AWARD NUMBER: W81XWH-20-1-0452

TITLE: NEUTROPHILS MODULATE DNA DAMAGE REPAIR TO PROMOTE SURVIVAL/PROGRESSION OF COLORECTAL CANCER

PRINCIPAL INVESTIGATOR: Triet Minh Bui

CONTRACTING ORGANIZATION: Northwestern University Feinberg School of Medicine

REPORT DATE: SEPTEMBER 2021

TYPE OF REPORT: Annual Report FY2021

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland, 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
SEPTEMBER 2021	ANNUAL	1SEPT2020 - 31AUG2021

4. TITLE AND SUBTITLE

Neutrophils modulate DNA damage repair to promote survival/progression of Colorectal Cancer.

5a. CONTRACT NUMBER
W81XWH-20-1-0452

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Northwestern University

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: Tumor-infiltrating neutrophils are a significant feature of colorectal cancer (CRC), where they can promote cytotoxicity or exacerbate disease outcomes. Leveraging human sporadic CRC biopsies, TCGA gene expression analyses, tumor xenografts and murine CRC models, we reveal that neutrophils exert a functional and phenotypic dualism in cancer cells, driving temporal modulation of the DNA Damage Response. Neutrophils were found to promote homologous recombination (HR)-deficiency in early CRC development by miR-155-dependent downregulation of RAD51. Importantly, neutrophil-mediated genotoxicity due to accumulation of double-strand breaks (DSBs) led to the induction of non-homologous end-joining (NHEJ), improving survival and promoting growth of advanced CRC. Importantly, our findings identify distinct HR-deficient and NHEJ-competent CRC therapeutic phenotypes. As such, CRC tumors featuring PMN presence, low RAD51 and low Ku70 levels could be effectively targeted by Olaparib and the resulting synthetic lethality. In contrast, treatment of CRC tumors featuring high Ku70 (and other NHEJ signature genes), indicating heightened NHEJ, should include NHEJ inhibition as monotherapy or in combination with Olaparib to restore sensitivity to synthetic lethality. Thus, our work delineates two mechanism-based translatable therapeutic interventions in sporadic CRC.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	**18**	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Tumor-infiltrating neutrophils (polymorphonuclear neutrophils [PMNs]) are a prominent feature of colorectal cancer (CRC), where the immune cells can promote cytotoxicity or exacerbate disease outcomes. Indeed, CRC microenvironment is frequently accompanied by chronic inflammation and *en masse* PMN infiltration. Increased PMN numbers are further associated with stage IV cancer and are predictive of poor clinical outcomes. In previous work, we showed that in acute colon injury, PMNs can increase DNA double-strand break (DSB) burden and promote genomic instability via microRNA-dependent inhibition of homologous recombination (HR) repair. In this current grant CA191071, we aim to establish whether neutrophils shape the DSB-repair responses and impact CRC progression and resistance to DNA-repair targeted therapy. If completed successfully, this DoD-funded project will outline mechanism-based therapeutic interventions that target aggressive/advanced CRC featuring PMN infiltration.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Neutrophils, colorectal cancer, double-strand breaks, DNA damage, miR-155, RAD51, HR-Deficiency Phenotype, NHEJ-Competent Phenotype, Olaparib, synthetic lethality

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Sub-task: Regulatory Review & Approval USAMRMC ACURO (ACURO CA191071.e001, *approved*)

Specific Aim 1: Determine whether neutrophils (PMNs) deregulate DNA repair machinery of CRC tumors through HR inhibition and NHEJ upregulation.

Sub-Aim 1.1/Major Task 1: Establish the contribution of PMN activity to HR inhibition and NHEJ upregulation during CRC tumor development (*timeline 4-6 months*; *100% complete*).
Sub-task 1: To determine PMN contribution to NHEJ upregulation in human tumor xenograft models (*100% complete*)
Sub-task 2: To validate PMN contribution to HR inhibition and NHEJ upregulation in Azoxymethane (AOM)/DSS CRC model (*100% complete*)

Sub-Aim 1.2/Major Task 2: Establish NHEJ upregulation in CRC patients or lack thereof in IBD patients and healthy individuals (*timeline 3-4 months*; *90% complete*)
Sub-task 3: IRB exemption approval via Human Research Determination Form: Not Human Research (*approved to exempt IRB requirement for de-identified clinical specimens*)
Sub-task 4: To determine NHEJ upregulation in CRC patient specimens and CRC patient-derived colonoids (*90% complete*, in the process of generating patient organoids for the remaining 10%)

Milestone(s) Achieved by Jan 2021 *(4 months)*: Successfully establish the role of PMN activity in upregulating NHEJ in two CRC mouse models and in de-identified CRC patients (via tissue microarrays and OCT-preserved patient tissues)

