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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.

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TABLE OF CONTENTS

1. INTRODUCTION:

Polymorphonuclear (PMN) leukocytes account for up to 70% and 25% of circulating immune cells in human and mice, respectively. IBDs, encompassing ulcerative colitis and Crohn's disease, as well as colitis-induced Colorectal Cancer (CRC) are characterized by recurring episodes of inflammation and tissue injury that are strictly associated with PMN pathologic activities. Unlike other tumor microenvironment that are often dominated by macrophages, CRC features *en masse* infiltration and prolonged PMN accumulation. Clinical observations indeed demonstrated that increased PMN numbers are further associated with stage IV cancer and are predictive of poor clinical outcomes.

In recently published works, we have reported that colitis- and CRC-associated PMNs respectively elicit severe tissue damage and anti-tumorigenic response during ulceration and at the early-stage of CRC. Under the current grant CA191071, we successfully established that PMNs shape the DSB-repair responses and impact CRC progression and resistance to DNA-repair targeted therapy. This final report summarized our findings that outline novel mechanism-based therapeutic interventions proposed to target aggressive/advanced CRC featuring PMN infiltration.

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

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 (*90% complete)*

Milestone(s) Achieved by Jan 2021 *(4 months)*: Successfully establish the role of PMN activity in upregulating NHEJ in two distinct 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 6 months, 90% 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 *(7-9 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, *20-30% complete*, *unable to finish due to emerging technical difficulties of long-term administration of ASO*).
Milestone(s) is not achieved due to timelines and technical difficulties: Consistent administration of expensive ASOs during long period of AOM/DSS model was very challenging and cannot yield reproducible outcomes.

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, including a xenograft model of human CRC cell line (HCT116) and a colitis-induced CRC model. First, an in vitro set of HCT116 CRC cell lines were treated with microparticle (MPs) from PMNs up to 12 population doublings. Measurement of HR and NHEJ activity using HR or NHEJ plasmid reporter confirmed that while HR activity was rapidly and significantly inhibited throughout PMN-MP treatment, NHEJ activity started to increase by PD5 and became markedly upregulated by PD12 (compared to control, non-treated cells) (**Figure 1A**). Second, I performed the same NHEJ reporter assays on single tumor cells dissociated from xenografted tumors of PMN-intact (PMN+) and PMN-depleted (PMN-) mice. I established that there exists an upregulation of NHEJ activity in tumor cells in response to persistent PMN accumulation and activity (**Figure 1B**, *ref. Bui et al. 2021* ***Main Figure 4A***). Quality control for PMN depletion in PMN- tumors by anti-Ly6G neutralizing mAb was shown in *Bui et al. 2021,* ***Supplemental Figure 1A***.
b. In addition, detailed transcriptional profiling of genes involved in classical and alternative NHEJ machinery was performed on xenografted tumors where I demonstrated that PMN+ tumor cells exhibited a transcriptional shift toward an 8-gene NHEJ gene (**Figure 1C** *ref. Bui et al. 2021* ***Main Figure 4F***). To further validate upregulation of NHEJ factors, I performed immunoblotting and immunofluorescence staining of Ku70 NHEJ regulator, proving the induction of Ku70 protein and the formation of active Ku70 foci (**Figure 1D-E**, *ref. Bui et al. 2021* ***Main Figure 4D-E***). All these critical findings were further reproduced in the xenografts derived from a different CRC cell line (SW48) (*not shown*).
c. Conclusion: *Major activity #1* has been accomplished and published in *Bui et al. 2021*.

