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14. ABSTRACT

This application proposes to inhibit the Discoidin Domain Receptor 1 (DDR1) both with a single agent and in conjunction with drugs targeting RAS-MEK-ERK as a new possible treatment for Pancreatic Ductal Adenocarcinoma (PDAC). We hypothesize that DDR1 mediate the crosstalk between mutant Kras "addicted" PDAC cells with collagen, and thereby activate signaling pathways that promote tumor cell survival and malignancy (MEK resistance). Thus, disrupting DDR1 function by pharmacological or genetic means may attenuate PDAC pro-survival/fibrotic pathways and enhance therapeutic efficacy drugs targeting Kras-driven (MEK) signaling networks. In our studies, we demonstrated that silencing of DDR1 enhances the sensitivity of PDAC cells to Trametinib, a MEK inhibitor. We characterized a novel selective DDR1 kinase inhibitor for its ability to dampen DDR1 activation by collagen. This inhibitor in combination with Trametinib reduced the proliferation of two PDAC human cell lines only in the presence of collagen, suggesting that DDR1 activation may synergize the MAPK pathway to confer resistance to MEK inhibition. These results are consistent with data indicating that DDR1 is critical for the development and progression of PDAC.

15. SUBJECT TERMS
Pancreatic cancer, discoidin domain receptors, collagen, drug resistance,

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Table of Contents

Page

1. Introduction……. 4-5

2. Keywords……. 5

3. Accomplishments……..……... 5-13

4. Impact……...…… 13

5. Changes/Problems...…..…… 13-14

6. Products…….……..…..……. 14

7. Participants & Other Collaborating Organizations…… 15

8. Special Reporting Requirements…… 15

9. Appendices…… N/A

1. INTRODUCTION

Pancreatic Ductal Adenocarcinoma (PDAC) is the ninth most common cancer but its dismal survival rate elevates it to the third leading cause of cancer-related death in the United States, and is on track to become the second most common cause of cancer related death before 2020. Chemotherapy and radiation therapy have little impact on PDAC, leaving surgery as the most effective treatment. Unfortunately, only ~20% of patients that undergo surgery survive past five years.

One of the hallmarks of PDAC is the intense pro-fibrotic response (collagen deposition). The extensive fibrosis in PDAC occupies a large area of the tumor mass, and is characterized by a dense matrix rich in interstitial collagen, hyaluronic acid, high number of stromal cells, hypovascularity, and hypoxic conditions, all of which have been postulated to contribute to the aggressive nature of PDAC tumors and their resistance to cytotoxic therapies. Collagen, the major component of fibrotic stroma, has been shown to elicit some of the pro-malignant effects of the desmoplastic stroma on the PDAC cells, including migration, invasion, survival, and drug resistance via regulation of epithelial-to-mesenchymal transition (EMT), protease production, and activation of TGF-β signaling, just to mention a few. These data point to an important pro-malignant effect of collagens in PDAC that may facilitate disease progression and resistance to treatment. Consistent with this notion, recent studies have postulated that therapies targeting the PDAC stroma or the crosstalk between PDAC cells and the collagen matrix may represent promising approaches for the treatment of PDAC.

The Discoidin Domain Receptors (DDRs) are unique RTKs because they are the only kinase receptors that recognize collagens as their ligands. Upon collagen binding, DDRs activate signaling pathways that regulate cell proliferation, migration, survival, and differentiation. Importantly, DDRs are emerging as new players in cancer progression because they mediate the interactions of tumor cells with their immediate collagen environment. The DDR family comprises two distinct members, DDR1 and DDR2, which undergo receptor autophosphorylation in response to fibrillar collagens (DDR1 and DDR2) or non-fibrillar collagen IV (DDR1), with distinctive activation kinetics and downstream effectors. DDRs regulate tumor cell migration and invasion, cell survival, and drug resistance. DDRs have been implicated in drug resistance to targeted therapies, and thus targeting DDRs may aid to increase drug sensitivity. Thus, DDRs are good candidates for mediating the pro-survival effects of the PDAC fibrotic stroma.