Specific Aim 2: Determine whether PMN-induced NHEJ upregulation is required for DSB resolution, survival, and CRC development.
Sub-Aim 2.1/Major Task 3: Investigate whether NHEJ is required for DSB resolution and survival of HR-inhibited CRC tumors (*timeline 3 months, 80% complete*).
Sub-task 5: To establish the necessity of NHEJ in DSB resolution and survival of CRC tumors using NHEJ-deficient CRC cell lines (*80% complete)*.
Sub-Aim 2.2/Major Task 4: Explore the therapeutic potential of NHEJ inhibitors in inducing DSB accumulation and tumor cell apoptosis (*timeline 4-6 months*, *100% complete*).
Sub-task 6: To determine therapeutic potential of NHEJ inhibitors to delay CRC development in human xenograft models (*100% complete*).
Sub-task 7: To determine therapeutic potential of NHEJ inhibitors to delay CRC development in AOM/DSS models (*100% complete*).
Milestone(s) Achieved by Mar-April 2021 *(7 months)***:** Successfully establish the dependency of PMN-infiltrated CRC tumors on NHEJ activity to resolve DSB and to maintain tumor survival/development.

Specific Aim 3: Determine the therapeutic potential of targeted miR-inhibition in preventing progression from colitis to CRC (timeline 5-6 months, *<5% complete*).
Milestone(s) to be achieved by May-June 2022: Expect to determine whether suppressing miR-23a/miR-155 by antisense oligonucleotides (ASOs) can restore colonic tissue homeostasis and prevent the transition from colitis to CRC.

What was accomplished under these goals?

Specific objective #1: Establish the contribution of PMN activity to HR inhibition and NHEJ upregulation during CRC tumor development
Major activity #1: To determine PMN contribution to NHEJ upregulation in human tumor xenograft models
Methods & Significant Results:
a. Specific objective #1 has been addressed thoroughly using both *in vitro* setup and two distinct *in vivo* models. First, I employed a long-term, in vitro co-culture setup of CRC cell line HCT116 with PMN-MPs (up to 12 population doublings, PDs) and measured HR or NHEJ activity using HR or NHEJ plasmid reporter that indicated DNA repair activity upon GFP reconstitution signal. I confirmed that HR activity was rapidly and significantly inhibited throughout PMN-MP treatment, whereas NHEJ activity started to increase by PD5 and became markedly upregulated by PD12 (compared to control, non-treated cells) (**Figure 1A**, *unpublished*).
b. Similarly, I conducted NHEJ reporter assays on dissociated HCT116 xenografted tumors and established in vivo upregulation of NHEJ activity in tumor cells extracted from PMN-infiltrated (PMN+) tumors compared to those extracted from PMN-depleted (PMN-) tumors (Tumors in PMN- mice were treated with anti-Ly6G neutralizing antibody every 48 hours) (**Figure 1B,** *ref. Bui et al. 2021* ***Main Figure 4A***).
c. In addition, I conducted a detailed profiling of NHEJ repair expression profile and confirmed a transcriptional shift toward a set of NHEJ gene signature (8 out of 11 NHEJ genes upregulated) in CRC tumors infiltrated by PMNs (**Figure 1C** *ref. Bui et al. 2021* ***Main Figure 4F***). NHEJ upregulation in PMN+ tumors was further substantiated by the induction of Ku70 level and presence of Ku70 nuclear foci, as shown tumor sections (**Figure 1D**, *ref. Bui et al. 2021* ***Main Figure 4D-E***) and dissociated tumor cells (not shown). Noticeably, there was a pronounced temporal difference in NHEJ activity/gene expression in early PMN+ tumors (tumor onset, week 2 since graft) versus late PMN+ tumors (week 8 since graft), as late PMN+ tumors have much higher NHEJ activity and display more robust shift toward NHEJ gene signature.

d. To supplement the data obtained from HCT116 cell lines/xenografts, I reproduced key experiments in another CRC cell line SW48. These SW48 xenograft studies successfully reproduced that the presence of PMNs led to increased tumor growth/survival as well as HR inhibition and NHEJ upregulation *(not shown)*

Major activity #2: To validate PMN contribution to HR inhibition and NHEJ upregulation in Azoxymethane (AOM)/DSS CRC model.

Methods & Significant Results:

a. As a complementary approach for human tumor xenograft model, I further utilized an AOM /DSS (carcinogen/colitis-driven) CAC/CRC model. Briefly, for AOM/DSS CRC model, mice will be pretreated with a single dose of AOM (10 mg/kg body weight) followed by 3 cycles of DSS (each cycle = 7 days 2% DSS + 14 days normal water). We defined early tumors as polyps appearing around week 8-9 whereas late tumors are bigger tumors with aggressive morphologies during experimental endpoint at week 13. Similarly to our findings in HCT116 and SW48 xenografted tumors, I confirmed persistent HR inhibition (**Figure 1E** *ref. Bui et al. 2021* ***Supplemental Figure 5J***) as well as the time-dependent upregulation of NHEJ DSB repair activity in advanced, PMN+ AOM/DSS tumors (compared to early polyps/tumors) (**Figure 1F** *ref. Bui et al. 2021* ***Supplemental Figure 7A***).

b. I also confirmed robust transcriptional shift toward NHEJ machinery as well as induction of Ku70 foci formation (**Figure 1G** and **1D,** *ref. Bui et al. 2021* ***Supplemental Figure 7F***). *As a result, the major activities reported in specific objective #1 have established PMN-driven upregulation of NHEJ repair in terms of DNA repair activity and NHEJ gene signature in in vitro and two distinct in vivo models.*

Specific objective #2: Establish NHEJ upregulation in CRC patients

Major activity #3: The study was determined as "Not Human Research" and allowed to use un-identifiable patient samples.