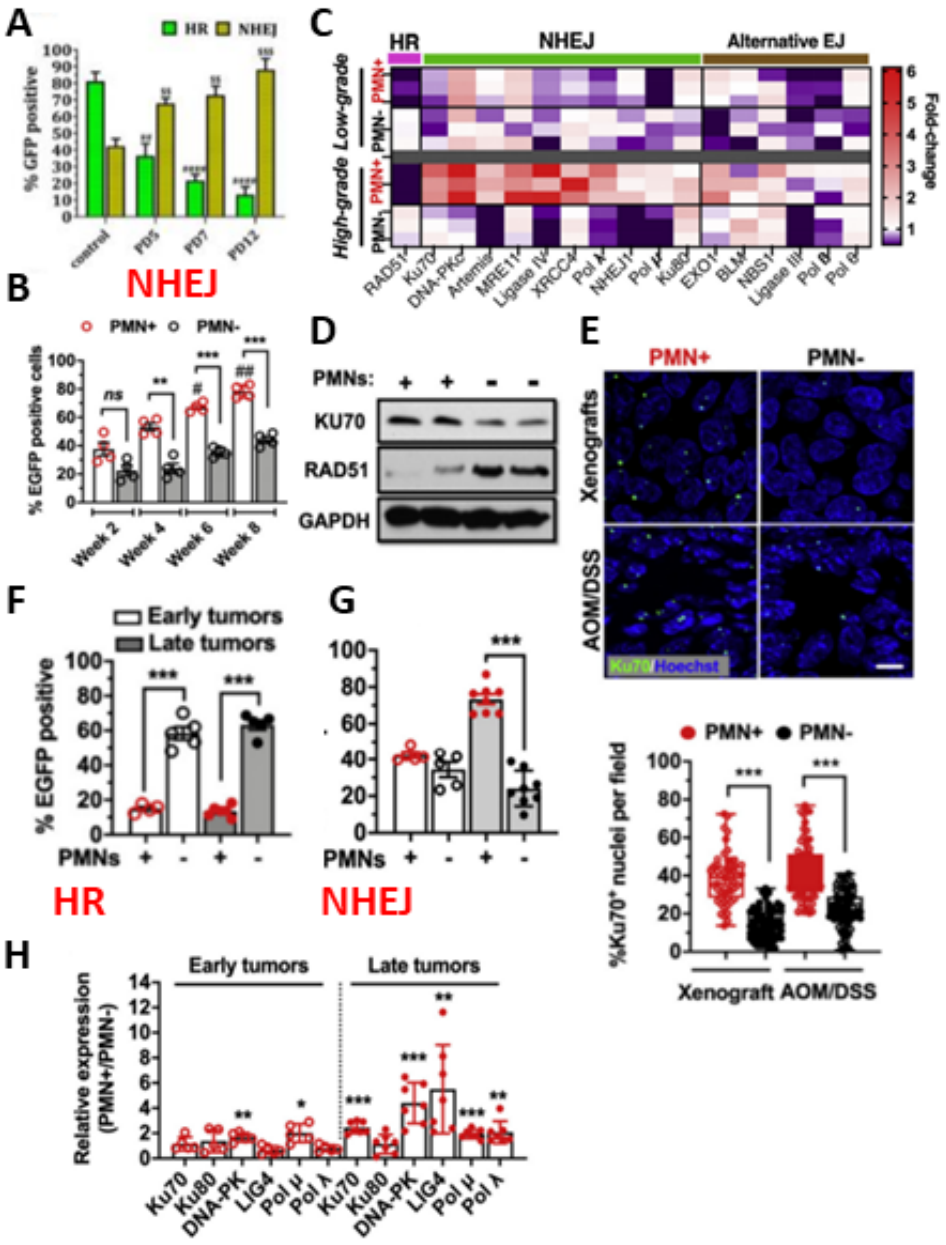

Figure 1. Prolonged PMN activity contributes to HR inhibition and NHEJ upregulation. A. Plasmid-based HR/NHEJ reporter assays based on EGFP reconstitution indicated NHEJ upregulation and HR inhibition in response to long-term co-culture with microvesicles from PMNs. **B.** NHEJ reporter indicating DSB repair activity and **C.** Expression analyses of NHEJ regulators in PMN+ versus PMN- tumors. **D.** Immunoblotting and **E.** Immunofluorescence of Ku70 indicating increased Ku70 protein levels and foci formation in PMN+ tumors as compared to PMN-. Quantification of %Ku70^{+} nuclei was shown below **E** where statistical comparison was done by unpaired student's t-test. **F.** HR and **G.** NHEJ reporter assays of AOM/DSS-induced tumors indicating suppressed HR activity and increased NHEJ activity in PMN+ tumors. **H.** Expression analysis of NHEJ genes (based on qRT-PCR) indicating increased NHEJ gene expression in PMN+ tumors compared to PMN-. Unpaired student's t-test was performed when two samples were directly compared. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, ns, non-significant, when compared between PMN+ and PMN-. #, $p < 0.05$; ##, $p < 0.01$, when compared advanced tumors with early tumors.

Major activity #2: To validate PMN contribution to HR inhibition and NHEJ upregulation in Azoxymethane (AOM)/DSS CRC model.
Methods & Significant Results:
a. A colitis-associated CRC (CAC) model featuring massive PMN infiltration was performed in parallel with the aforementioned xenograft models. Briefly, I injected AOM carcinogen (12.5 mg/kg body weight) and treated the mice with 3 cycles of DSS (each cycle = 7 days 2% DSS + 14 days normal water). In this model, we defined early tumors (week 8-9) as low-grade based on well-differentiation score and advanced tumors aggressive morphologies and poor differentiation scores (week 13-14) as high-grade.
b. Like our findings in HCT116 and SW48 xenografted tumors, I confirmed persistent HR inhibition (**Figure 1F** *ref. Bui et al. 2021* ***Supplemental Figure 5J***) as well as the upregulation of NHEJ DSB repair activity and robust NHEJ gene expression in advanced, PMN+ AOM/DSS tumors (compared to early polyps/tumors) (**Figure 1G and 1H** *ref. Bui et al. 2021* ***Supplemental Figure 7A***).

c. Conclusion. *Major activity #2* has been accomplished and published in *Bui et al. 2021*.

Per this objective, I have established PMN-driven upregulation of NHEJ repair in terms of DNA repair activity and NHEJ gene signature using 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, a tissue microarray of 60 CRC patients was purchased from BioMax and stained for assessment of Ku70 foci formation (a direct index of NHEJ repair activity). I performed 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 (Co-mentor of the current grant), we obtained un-identifiable patient samples from Northwestern University Biorepository and performed transcriptional analysis. Similar to our findings in animal models, NHEJ gene transcription signature was enriched only in grade III CRC patient biopsies (**Figure 2B,** *ref. Bui et al. 2021* ***Main Figure 5A***), which were in parallel enriched with neutrophil gene signatures (**Figure 2C,** *ref. Bui et al. 2021* ***Supplemental Figure 9A***). We further performed immunofluorescence staining in these patients and confirmed ongoing Ku70 foci formation in high-grade patients (**Figure 2D,** *ref. Bui et al. 2021*).

c. We were unable to obtain fresh biopsies from CRC patients directly after surgery due to the rarity of cases during COVID19. OCT-frozen biopsies obtained via Biorepository were not able to form cancerous colonoids for measurement of NHEJ activity. As a result, we primarily used Ku70 foci levels in tumor tissue sections as an indication of NHEJ activity in CRC patients.

d. Conclusion: *Specific Objective #2* has been 90-95% accomplished and published in *Bui et al. 2021*.