New therapeutic approaches are urgently required to target the pro-oncogenic signaling networks activated in PDAC. In excess of 90% of PDAC harbor oncogenic Kras mutations that drive tumorigenesis and disease progression. Mutant Kras signals via the downstream components the RAS/RAF/MEK/ERK pathway, and major efforts have been invested to target Kras and its effectors. Direct inhibition of Kras has proven to be challenging. Thus, most approaches target downstream effectors such as MEK1/2 for the treatment of Kras-ERK driven PDAC. However, single agents targeting Ras and/or RTK downstream effectors have yielded disappointing results. In the case of MEK inhibitors, feedback reactivation of ERK or PI3K signaling was shown to be mediated in part by compensatory RTK activation pathways (kinome reprograming) leading to MEK inhibitor resistance.

Kinome reprograming is a process in which cancer cells can rewire their signaling networks to restore ERK activity and override the actions of MEK inhibition by reactivating MEK2, resulting in c-myc degradation, and transcriptional activation of several RTKs. Importantly, studies in breast cancer cells showed that DDR1 is one of the RTKs that appears to compensate for the inhibition of MEK1/2 in AZD6244-resistant triple negative breast cancer cells. Consistently, downregulation of DDR1 restored MEK inhibitor sensitivity. As we described in our application, we searched for resistance to AZD6244, a MEK1/2 inhibitor, in several human PDAC cell lines harboring mutant Kras using the COSMIC database (http://cancer.sanger.ac.uk/cosmic), and compared the extent of MEK inhibitor resistance in the cells in relation to the expression levels of DDR1. Interestingly, we observed a direct relationship between higher levels of DDR1 and resistance to AZD6244, suggesting that DDR1 expression is associated with MEK inhibitor resistance. MiaPaCa-2 cells, a mesenchymal PDAC cell line, which only express DDR2, is the most sensitive to AZD6244. Based on this preliminary, yet interesting association, and the potential role of DDR1 in kinome reprograming we hypothesized that DDR1, but not DDR2, expression may be part of the genomic make up of Kras-mutated PDAC tumors displaying greater MEK inhibitor resistance. Thus, a combination of DDR1 and MEK inhibition may produce synthetic lethality in MEK-dependent mutated Kras-driven PDAC tumors thriving within the collagen-rich environment.

2. KEYWORDS

Discoidin domain receptors, pancreatic cancer, receptor tyrosine kinases, collagen, chemotherapy, drug resistance, MEK inhibitors, kinome reprograming

3. ACCOMPLISHMENTS

- **What were the major goals of the project?**

Specific Aim 1: Evaluate the Role of DDRs in Resistance to MEK Inhibition and their Effectiveness as Potential Therapeutic Targets in the KPC Mouse Model.

> **Major Task 1:** Evaluate roles of DDRs in KPC cell lines in *in vitro* studies
>
> **Major Task 2:** Evaluate role of DDRs in KPC cell lines in the orthotopic syngeneic mouse model
>
> **Major Task 3:** Evaluate the therapeutic effect of a pan-DDR kinase inhibitor (Compound A) in the KPC model of pancreatic cancer

Specific Aim 2: Establish the Anti-Tumor Effect of Single or Combinatorial Lethality of DDR1 Inhibition on Human PDX and Matched Organoid Cultures.

> **Major Task 4:** Evaluate Compound A in Organoids from Primary Tumor Lines Derived from PDAC Patients (PDX, currently in hand)
>
> **Major Task 5:** Evaluate the Therapeutic Response of human PDX to DDR plus MEK

Inhibition

- **What was accomplished under these goals?**

1) Major activities:

Specific Aim 1: Evaluate the Role of DDRs in Resistance to MEK Inhibition and their Effectiveness as Potential Therapeutic Targets in the KPC Mouse Model.

Task 1. During this period we continue investigating the sensitivity of Kras-mutated mouse KPC and human PDAC cell lines to pharmacological or genetic inhibition of DDRs and MEK inhibitor response.

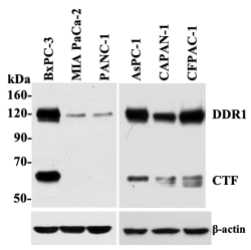

Figure 1. Expression of DDRs in selected human PDAC cell lines. CTF, C-Terminal fragment of DDR1.