Major activity #4: To determine NHEJ upregulation in CRC patient specimens and CRC patient-derived colonoids.

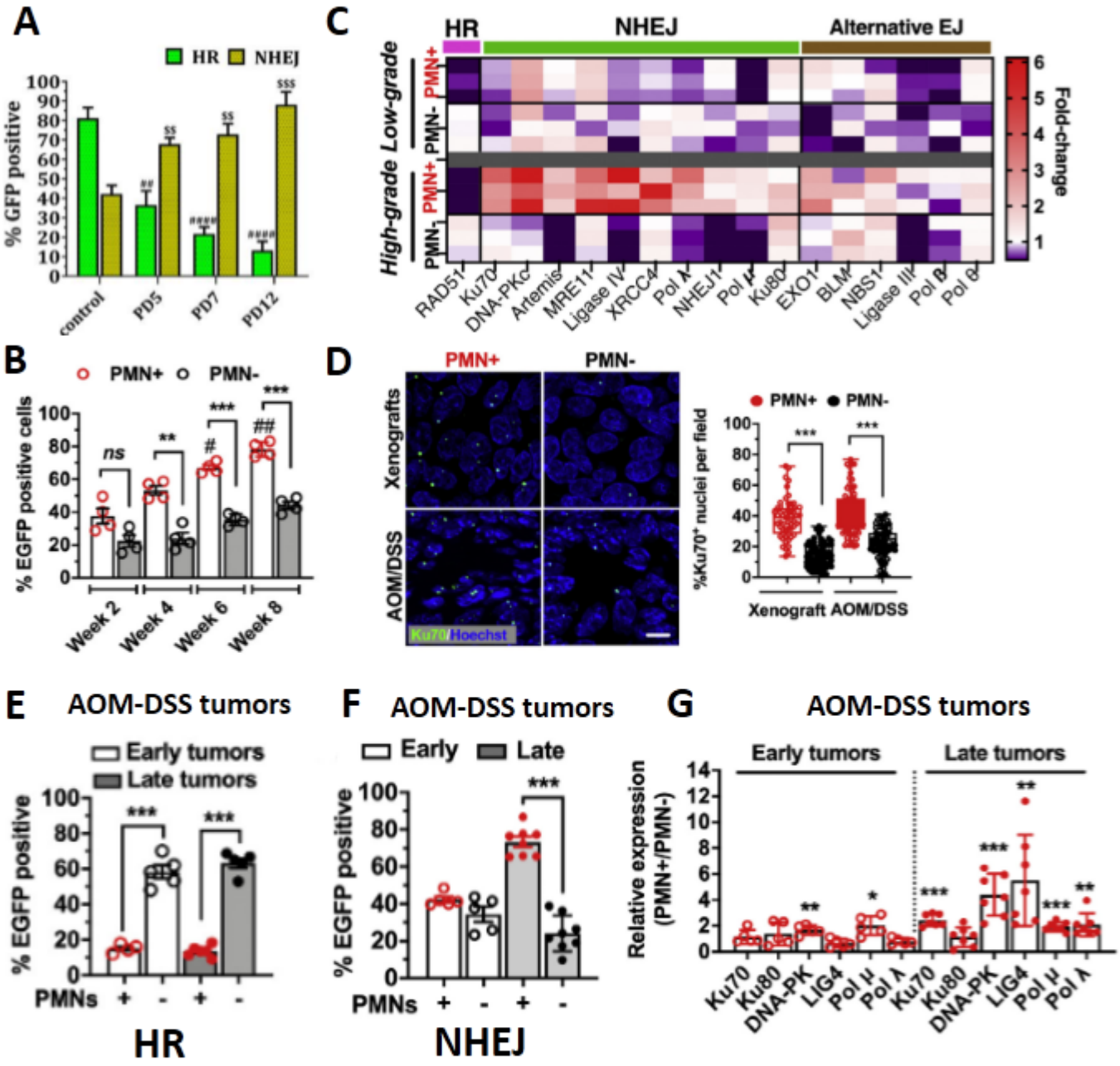
Methods & Significant Results:

a. For this major activity, I employed a tissue microarray of 60 CRC patients and confirmed increase in Ku70 foci formation (a direct index of NHEJ repair activity) as tumor grade increased. Most importantly, correlative analyses established a strict inverse correlation between the number of Ku70 foci and gH2AX foci (an indicator of DSB burden) (**Figure 2A** *ref. Bui et al. 2021* ***Main Figure 5H-5I***).