Per this objective, I have established increased NHEJ activity by means of Ku70 foci formation in two independent sources of CRC patient tissues.

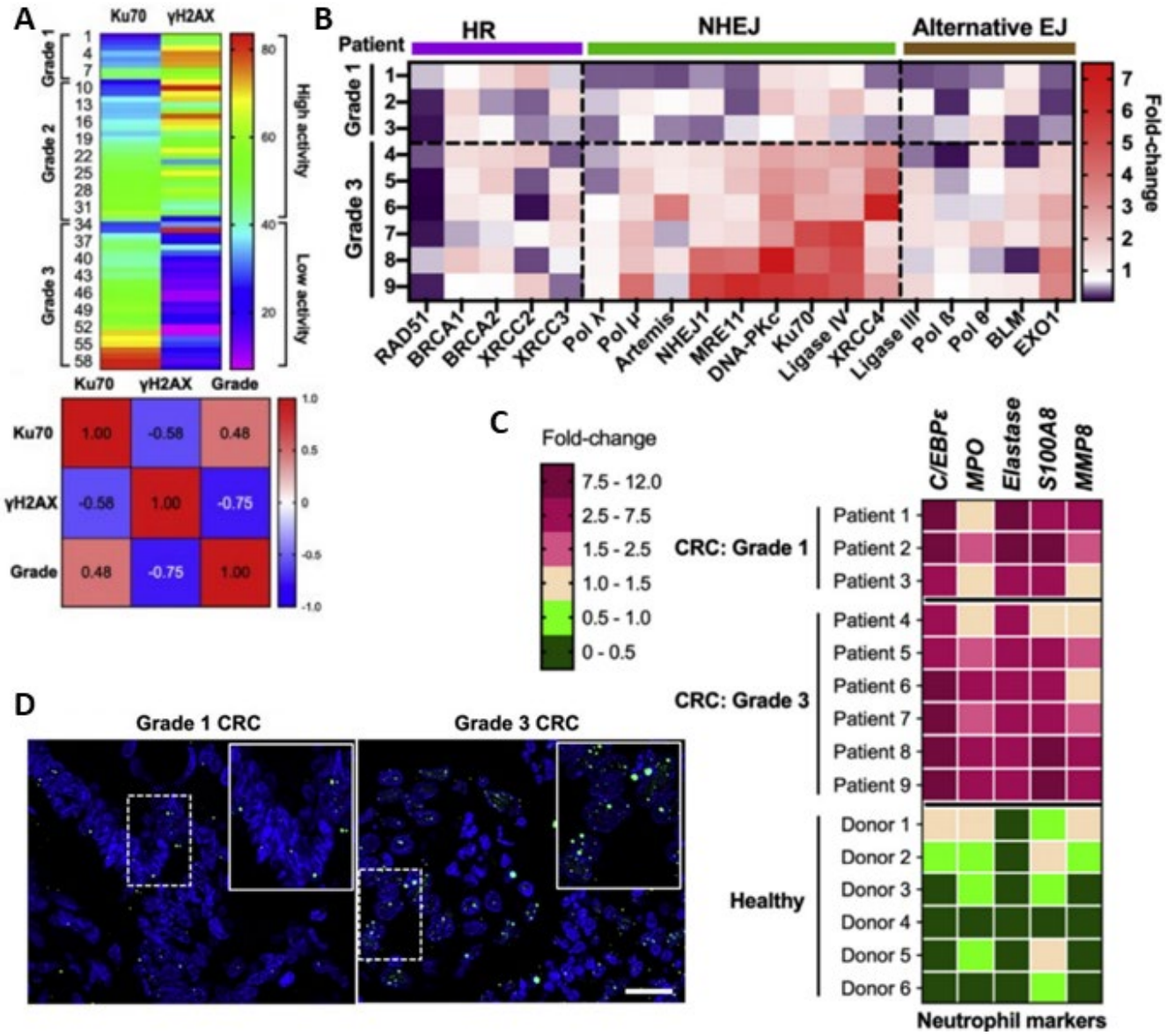

Figure 2. Robust NHEJ activation in high-grade CRC patients. A. Heatmap representation depicting paired Ku70 and gH2AX foci distribution among 59 patients with CRC, grades 1–3. Assigned values of the heatmap are means of percent positive nuclei for each patient biopsy averaged across 10 to 15 randomly acquired fields of view. High versus low activity was defined as 40% to 100% and 0% to 39%, respectively. Below was Pearson's correlation analysis of Ku70 foci, gH2AX foci, and tumor grade. **B.** Heatmap representation depicting relative expression of genes involved in HR, NHEJ, and alternative EJ DSB repair pathways. Fresh CRC biopsied tissues grade 1 (n = 3 patients) and grade 3 (n = 6 patients) were analyzed by qPCR for HR or NHEJ-regulating genes. **C.** Heatmap representation show expression analyses by qRT-PCR of canonical PMN genes in cancer biopsies (grade 1, n = 3; grade 3, n = 6) and in healthy tissue (n = 6). Expression values of the indicated genes were normalized to averaged value of 3 control healthy biopsied tissue. **D.** Representative images of Ku70 foci in grade 1 (left) and grade 3 (right) CRC-biopsied tissues. Insets show magnified view of the outlined tissue regions (scale bar, 25 μm).