As we reported, we selected PDAC cell lines based on their profile of endogenous DDR1 expression (**Figure 1**). MiaPaCa-2 and PANC1 cells, which are reported to be sensitive to the MEK inhibitor AZD6244, express low levels of DDR1, as we showed in the original application. In contrast, resistant cell lines usually express high levels of DDR1. To examine the role of DDR1 in MEK inhibitor response, we decided to utilize MiaPaCa and PANC-1 with modulated expression of recombinant DDR1. Thus, during the first funding period, we generated human PDAC MiaPaCa and PANC-1 cells overexpressing DDR1.

Figure 2 shows the expression and activation of two DDR1 isoforms, DDR1a and DDR1b, in the human MiaPaCa cell line. These stable transfectants were generated to express wild type (WT) DDR1a or DDR1b or DDR1 mutants. Specifically, the K618A (DDR1a) and K655A (DDR1b) mutants, which are unable to display kinase activity (referred to as kinase dead, KD), and the mutant R105A, which dampens binding to collagen, resulting in lack of ligand-stimulated activation.

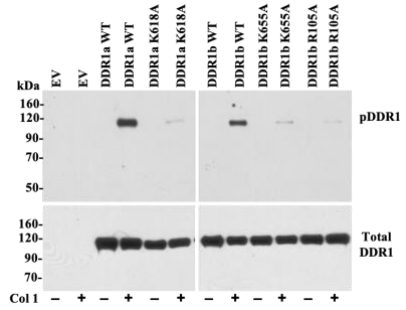

Figure 2. Expression and activation of DDR1 (DDR1a or DDR1b) wild type (WT) and mutants in human MiaPaCa-2 cells. EV, empty vector control cells. pDDR1: phosphorylated DDR1.

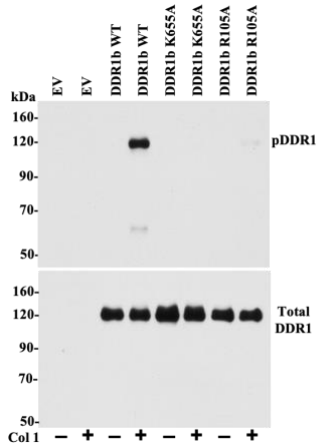

Figure 3. Expression and activation of DDR1b wild type (WT) and mutants in human PANC-1 cells. EV, empty vector control cells. pDDR1: phosphorylated DDR1.

Figure 3 shows the expression and activation of DDR1b, WT and K655A, and R105A mutants in human PANC-1 cells. As shown in **Figures 2 and 3**, WT DDR1 isoforms were activated in response to collagen I (COL1), a ligand for DDR1. In contrast, the K618A, K655A, and R105A mutants were not responsive, as expected. These figures also show that the level of total recombinant DDR1 expression in the transfectants is comparable.

Next, we tested the response of the human PDAC cell lines to MEK inhibition. To this end, we used Trametinib (Selleck Chemicals Cat. #: S2673), an FDA approved MEK inhibitor. First, we verified that the inhibitor inhibits constitutive ERK phosphorylation in MiaPaCa and ASPC-1 cells. These studies showed complete ERK1/2 phosphorylation by Trametinib, as expected (**Figure 4**).

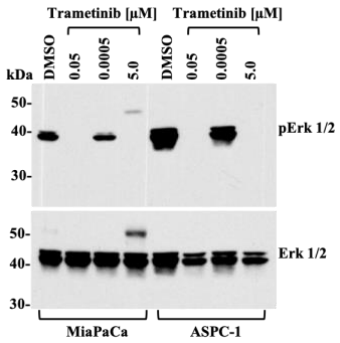

Figure 4. Trametinib inhibits ERK phosphorylation in human MiaPaCa-2 and ASPC-1 cells.

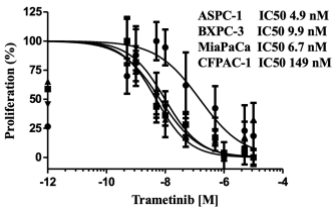

Figure 5. Sensitivity of human PDAC cell lines to Trametinib. BXPC-3 (▼), ASPC-1 (▲), MiaPaCa (■), and CFPAC-1 (●)

Next, we determined the sensitivity of ASPC-1, BXPC-3, CFPAC-1 and MiaPaCa-2 cell lines to Trametinib. With the exception of MiaPaCa-2 cells, the other cell lines express high levels of DDR1 (Fig. 1). The cells were incubated for 72 h with various doses of Trametinib in complete media and then analyzed with the MTT assay. No collagen was added in this experiment. As shown in **Figure 5**, CPFAC-1 cells were highly resistant to Trametinib with an IC50 of 149 nM. In contrast, ASPC-1, BXPC-3, and MiaPaCa-2 cells displayed a comparable IC50: ~5-10 nM. From these preliminary results we conclude that sensitivity to Trametinib is not directly related to the levels of DDR1 expression in the absence of collagen stimulation.