b. In collaboration with Dr. Guang-Yu Yang, we obtained un-identifiable patient samples from Northwestern University and performed transcriptional analysis. Similar to our findings in animal models, I found an upregulation of NHEJ gene transcription signature only in grade III CRC patient biopsies (**Figure 2B,** *ref. Bui et al. 2021* ***Main Figure 5A***), which were also enriched with neutrophil gene signatures (**Figure 2C,** *ref. Bui et al. 2021* ***Supplemental Figure 9A***).

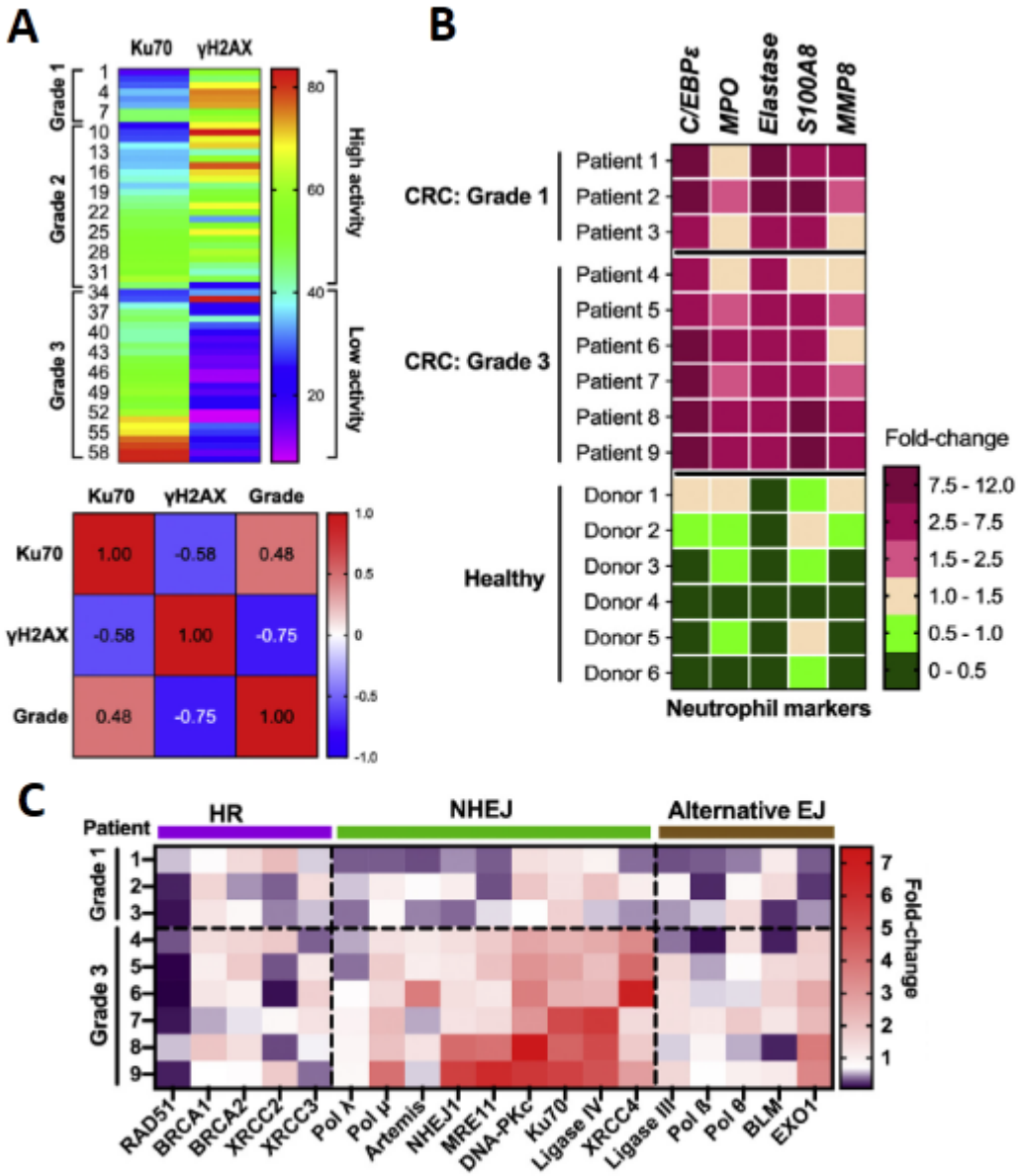
Unfinished goal(s): Although our team managed to obtain cryopreserved CRC tissues (by OCT), it has been challenging to create patient-derived organoids from these tissues. Tumor organoids failed to grow with these patient samples. We plan to obtain fresh and un-identified patient biopsies which have been well optimized for generation of organoids, which will be used to directly test NHEJ repair activity.