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 was 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 after the utilization of one sgRNA or dual sgRNA (**Figure 3A**,) and engrafted into Rag-/- mice to generate tumors. Following this set of in vivo study, we have confirmed that Ku70-/- tumor cells failed to for Ku70 foci and suffer from tremendous DSB burdens (**Figure 3B**,), exhibited reduced survival (**Figure 3C**) and hypersensitive to synthetic lethality by Olaparib (**Figure 3C**,). However, the knockout of Ku70 severely compromised cellular fitness and nuclear morphology even during passage in tissue cultures (**Figure 3D**), partly due to the key role of Ku70 in maintaining telomere stability and ensuring proper cellular mitosis. Thus, we did not include the Ku70-/-tumors data in our publication in *Bui et al. 2021.*

b. Unfinished goal(s): As for MRE11A-/-, we were not able to generate viable clones with complete MRE11 KO (*not shown*). We concluded that MRE11 is an essential gene, and its complete knockdown will compromise cell viability (even without neutrophils or DNA damage insults). Due to the strong impact of NHEJ gene knockout on basal cellular fitness, we only addressed the necessity of NHEJ activity in DSB resolution and CRC survival using small-molecule inhibitors, not by CRISPR cell lines where the gene(s) are permanently deleted.

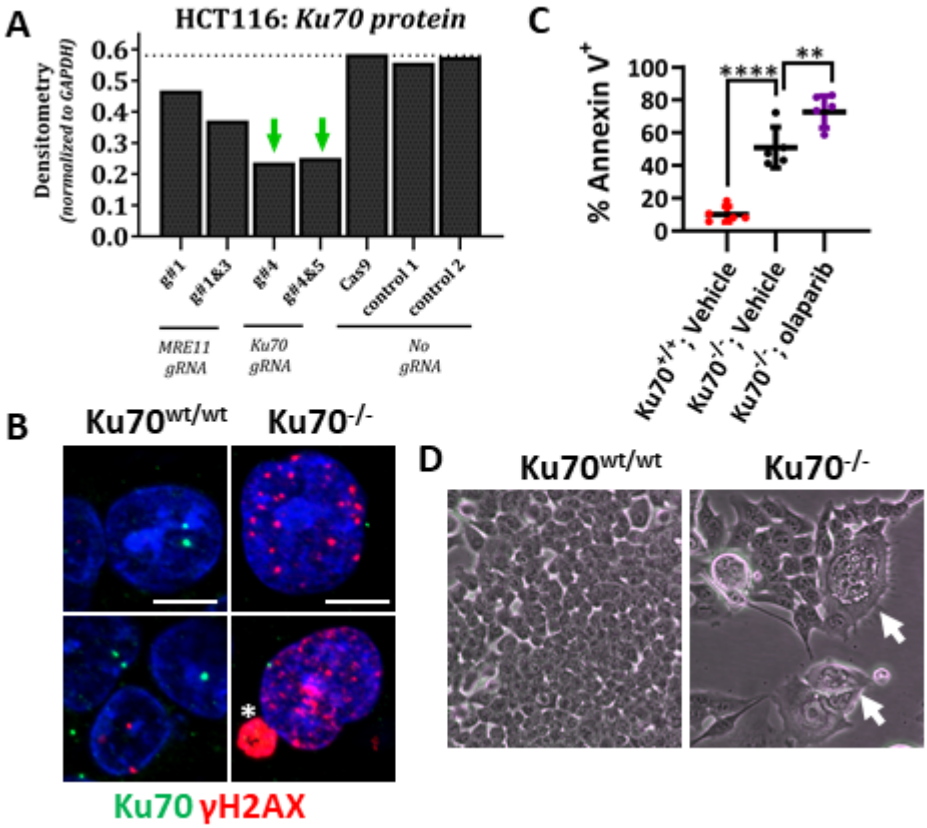

Figure 3. Permanent Ku70 knockout induces massive DSB burden and aberrant nuclear morphology even under homeostasis. A. Immunoblotting densitometry analysis of CRISPR clones knocked out by a single or two guide RNAs (gRNA) targeting MRE11A or Ku70 genes in HCT116 CRC cell line. The analysis indicated decent knockout in initial clones. **B.** Immunofluorescence analyses of $Ku70^{wt/wt}$ (left) and $Ku70^{-/-}$ subclones indicated absence of Ku70 foci formation in $Ku70^{-/-}$ subclones while showed drastic DSB burdens and nuclear blebbing (asterisk) even in homeostatic culturing condition. Scale bar, 5 μm. **C.** Engraftment of $Ku70^{wt/wt}$ and $Ku70^{-/-}$ HCT116 into PMN-intact mice resulted in tumors with high apoptotic ratio even without treatment. The treatment with PARP1 inhibit Olaparib further heightened tumor cell apoptosis. Comparison between three groups was done by one-way ANOVA with post hoc multiple comparison test. All data are presented as mean ± SEM **, $p < 0.01$; ****, $p < 0.0001$. **D.** Bright-field microscopy showed normal cell size and cell morphology of $Ku70^{wt/wt}$ HCT116 while confirmed aberrant giant-cell morphology with multiple nuclei (per cell) in $Ku70^{-/-}$ HCT116, indicating the unstable genome and cellular fitness in the absence of Ku70.