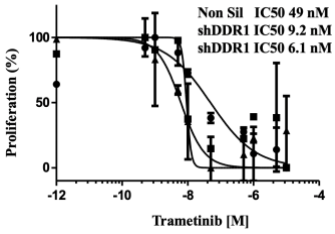

Figure 6. Effect of DDR1 silencing on Trametinib sensitivity in collagen I-treated CFPAC-1 cells. Non-silencing control (●), shDDR1 44b (■), and shDDR1 45b (▲).

Because CFPAC-1 cells express DDR1 and are highly resistant to Trametinib when compared to the other tested PDAC cell lines, we utilized two DDR1 silencing shRNAs (shDDR1 44b and 45b) to downregulate DDR1 in these cells. These stable cell lines with control non-silencing shRNA or shDDR1 were treated with various Trametinib concentrations in the presence of collagen I to activate the receptor. As shown in **Figure 6**, DDR1 downregulation with two different shRNA constructs reduced the IC50 of Trametinib by 5-7-fold compared to cells with the non-

silencing shRNA, suggesting that expression of DDR1 is associated with increased resistance to Trametinib, at least in CFPAC-1.

Based on our results with CPFAC cells, which under our conditions exhibit the most resistance phenotype to Trametinib (IC_{50} = 149 nM), we decided to use these cells to test whether the selective DDR1 inhibitor RO8807 in combination with Trametinib will reduce the resistance of CFPAC cells to MEK inhibition. R08807 is a potent DDR1 inhibitor (binding IC_{50} = 0.026 μM, phosphorylation IC50 = 0.018 μM) developed by Roche and provided to our lab via a Material Transfer Agreement. In contrast, R08807 displays a binding IC50 = 2.3 μM for human DDR2 (Roche data). As shown in **Figure 7**, using MiaPaCa-2 cells overexpressing recombinant human DDR1b (these cells are DDR1 deficient), R08807 inhibits the activation of DDR1b in response to collagen I with an IC_{50} between 0.05-0.1 μM, under our conditions. R08807 was also effective inhibiting the phosphorylation of endogenous DDR1 after collagen I stimulation in other PDAC cell lines including CFPAC-1, PANC-1 cells (data not shown).

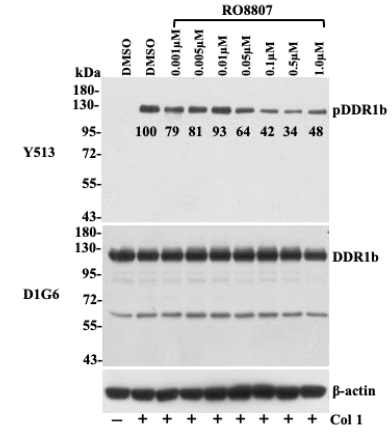

Figure 7. RO8807 inhibits collagen-induced DDR1b phosphorylation in human MiaPaCa-2 cells.

Next, we tested the combination of Trametinib with RO887 in various human PDAC cell lines expressing endogenous DDR1. ASPC-1 and CFPAC-1 cells display mutated Kras whereas BXPC-3 harbors a wild type Kras. The cells were seeded in 96-well plates in medium supplemented with 2% FBS. The next day, half of the medium was replaced with fresh medium containing 0.1 μM (final concentration) of R0887 in DMSO, alone or in combination with Trametinib (at the IC_{25} concentration foe each cell line, as indicated in the legend of Fig. 8). Each well also received 20 μg per ml of collagen type I to induce DDR1 activation. Control wells received DMSO or 0.02N acetic acid. After 3 days, cell proliferation was evaluated using the XTT assay as described by the manufacturer. As depicted in **Figure 8**, in the absence of collagen, Trametinib alone inhibited the proliferation of ASPC-1 and BXPC-3 cells by 47 and 30% when compared to controls. As expected, CFPAC-1 cells were resistant to Trametinib. R0887 alone had minimal impact on cell proliferation, suggesting that DDR1 inhibition alone is not sufficient to alter the proliferation of these cell lines. The combination of Trametinib and RO887,