Figure 1



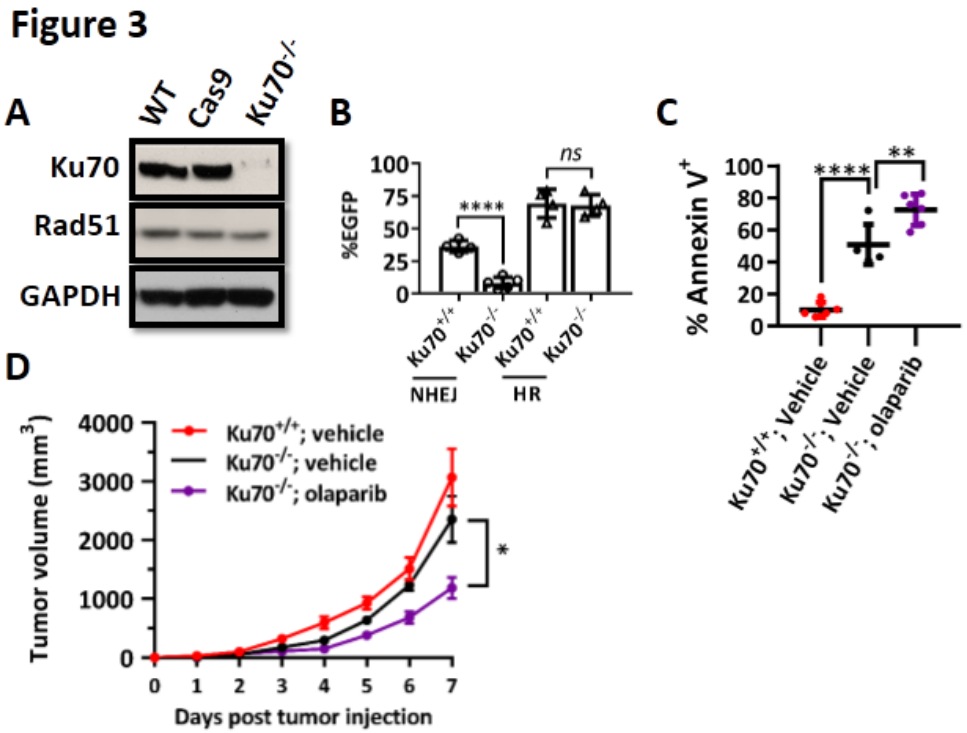
(A) DSB repair reporter assays of in vitro co-culture and **(B)** in tumor xenografts indicated NHEJ upregulation with long-term exposure to PMNs.
(C) Upregulation of NHEJ gene signature in high-grade CRC xenografted tumors exposed long-term to PMNs.
(D) Representative images *(left)* and quantification *(right)* of Ku70 foci induction in high-grade xenografts and colitis-induced AOM/DSS tumors.
(E-F) DSB repair reporter assay and **(G)** Transcriptional analyses of NHEJ factors in AOM-DSS tumors.

Figure 2



(A) Correlative analyses of Ku70 and gH2AX foci across 60 CRC patients of 3 grades obtained from patient tissue microarray.
(B) Enrichment of neutrophil gene signature in cohort of CRC patients at Northwestern Hospital
(C) Upregulation of NHEJ gene signature in 6 patients of grade 3.

Specific objective #3: Investigate whether NHEJ is required for DSB resolution and survival of HR-inhibited CRC tumors.
Major activity #5: To establish the necessity of NHEJ in DSB resolution and survival of CRC tumors using NHEJ-deficient CRC cell lines
Methods & Significant Results:
a. To address the necessity of NHEJ activity (and NHEJ regulators Ku70 and Mre11) in survival of CRC tumors in response to PMN activity, I have been working with the Northwestern Genetic Core to generate CRISPR/Cas9-mediated MRE11-/- (involved in both HR and NHEJ) and Ku70-/- (NHEJ repair-specific deficient) human HCT116 and SW48 CRC cell lines. Knock-out subclones for Ku70-/- cell lines were successfully selected (5 sub-clones) (**Figure 3A**, *unpublished*) and engrafted into Rag-/- mice to generate tumors. Following this set of in vivo study, we have confirmed that Ku70-/- tumors failed to upregulate NHEJ activity (**Figure 3B**, *unpublished*), exhibited reduced survival (**Figure 3C**) and hypersensitive to synthetic lethality by Olaparib (**Figure 3C** and **3D**, *unpublished*).
Unfinished goal(s): As for MRE11A-/-, we were not able to generate viable clones with complete MRE11 KO. We concluded that MRE11 is essential gene and its complete knockdown will compromise cell viability (even without neutrophils or DNA damage insults). We are planning to knock out other NHEJ regulators such as Ligase IV or Pol lamda or Pol mu (instead of Mre11) for this purpose.

