinical trials in patients with cancer, but studied in focal segmental glomerulosclerosis, pulmonary arterial hypertension, and asthma (NCT03422510, NCT04053543, NCT03762395)	[50]
Chicago sky blue	Unknown	No in-human studies to date, but potential cardiac effects may limit safety and tolerability.	[51]
Halenaquinone	Inhibit RAD51 binding to dsDNA	–	[53]
RI-1	Binding to Cysteine 31	–	[54]
IBR2	Binding to Alanine 192	–	[55]
CAM 833	FxxA motif	–	[64]
RS-1	Stabilize RAD51 nucleoprotein filament	–	[65]
CYT-0851	Not reported	NCT03997968 – Ongoing phase I trial of CYT-0851 with preliminary results demonstrated well-tolerated oral drug.	[72]
Antibodies			
3E10 autoantibody	N-terminus of RAD51	–	[75]
Fab-F2-iPTD	Inhibit RAD51 binding to ssDNA	–	[77]

receiving amuvatinib, however without a clear trend in RAD51 modulation. One patient with a gastrointestinal stromal tumor (GIST) had stable disease and demonstrated decreased RAD51 expression in serial skin punch biopsies. A further phase I trial evaluated amuvatinib in combination with five chemotherapy regimens, including carboplatin/paclitaxel, carboplatin/etoposide, topotecan, docetaxel, and erlotinib.[39] Similarly to the monotherapy, serial skin biopsies suggested a decrease in RAD51 expression in five of six patients. The tolerability and preliminary results of this combination approach prompted a subsequent phase II study. However, ESCAPE (NCT01357395), a subsequent study combining amuvatinib with platinum and etoposide in 23 patients with platinum-refractory small cell lung cancer was terminated early for missing its prespecified endpoint of two confirmed partial responses.[40] In this study, no association was observed between baseline RAD51 expression levels by IHC and response to study treatment. A planned fourth study had never been activated and further clinical development of amuvatinib has since been halted.

DIDS (4,4′-Diisothiocyanostilbene-2,2′-Disulfonic Acid)

During a library screen of 185 compounds, DIDS was identified for its ability to inhibit RAD51 signaling, interfering with DNA strand exchange during DNA damage repair.[41] Preclinical studies suggested that DIDS may be more effective at inhibiting RAD51 from binding to dsDNA compared to ssDNA. However, the profound cell death induced by DIDS in cultured cells confounded the precise assessment of DIDS-related RAD51 modulation. DIDS was studied in chronic lymphocytic leukemia cell lines and showed enhanced cytotoxicity in the setting of activation-induced cytidine

deaminase induced DNA breaks.[42] Less toxic DIDS derivatives have been studied in prostate cancer models. However, despite showing efficacy in cultured prostate cancer cells treated with cisplatin and decreased RAD51 foci, no clinical data are available to date with DIDS or DIDS derivatives.[43]

B02 and Related Derivatives

High-throughput screening of greater than 200,000 compounds within the NIH Small Molecule Repository identified a compound named B02 that could effectively inhibit human RAD51.[44] When combined with various chemotherapy agents, B02 in combination with cisplatin demonstrated considerable cytotoxic activity in both in vitro cell culture and in mouse xenograft models.[45] B02 analogs have been further developed with the goal to increase affinity for RAD51 compared to the parent compound.[46] In vitro studies of such compounds suggested a sensitization to the PARP inhibitor olaparib in triple negative breast cancer cell lines. The need for prolonged incubation period in preclinical models and limited target specific binding due to its flat hydrophobic structure may outweigh the benefits over the parent compound. A translation to early phase clinical studies of B02 or its derivatives has not been reported.[47,48]

Fatty Acid Nitroalkenes

Fatty acid nitroalkenes are products from the metabolic and inflammatory reactions between nitric oxide and unsaturated fatty acids. Their electrophilic properties allow for post-translational modifications of cysteine thiols, and subsequent alteration of protein structure and function. The role of fatty acid nitroalkenes in triple negative breast cancer cell lines was evaluated alone and in combination with several known anti-cancer therapeutics, including doxorubicin, cisplatin, olaparib, and γ-radiation.[49] The fatty acid nitroalkene OA-NO_2 was found to alkylate cysteine 319 in RAD51 with subsequent inhibition of RAD51 binding to ssDNA and causing defective homologous recombination. Thus, the effects of doxorubicin, cisplatin, olaparib, and γ-radiation were augmented in the presence of OA-NO_2. To our knowledge, OA-NO_2 has not yet been studied clinically in the context of cancer. So far, the development of OA-NO_2 has been focused on in inflammatory conditions, including focal segmental glomerulosclerosis, pulmonary arterial hypertension, and asthma (NCT03422510, NCT04053543, NCT03762395).[50]