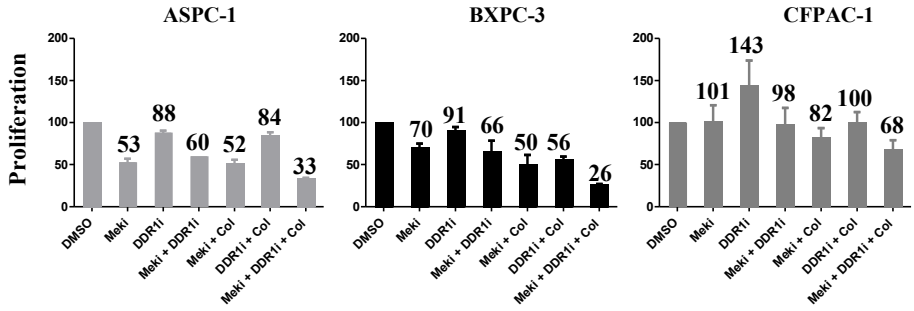

Figure 8. Effect of Trametinib (MEKi) and RO887 (DDR1i), alone or in combination, on cell proliferation in the presence or absence of collagen I. Trametinib was used at IC_{25} concentrations (determined earlier), as follows: 1.2 nM for ASPC-1, 2.2 nM for BXPC3 and 24 nM for CFPAC-1 cells. RO887 was used at a concentration of 0.1 μM. Numbers on top of the bars indicate the percentage of cell proliferation relative to the controls (100%)

in the absence of collagen, did not alter the effects of Trametinib alone.

In the presence of collagen, sensitivity of Trametinib was not altered in ASPC-1 cells but collagen + Trametinib elicited a stronger effect (~20%) on cell proliferation over Trametinib alone in BXPC-3 and CFPAC-1 cells, suggesting that collagen synergized with Trametinib to inhibit cell proliferation. R0887 inhibited growth of BXPC-3 by ~40% only in the presence of collagen. Thus, in wild type Kras-expressing PDCA cells DDR1 activation is associated with inhibition of cell proliferation. These studies also revealed that the combination of Trametinib and RO887 increased lethality in the presence of collagen in both ASPC-1 (from 60 to 33%) and BXPC-3 (from 66 to 26%) cells when compared to the combination in the absence of collagen. Thus, inhibition of activated DDR1 by RO887 increased the sensitivity of these cells to Trametinib.

Task 2: Due to our focus on Task 1, we have not conducted the studies in the mouse model, as of yet. Therefore, we have Nothing to Report at this junction.

Task 3: This task was intended to test the DDR1 inhibitor in the KPC model. The goal was to determine the role of DDR1 in PDAC progression using the genetically engineered mice that develop PDAC in a similar process that the human disease, leading to tumor growth and metastases. To define the role of DDR1 in PDAC progression, we crossed DDR1-null (*DDR1*$^{-/-}$) mice into the *KPC* model. DDR1$^{-/-}$ animals have been previously characterized and do not display evident defects in pancreas development. To study the effects of DDR1 ablation in PDAC, *KPC* and *DDR1*$^{-/-}$*; KPC* mice were aged until moribund, then sacrificed. The results of these comprehensive studies, supported in part by this award, were recently published in the ***American Journal of Pathology, Volume 190, Issue 8, August 2020, Pages 1622-1624; PMID: 32339496***.

To report these comprehensive results, we provide here the Abstract of the publication, which summarizes the major findings: From Ruggieri et al AJP 190, 1622-1624, 2020.