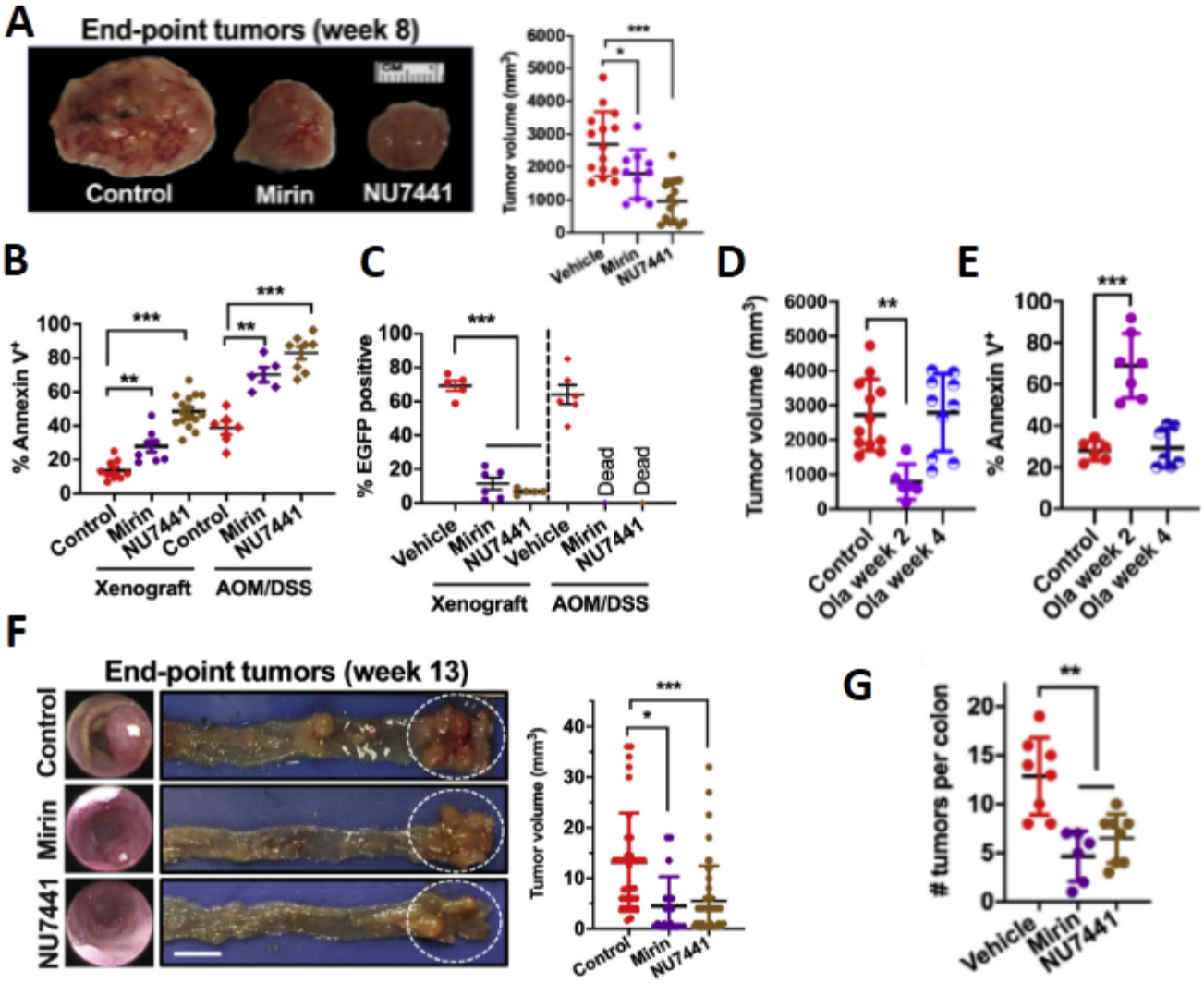


(A) Immunoblotting of Ku70 and RAD51 and **(B)** HR and NHEJ reporter assays in WT and in Ku70-/- CRISPR HCT116.
(C) Apoptotic analysis by Annexin V/PI and (**D**) Tumor volume measurements of Ku70-/- xenografted tumors in exposure to PMNs.