Chicago Sky Blue

In an attempt to identify small molecules with RAD51 inhibitory activity, a library of 1120 molecules was screened. Chicago Sky Blue (CSB), a promising compound for its potency of RAD51 inhibition emerged from this library.[51] CSB has been otherwise found in colorants and ink pigments. However, CSB was further shown to interrupt the association between RAD51 and ssDNA. Due to the potential to disrupt the formation of nucleoprotein filaments, CSB has been investigated in cardiomyocyte proliferation. CSB administered to mice after induction of myocardial infarctions showed enhanced cardiomyocyte contractility and a dampened acute inflammatory response manifesting as reduced scarring.[52] CSB has not yet been studied in humans in the setting of malignancy, but its potential concurrent cardiac effects will need to be considered carefully.

Halenaquinone

In addition to screening chemical libraries, several novel compounds have been identified by screening natural compounds. Evaluation of 160 crude extract fractions from marine sponges identified halenaquinone as an effective RAD51 inhibitor.[53] Halenaquinone inhibits binding of dsDNA to RAD51 which was further explored in human GM0637 cell lines exposed to ionizing radiation. Suppression of RAD51 foci formation in these cells was seen after treatment with halenaquinone. Despite promising preclinical studies, to date, halenaquinone has not yet been studied in clinical cancer trials.

RI-1

Another compound identified by high-throughput screening of 10,000 molecules is RI-1. This molecule binds irreversibly to the RAD51 protein at cysteine 319. This was shown to disrupt the interface between protein units within RAD51 monomers used to form a filament.[54] Unfortunately, the short half-life of RI-1 precluded further development of this compound in animal models. Further development of similar compounds identified an analogue, RI-2. RI-2 still targets the same binding site as RI-1, but was found to have a longer half-life and increased bioavailability, albeit at the cost of reversible binding.[48] In-human studies have not yet been conducted with this novel compound.

IBR2

The compound IBR2 was identified in a library screen of 24,000 compounds for its direct binding to RAD51.[55] Decreased RAD51 foci were observed in cells treated with IBR2, suggesting effective inhibition of homologous recombination mediated by RAD51. Cell growth inhibition with IBR2 was observed in multiple cell lines including breast cancer mouse models and imatinib-resistant chronic myeloid leukemia cells. Several analogues with the goal to increase the potency of RAD51 inhibition were synthesized based on the chiral center of IBR2. One of these analogues, IBR120, showed a 4.8-fold increase in RAD51 inhibition with demonstrated anti-proliferative activity in triple negative breast cancer cell lines.[56] The clinical testing of this compound has not yet begun.

CAM 833

RAD51 and BRCA2 are intricately linked in homologous recombination repair after DNA damage. Various molecules have been developed in an attempt to modulate the interaction between RAD51 and BRC repeats on BRCA2. However, detailed reports regarding the exact chemical structures have been limited.[57–63] To this effect, a novel molecule, CAM833 was recently introduced after discovery by a structure-led fragment-based approach.[64] CAM 833 has been reported to decrease RAD51 clustering, thus inhibiting the HR repair pathway. In *BRCA2* wild-type cell lines treated with CAM 833 and the PARP inhibitor AZD2461, decreased growth was observed in a dose-dependent fashion. Further preclinical and clinical development for this class of compound type is awaited.

RS-1

RS-1, another small molecule RAD51 inhibitor identified from screening a library of 10,000 compounds, serves to disrupt genomic stability by upregulation of RAD51 and stimulation of RAD51-medicated HR activity with D-loop stimulation and binding to DNA.[65] Initial studies in prostate cancer cell line models showed that treatment with RS-1 induced RAD51 foci accumulation irrespective of cell cycle stage, suggesting DNA damage repair inhibition as its putative mechanism of action.[66] RS-1 showed anti-tumor activity in mouse xenograft models, particularly in tumors with altered levels of RAD54B and RAD54L proteins. Both may have a role as a potential predictive marker in future investigations; however, upregulation of RAD51 by this compound has not yet been tested in human studies.