Pancreatic ductal adenocarcinoma (PDA) and chronic pancreatitis are characterized by a dense collagen-rich desmoplastic reaction. Discoidin domain receptor 1 (DDR1) is a receptor tyrosine kinase activated by collagens that can regulate cell proliferation, migration, adhesion, and remodeling of the extracellular matrix. To address the role of DDR1 in PDA, Ddr1-null (Ddr1$^{-/-}$*) mice were crossed with the Kras*$^{G12D/+}$*; Trp5*$^{R172H/+}$*; Ptf1a*$^{Cre/+}$ *(KPC) model of metastatic PDA. Ddr1*$^{-/-}$*; KPC mice progress to differentiated PDA but resist progression to poorly differentiated cancer compared with KPC control mice. Strikingly, severe pancreatic atrophy accompanied tumor progression in Ddr1*$^{-/-}$*; KPC mice. To further explore the effects of Ddr1 ablation, Ddr1-/- mice were crossed with the Kras*$^{G12D/+}$*; Ptf1a*$^{Cre/+}$*neoplasia model and subjected to cerulein-induced experimental pancreatitis. Similar to KPC mice, tissue atrophy was a hallmark of both neoplasia and pancreatitis models in the absence of Ddr1. Compared with controls, Ddr1*$^{-/-}$ *models had increased acinar cell dropout and reduced proliferation with no difference in apoptotic cell death between control and Ddr1*$^{-/-}$ *animals. In most models, organ atrophy was accompanied by increased fibrillar collagen deposition, suggesting a compensatory response in the absence of this collagen receptor. Overall, these data suggest that DDR1 regulates tissue homeostasis in the neoplastic and injured pancreas.*

These studies demonstrated the critical role that DDR1 plays in the progression of PDAC using a relevant mouse model that resembles in many aspects the development and progression of human PDAC. *DDR1* loss significantly reduced the tumor mass and also the progression of the tumor to a metastatic phase, as shown by the reduced metastases in the liver (**Fig. 9**). Therefore, although we were not able to test the DDR1 inhibitor at this junction, as originally proposed in Task 3, our studies provides demonstrate the therapeutic relevance of targeting DDR1 in PDAC. We are planning to conduct the studies with a DDR1 inhibitor in the near future. For this, we need to overcome the issues with mice, as we described in our previous report. Nevertheless, the published studies provide a new insight on the role of DDR1 in PDAC that support its potential as therapeutic target.

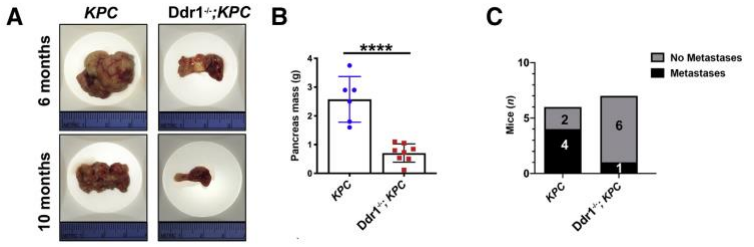

Figure 9. *DDR1* ablation reduces tumor growth and delays pancreatic ductal adenocarcinoma (PDA) progression. **A**: Gross anatomy of pancreata from *Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$; *Ptf1a*$^{Cre/+}$ (KPC) and *Ddr1*-null (*Ddr1*$^{-/-}$); KPC mice aged 6 and 10 months. B: Pancreatic mass of all KPC and *Ddr1*$^{-/-}$; KPC mice. C: Graphical representation of mice with macrometastases to the liver. From Ruggieri *et. al.*, AJP 190, *1622-1624*, 2020.

Importantly, our results uncovered a novel role of DDR1 in regulation of the tumor microenvironment in PDAC, namely the ability of DDR1 to influence the expression of the stromal fibrillar collagen within the tumors. Indeed, ablation of *Ddr1* resulted in a significant increase on the levels of collagen deposition within the stroma, as indicated by Trichrome staining (**Fig. 10A**). Second harmonic generation (SHG) microscopy of the tissues demonstrated that the overall SHG integrated intensity was higher in *Ddr1*$^{-/-}$*;* KPC mice consistent with the findings of enhanced Trichrome staining (**Fig. 10B**). Collectively, these data suggest that DDR1 is essential for pancreatic tissue homeostasis and consequently plays a role in support of PDAC progression