Specific objective #4: Explore the therapeutic potential of NHEJ inhibitors in inducing DSB accumulation and tumor cell apoptosis.
Major activity #6: To determine therapeutic potential of NHEJ inhibitors to delay CRC development in human xenograft models.
Methods & Significant Results:
a. To determine whether DSB resolution and CRC survival in the presence of PMNs is dependent on NHEJ, I administered a novel small-molecule NHEJ inhibitor (Mirin, 25 mg/kg) or vehicle control to CRC tumor xenografts. Here I confirmed that pharmacological inhibition of NHEJ by Mirin and a classic NHEJ inhibitor NU7441 (DNA-PKc inhibitor) substantially decreased tumor size (**Figure 4A,** *ref. Bui et al. 2021* ***Main Figure 7B-7C***) and increased apoptotic cell death despite prolonged PMN infiltration (**Figure 4B,** *ref. Bui et al. 2021* ***Main Figure 7J***). Reduction in NHEJ repair activity was confirmed by reporter assay (**Figure 4C** *ref. Bui et al. 2021* ***Main Figure 7H***) as well as by the diffuse localization of Ku70 in Mirin- and NU7441-treated tumor cells (*not shown*).
b. To further demonstrate that the survival of CRC tumors is correlative with the level of NHEJ activity in PMN-infiltrated tumors, I utilized the concept of synthetic lethality. By using HR and NHEJ reporter assays and transcriptional analyses, I already knew that early PMN+ tumors (~week 2) had low HR and low NHEJ activity, and as a result, early treatment with PARP inhibitor Olaparib (50 mg/kg/day, starting at week 2 – tumor onset) delayed tumor growth and induced cell death (**Figure 4D-E** *ref. Bui et al. 2021* ***Main Figure 6G, 6H, and 6I***). In stark contrast, starting Olaparib treatment at later stage PMN+ tumors (~week 4), where NHEJ activity is significantly upregulated, had no effect on tumor size and tumor apoptosis (**Figure 4D** and **4E** *ref. Bui et al. 2021,* ***Main Figure 6G, 6H, and 6I***).

Major activity #7: To determine therapeutic potential of NHEJ inhibitors to delay CRC development in AOM/DSS models.
Methods & Significant Results:
a. To test whether PMN-induced NHEJ upregulation facilitates the progression from colitis to CRC, NHEJ inhibitors Mirin and NU7441 were also used in the AOM/DSS CAC model. I confirmed that NHEJ inhibition starting from early tumor development (week 7) significantly prevented tumor formation as well as tumor progression, characterized by size, tumor number per colon, survival and DB burdens). (**Figure 4F**, **5G**, and **5B** *ref. Bui et al. 2021,* ***Main Figure 7E, 7F, and 7G)***

Figure 4



(A) Representative images *(left)* and quantification of tumor volumes *(right),* **(B)** Apoptosis analysis, and **(C)** NHEJ reporter assay of xenografted tumors treated with MRE11 (Mirin) and DNA-PKc inhibitors (NU7441).
(D) Quantification of tumor volumes and **(E)** Apoptosis analysis of tumors treated with Olaparib at early or late timepoints to induce synthetic lethality with HR deficiency driven by PMNs.
(F) Representative images *(left)* and quantification *(right)* of tumor volumes, and **(G)** the number of tumors per colon of colitis-induced AOM/DSS tumors.

What opportunities for training and professional development has the project provided?

During the first fiscal year of the training grant, the PI continues his PhD education under the supervision of Dr. Ronen Sumagin. He also had biweekly with Dr. Guang-Yu Yang as part of the co-mentoring system under the DoD Horizon Award. Importantly, the PI has been extensively by the mouse facility and a number of equipment facilities within Northwestern University to increase his expertise in animal surgery, high-resolution imaging, bioinformatics analysis, and genetic manipulation by CRISPR/Cas9 technology. On special occasion, the PI attended workshops on data mining and learned how to make use of the TCGA cancer database for his project. With the connections of Drs. Sumagin and Yang, the PI had access to individuals with expertise in making patient-derived organoids that will be used in the next steps of the project (next fiscal year).

Finally, the project has allowed the PI to attend a number of international, domestic and virtual conferences (Experimental Biology 2020, Digestive Disease Week - DDW, Guts & Bugs, PISA2020, Lurie Cancer Symposium) and present the works to different audiences. This funded project was also presented in several departmental and interdepartmental seminars and was well-received by Northwestern faculty and scientists.

How were the results disseminated to communities of interest?

The findings of the projects have been presented to scientists and lay audiences attending seminars within Northwestern University and conferences within the US (as international traveling is limited in 2020-2021).

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

I plan to complete Specific Aim 3 and the final Milestone of the funded project. This will bring forth a novel preventive "cure" to significantly diminish the risk of CRC of colitis/IBD patients. In addition, I plan to make use of the funding from the Horizon Award to attend several international/domestic conferences to present and spark the interest of scientific community in neutrophils and cancer inflammation.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

The work so far has followed the basic science-to-clinic trajectory where the PI and the research team have discovered novel biological processes in cancer-bearing animals and found similarities/correlations to specimens acquired from colon cancer patients. The findings emphasized that neutrophils – the most abundant white blood cells in human body – function as a double-edged sword during cancer progression.

At the early stage of cancer, these immune cells were sent to the tumor to kill cancer cells, rendering them vulnerable to lethal genomic damages. The work took advantage of this understanding and made use of Olaparib – an FDA-approved drug for breast cancer – to push the DNA damage beyond the tolerance point of early cancer cells. This attempt to repurpose a standard breast cancer drug for early colorectal cancer showed superior efficacy in immortalized human cancer cells and in mouse models of colorectal cancers. As a result, the work demonstrates strong generalizability and brings forth the promising use of Olaparib for colon cancer patients. This hopefully aims at the treatments for inflammation-driven cancers featuring neutrophil infiltration, which include colorectal, gastric, pancreatic, hepatocellular cancers, and inflammatory breast cancers.