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 two small-molecule NHEJ inhibitors (Mirin, MRE11 inhibitors, 25 mg/kg and NU7441, DNA-PKc inhibitor, 10 mg/kg) or vehicle control to CRC tumor xenografts. Here I confirmed that pharmacological inhibition of NHEJ by Mirin and NU7441 substantially decreased tumor size (**Figure 4A,** *ref. Bui et al. 2021* ***Main Figure 7B-7C***) and increased apoptotic cell death considering 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***). On the other hand, 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:
c. 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 DSB burdens). (**Figure 5A**, **5B**, **5C** and **5D** *ref. Bui et al. 2021,* ***Main Figure 7E, 7F, and 7G)***. Also, the effects of NHEJ inhibition in AOM/DSS-induced tumors were more drastic than that observed in xenografted tumors, partly due to a more robust infiltration of PMNs to AOM/DSS-induced tumors (**Figure 5E** *ref. Bui et al. 2021*).

d. Conclusion: *Specific Objective #4* has been 100% accomplished and published in *Bui et al. 2021*. Here we prove that NHEJ activity is required for DSB resolution and CRC survival in the presence of PMNs. We also showed the potential of NHEJ inhibitors in attenuating tumor burdens in two distinct CRC models.

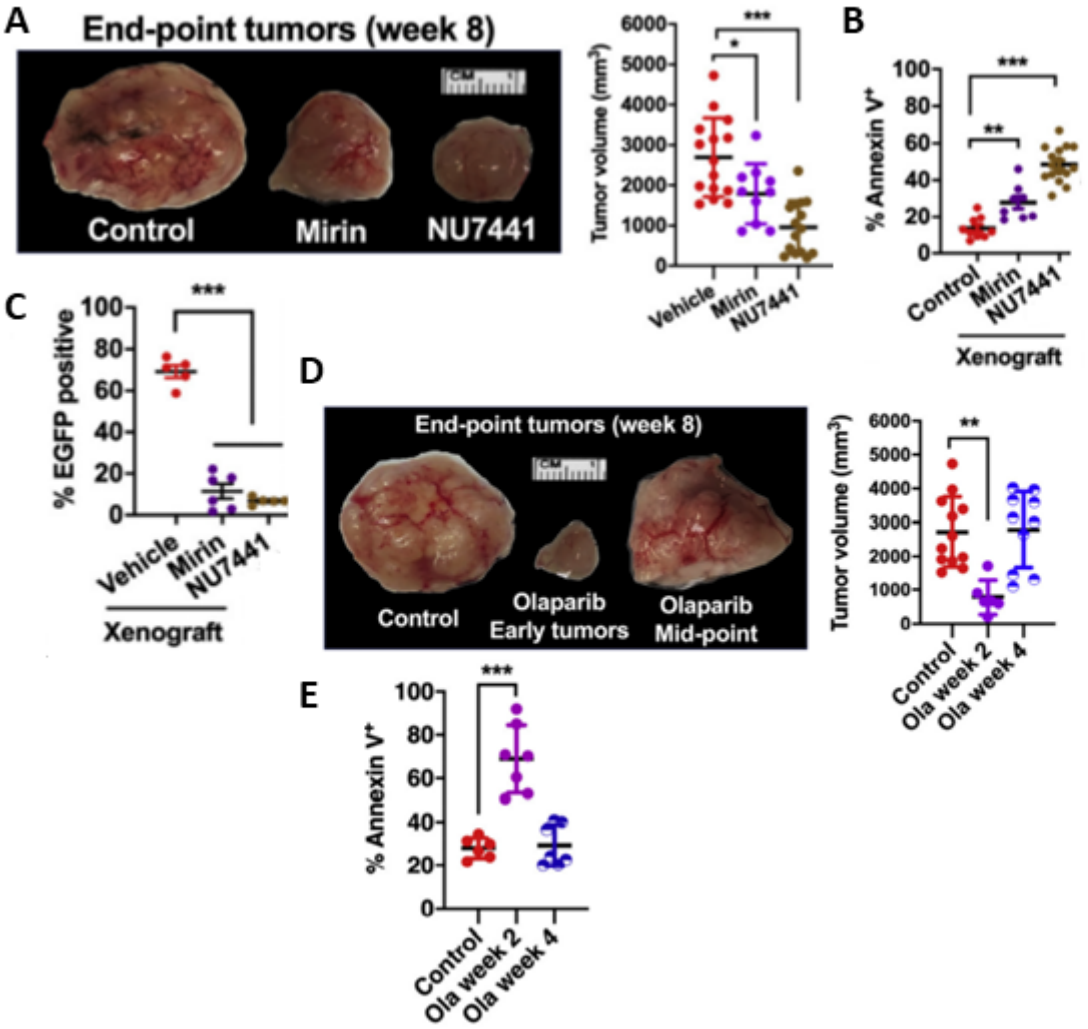

Figure 4. NHEJ inhibitors effectively delayed CRC development in human xenograft model. Pharmacological inhibitors (NU7441, 10 mg/kg and Mirin, 25 mg/kg, biweekly) were used to prevent NHEJ upregulation in HCT116 xenograft model. **A.** Images (left) and volume quantification (right) of treated tumors (Vehicle, n = 15 tumors; Mirin, n = 10 tumors; NU7441, n = 15 tumors). **B.** Tumor cell apoptosis in human xenografts indicating heightened cell death upon NHEJ inhibition. **C.** Analyses of NHEJ activity in tumors dissected at endpoint indicating complete shutdown of NHEJ-mediated DSB repair. **D.** Images (left) and volume quantification (right) and **E.