CYT-0851

Cytidine deaminase overexpression has been demonstrated in several cancer types, including breast and squamous cell skin cancers.[67,68] Cytidine deaminase overexpression has been suggested to increase DNA damage and reliance on RAD51 dependent homologous recombination repair mechanism. Several preclinical studies suggested CYT-0851 to be a novel oral RAD51 inhibitor as it showed effective reduction of RAD51 foci with increased unrepaired DNA damage by γH2AX. Single agent activity was observed in patient-derived xenograft models of Burkitt's lymphoma and pancreatic cancer, creating much interest in this compound.[69,70] CYT-0851 further showed enhanced activity when given with the PARP inhibitor olaparib in breast cancer cell line models.[71] Recent data however has demonstrated that CYT-0851 acts more likely as a monocarboxylate transporter (MCT) 1 inhibitor, with indirect effects on RAD51 expression accounting for the initial characterization as a RAD51 inhibitor. Additional studies are being conducted to further understand the mechanism of MCT inhibition (www.cyteir.com).

While no longer believed to be a direct RAD51 inhibitor, this compound is currently being explored in a phase I/II clinical study and the preliminary results from 35 patients enrolled in the dose escalation cohort of CYT-0851 were recently reported (NCT03997968).[72] Treatment was well-tolerated, with no reported dose-limiting toxicities and no treatment-related discontinuation. 37% of patients reported any grade fatigue (3% with grade 3), nausea, or increased alkaline phosphatase. Of 21 evaluable patients, three partial responses were observed, and 10 patients demonstrated stable disease. Four patients continued treatment for over six months with no cumulative toxicity seen. Determination of the maximum tolerated and recommended phase II doses are ongoing, as well as studies involving combination treatments. The preclinical and clinical development of this compound and the challenges with many of the other described compounds underscore the difficulties in separating the indirect effects on RAD51 from truly specific RAD51 inhibitors.

Antibodies as the Next Frontier?

3E10 Autoantibody

One of the major hopes of targeted therapy lies in the potential for selective cytotoxicity with minimal toxicity to normal cells. In recent years, antibody-based therapies have emerged as effective therapeutic delivery mechanisms. One such antibody, 3E10, an anti-DNA systemic lupus erythematosus autoantibody does not appear to affect normal cells but can inhibit HR repair in *BRCA2* deficient cancer cells.[73,74] 3E10 penetrates into cells through interaction with RAD51 and subsequent inhibition of filament formation.[75] When tested in *PTEN*-deficient glioma and melanoma cell lines, synthetic lethality was observed with increased cell death and in vitro assays demonstrating increased sensitivity to concomitant treatment with an ATR inhibitor.[76] Clinical testing is awaited but has not yet started.

Fab-F2-iPTD

Another novel antibody approach involves the fusion of a cell-penetration peptide (iPTD) to an antigen-binding fragment that inhibits RAD51.[77] Together, this synthetic antibody demonstrated decreased RAD51 ssDNA binding. In vitro assays demonstrated increased cell death, specifically in cells co-treated with the alkylating agent methylmethane sulfonate. Similar to the 3E10 antibody, Fab-F2-iPTD also penetrates the cell and effectively targets RAD51. To date, these antibodies have not been studied in human trials albeit a strong interest in their development.

Conclusions and Future Directions

RAD51 has been well recognized as a critical contributor to effective DNA repair with homologous recombination and thus has garnered much interest as a therapeutic target and predictive marker of response to DNA damaging agents and PARP inhibitors. However, the identification of direct RAD51 inhibitors remains challenging and maybe even elusive, although indirect RAD51 inhibition appears to be of clinical relevance. While a substantial number of molecules have shown promise in preclinical studies, only a very select group of molecules have been advanced to clinical testing. While some of the agents have shown early promise, overall the clinical findings suggest that there is a considerable need for more potent agents, a better understanding in the mechanism of Rad51 inhibition and selective biomarker to enrich for patients more likely to respond.

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Disclosure

The authors report no conflicts of interest in this work.

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