We further found that as the cancer cells become more aggressive, they hijack neutrophils to counteract the cancer treatments. Following this trend, the work discovered that colorectal cancer cells that were educated by inflammatory neutrophils became highly adaptive and drug-resistant. The team has identified the biological factors that are responsible for this process. In addition, we revealed that the abnormal genomic repair following such event has provided the cancer cells with an "accelerated track" to tumor evolution. The resulting publication in *Gastroenterology* – the most highly-cited scientific journal in the field of gastroenterology and hepatology – has further highlighted the potential of the studied cancer drugs in shutting down cancer drug resistance and thus evoking the death of aggressive colon cancer cells.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Nothing to Report

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations
Report only the major publication(s) resulting from the work under this award.

Journal publications.

Journal publications:
1. Bui TM, Butin-Israeli V, Wiesolek HL, Zhou M, Rehring J, Yang GY, Wiesmuller L, Hanauer SB, Sebag J., Sumagin R. Neutrophils alter DNA repair landscape to impact survival and shape distinct therapeutic phenotypes of colorectal cancer. Gastroenterology. 2021 Jul; 161(1):225-238. PMID: 33753103 (*published, with acknowledgement of federal support*).

2. Bui TM, Yalom LK, Sumagin R. Tumor-associated neutrophils: orchestrating cancer pathobiology and therapeutic resistance. Expert Opinion on Therapeutic Targets. 2021 Jul; 25(7): 573-583. PMID: 34236924 (*published, with acknowledgement of federal support*).

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.

Conference abstracts:
1. Bui TM, Butin-Israeli V, Hanauer S, Sebag J, Sumagin R. Gastroenterology. Vol 160, Issue 3, 2021. Page S44-S45.
2. Bui TM, Butin-Israeli V, Hanauer S, Sebag J, Sumagin R. Inflammatory Bowel Diseases, Vol 27, Issue Supplement_1, Page S33.
3. Bui TM, Wiesolek HW, Butin-Israeli V, Sumagin R. Gastroenterology. Vol 158, Issue 6, 2020. Page S1044–S1045.
"Tu1288 Neutrophils regulate progression of colon cancer by temporally modulating the genomic landscape of DNA Damage Repair"

Presentations:
1. Trailblazer's Talk at Lurie Cancer Center Symposium, Northwestern University. 2021
"Neutrophils re-sculpture the DNA repair landscape to impact survival and shape distinct therapeutic phenotypes of colorectal cancer".
2. Gut&Bugs: Virtual GI Seminar, Rutger New Jersey Medical School. 2020.
"The Pathobiology of neutrophils in gut inflammation, injury, and carcinogenesis"

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

Other Products

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Name: Triet Bui
   Project Role: PI, Ph.D. student
   ORCID: 0000-0002-1092-9820
   Nearest person month worked: ~14
   Contribution to Project: Mr. Bui has executed leading role in project conceptualization, experimental designs, data curation and analysis. He also performed a majority of experiments related to the funded grant/project and was directly involved in generating the published manuscript.
   Funding Support: The CDMRP Horizon Award: Colorectal Cancer.

2. Name: Ronen Sumagin, Ph.D.
   Project Role: Supervisor; IACUC Protocol PI
   ORCID: 0000-0002-5689-1100
   Nearest person month worked: 8
   Contribution to Project: Dr. Sumagin plays the primary role in project conceptualization and funding acquisitions to support the project. He also assisted with experimental designs and supported key in vivo experiments. In addition, he provided insights into project construction and performed extensive edits of the original draft and the published manuscript.
   Funding Support: The Digestive Health Foundation, The American Cancer Society Research Scholar Award, The Crohn's & Colitis Foundation Senior Research Award.

3. Name: Veronika Butin-Israeli, Ph.D.
   Project Role: External Consultant
   ORCID: N/A
   Nearest person month worked: 4
   Contribution to Project: Dr. Butin-Israeli plays important role in conceptualization and experimental designs. She supported the PI with technical advice and provided insights into data analyses. She was involved in the extensive edits of the original draft and the published manuscript.
   Funding Support: N/A

4. Name: Guang-Yu Yang, M.D., Ph.D.
   Project Role: Co-mentor
   ORCID: 0000-0002-5987-7750
   Nearest person month worked: 2
   Contribution to Project: Dr. Yang provided insights and directly performed histological analyses of tumor specimens. He also played supporting role in project conceptualization and was involved in the edits of the published manuscript
   Funding Support: NCI R01 CA172431, NIDDK R01DK107767.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

Not applicable.

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Not applicable.

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

See attached published manuscripts.