** Apoptotic analysis of tumors treated with PARP1 inhibitor Olaparib at early timepoint (week 2, $NHEJ^{low}$ tumors) and later timepoint (week 4, $NHEJ^{high}$ tumors) indicating synthetic lethality effects on $NHEJ^{low}$ tumors and resistance in $NHEJ^{high}$ tumors (Vehicle, n = 12 tumors; Olaparib week 2, n = 7 tumors; Olaparib week 4, n = 10 tumors). Comparison between three groups was done by one-way ANOVA with post hoc multiple comparison test. All data are presented as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

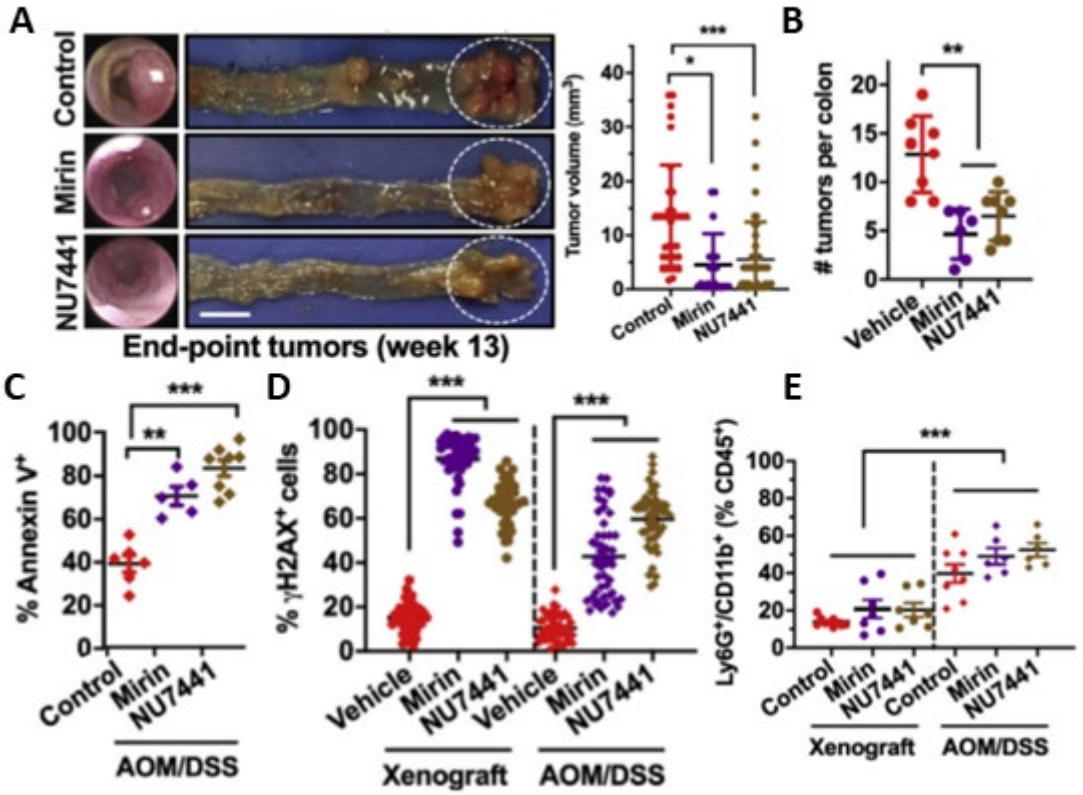

Figure 5. NHEJ inhibitors effectively delayed CRC development in colitis-induced AOM/DSS model. Pharmacological inhibitors (NU7441, 10 mg/kg and Mirin, 25 mg/kg, biweekly) were used to prevent NHEJ upregulation in HCT116 xenograft model. **A.** Images (left) and volume quantification (right) and **B.** tumor numbers of treated tumors (Vehicle, n = 15 tumors; Mirin, n = 10 tumors; NU7441, n = 15 tumors). For tumor volume: vehicle, n = 36 tumors; Mirin, n = 19; NU7441, n = 49 tumors. For total tumor numbers per colon: n = 6-8 mice/condition. **C.** Tumor cell apoptosis in colitis-induced AOM/DSS tumors indicating heightened cell death upon NHEJ inhibition. **D.** DSB levels (γH2AX foci) (n = 3–5 tumors per condition with >50 fields per condition analyzed) following each treatment in xenograft and AOM/DSS models. **E.** Flow cytometry analyses of PMN infiltration in advanced human xenografts and murine AOM/DSS-induced tumors with/with treatment with NHEJ inhibitors (for xenografts: n = 7–11 mice per condition; for AOM/DSS: n = 6–8 mice per condition). Comparison between three groups was done by one-way ANOVA with post hoc multiple comparison test. All data are presented as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Specific objective #5: Determine the therapeutic potential of targeted miR-inhibition in preventing progression from colitis to CRC
Major activity #8: 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.

Methods & Significant Results:
a. We proved that there is a strong and persistent infiltration of PMNs during a colitis-to-CRC transition (**Figure 6A** and **6B**, not published). Neutrophils sorted out from the AOM/DSS-induced tumors expressed high levels of miR-23a and miR-155 (**Figure 6C**), as we previously reported in acutely injured/inflamed colon tissues (*ref Butin-Israeli and Bui et al., Journal of Clinical Investigation, 2019*).

b. We attempted to administer antisense oligonucleotides (ASOs) against these two miRNAs during the course of AOM/DSS. However, the cost for each preparation of ASO was inhibitory at the time. In addition, with the constant turnover of PMNs (shown in **Figure 6A**), temporary inhibition of miR-23a/miR-155 at low dose seemed not to yield meaningful effects.

c. Conclusion: *Specific Objective #5* has not been accomplished. There were multiple attempts by our team to address the technical feasibility but were not very successful. However, the majority body of work from the grant CA191071 has been accomplished and resulted in publication in the first-tier journal within the field of gastroenterology (*Gastroenterology*, impact factor: 22.682).

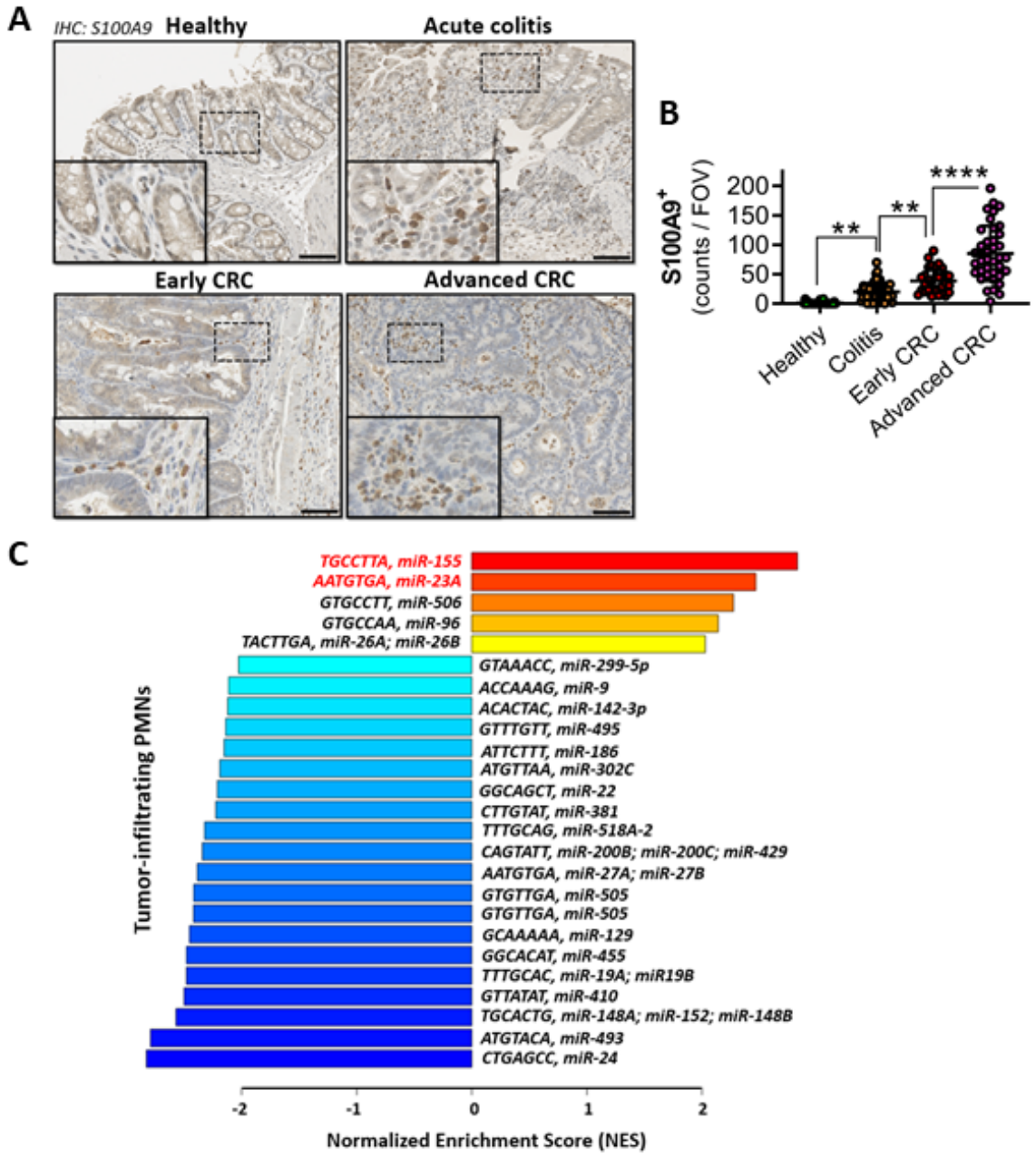

Figure 6. Constant PMN infiltration and tissue accumulation posed remarkable challenge in inhibition of miR-23a and miR-155 with single administration of ASOs. A. Immunohistochemical (IHC) staining and **B.** Quantification of S100A9 in healthy, inflamed colitis regions, and tumor regions in early and advanced CRC in AOM/DSS model (n=3-4 mice per condition). Inset shows focused images of PMN tissue accumulation and interaction with the colon or cancerous epithelium. **C.** Enrichment score of miRNAs in FACS-sorted tumor-infiltrating PMNs (analyzed by total-input RNAseq) indicating top enrichment of miR-155 and miR-23A strands. Comparison between three groups was done by one-way ANOVA with post hoc multiple comparison test. All data are presented as mean ± SEM. **, $p < 0.01$; ****, $p < 0.0001$. The constant infiltration and turnover of miR-155^{high}/miR-23a^{high} PMNs rendered complete inhibition of these two miRNAs by ASOs very challenging. It required daily administration of ASOs, whose synthesis and costs are very expensive.

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

Throughout two years of training under the Horizon Award, the PI has finished his PhD education under guidance of Dr. Ronen Sumagin. In addition, he also had biweekly with Dr. Guang-Yu Yang to further learn about clinical pathology as part of the co-mentoring system under the DoD Horizon Award. Importantly, the PI has been extensively trained in the mouse facility and learned to operate a number of capital equipment (confocal microscopy Nikon A1R, spinning-disk confocal microscopy, FACS Melody and FACS Aria, immunohistochemistry staining platform, etc.) in different facilities within Northwestern University. In addition, the PI has attended a number of workshops and seminars to increase his expertise in animal surgery, high-resolution imaging, bioinformatics analysis of RNA-sequencing data, and genetic manipulation by CRISPR/Cas9 technology. On special occasion, the PI attended workshops on career development and NIH pre/doctoral training seminars.

In parallel, the project has allowed the PI to attend a number of international, domestic and virtual conferences (Experimental Biology 2020-2022, Digestive Disease Week - DDW, Guts & Bugs, PISA2020, Lurie Cancer Symposium, American Association of Immunology, American Association of Cancer Research meetings) 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. In parallel, the DoD has chosen the current project as CDMRP Research Highlight for funded grant. *https://cdmrp.army.mil/prcrp/research_highlights/22bui_highlight.aspx*

Finally, the publication resulted from the funded grant has enable the PI to graduate with his PhD and proceed to pursue his post-doctoral research in breast cancer heterogeneity at Dana-Farber Cancer Institute and Harvard Medical School in the coming year.

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).

In parallel, the DoD has chosen the current project as CDMRP Research Highlight for funded grant. *https://cdmrp.army.mil/prcrp/research_highlights/22bui_highlight.aspx*

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to Report

4. IMPACT:

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?

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?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

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:

- **Publications, conference papers, and presentations**

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*).

3. Bui TM, Sumagin R. Neutrophils and micronuclei: An emerging link between genomic instability and cancer-driven inflammation. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2022. Mar 18;824:111778. doi: 10.1016/j.mrfmmm.2022.111778. PMID: 35334355 (*published, with acknowledgement of federal support*).

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"

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

Research Highlight by CDMRP
https://cdmrp.army.mil/prcrp/research_highlights/22bui_highlight.aspx

Research Highlight by American Cancer Society
https://www.cancer.org/research/acs-research-highlights/colon-and-rectal-cancer-research-highlights/taming-gemini-immune-cells-called-tumor-infiltrating-neutrophils.html

Research Highlight by Northwestern News
https://news.feinberg.northwestern.edu/2021/04/study-discovers-dual-function-of-neutrophils-in-cancer-cells/

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Name: Triet Bui, Ph.D., MSCI
   Project Role: PI
   ORCID: 0000-0002-1092-9820
   Nearest person month worked: ~20
   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: 14
   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: 6
   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: 4
   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?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

References

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.e15. doi: 10.1053/j.gastro.2021.03.027. Epub 2021 Mar 19.

2. **Bui TM**, Yalom LK, Sumagin R. Tumor associated neutrophils orchestrate tumor immunology and cancer therapeutic resistance. Expert Opinion on Therapeutic Targets. 2021. Jul;25(7):573-583. doi: 10.1080/14728222.2021.1954162. Epub 2021 Jul 16.

3. **Bui TM**, Sumagin R. Neutrophils and micronuclei: An emerging link between genomic instability and cancer-driven inflammation. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2022. Mar 18;824:111778. doi: 10.1016/j.mrfmmm.2022.111778.

4. Butin-Israeli V, **Bui TM**, Wiesolek HL, Mascarenhas LA, Lee JJ, Adam SA, Goldman RD, Beyder A, Wiesmuller L, Hanauer SB, Sumagin R. Neutrophil-induced genomic instability impedes resolution of inflammation and wound healing. Journal of Clinical Investigation. 2019. Feb 1;129(2):712-726. doi: 10.1172/JCI122085. Epub 2019 Jan 14.