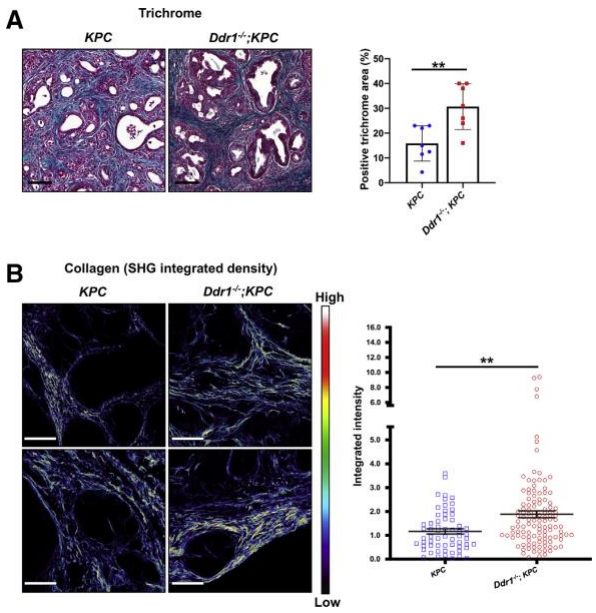

Figure 10. Ablation of *Ddr1* alters the fibrotic response in PDAC progression in the KPC model. **A**: Representative Trichrome staining (blue) of *Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$; *Ptf1a*$^{Cre/+}$ (KPC) (left panel) and *Ddr1*$^{-/-}$); KPC mice (right panel). Quantitative analysis of the percentage of positive Trichrome staining per tissue area shows *Ddr1*$^{-/-}$; KPC mice have significantly more fibrosis (graph on right). **B**: Representative images showing second harmonic generation (SHG) microscopy signatures from KPC mice (left column) and *Ddr1*$^{-/-}$; KPC (right column). Color scale indicates intensity of polymerized collagen fibers showing warm colors for high (white) and cold for low (black) SHG intensity levels. Quantitative analysis of polymerized collagen signatures (graph on right) obtained as SHG signal intensities divided by positive SHG signal areas (e.g., integrated intensity). Values presented are relative (e.g., normalized) to KPC. Data are expressed as means ± SD. n > 3 (5 for KPC and 6 for *Ddr1*$^{-/-}$; KPC) (A); n = 9 (3 selected areas from an H&E slide from each condition and 3 images/regions) (**B**). **P < 0.01. Scale bars = 100 mm (**A** and **B**). From Ruggieri *et. al.*, AJP 190, *1622-1624*, 2020.

through the interacts with collagens, possibly by promoting a fibrotic response that is conducive to tumor progression. Altogether, our data indicate that DDR1 is an important signaling factor during pancreatic injury, tumor development, and tumor progression. These studies show that DDR1 is necessary for tissue homeostasis after an injury whether it be physical or oncogenic. Indeed, the ablation of DDR1 in pancreatic disease models in the current study had an impact on the surrounding desmoplasia, which usually exhibited a more persistent stromal response and altered rates of ECM remodeling. Thus, the overexpression of DDR1 and the extensive collagen production in pancreatic diseases suggest that DDR1 is a putative therapeutic target. However, these results also suggest that long-term systemic inhibition of DDR1 could induce organ atrophy in an environment of chronic cell stress, a factor that should be taken into account when targeting DDR1.

Specific Aim 2: Establish the Anti-Tumor Effect of Single or Combinatorial Lethality of DDR1 Inhibition on Human PDX and Matched Organoid Cultures.

Tasks 4 and 5: These studies are still pending. Due to our focus on the *in vitro* studies with the human cell lines attempting to identify the combination of DDR1 inhibition with MEK inhibition, these studies were delayed. The progress in this Aim was also delayed by our studies to establish the role of DDR1 in the KPC model, as described above. However, we will be pursuing these studies with our collaborator, Dr. Howard Crawford.

2) Specific objectives:

The objectives during the period covered by this report (Aug 2019- Aug 2020) were:

a. Investigate the effects of Trametinib or Gemcitabine in combination with DDR1 inhibitors on the proliferation and survival of PDAC cell lines. Examine effects of DDR1 activation and signaling (MAPK and PI3K/AKT pathways.

b. Examine the role of DDR1 in PDAC progression in the KPC model of PDAC. Characterize the pathological consequences of DDR1 ablation on PDAC growth and metastasis. Determine effects on the tumor microenvironment, particularly on the development of fibrosis, as determined by collagen levels and organization.

3) Significant results or key outcomes:

In this period we learn:

1. PDAC cell lines display differential sensitivity to the MEK inhibitor Trametinib, which is unrelated to the levels of DDR1 expression.

2. A new inhibitor to DDR1, RO887, is effective in inhibiting collagen-stimulated DDR1 in PDAC cell lines.

3. Inhibition of DDR1 has no impact on PDAC proliferation

4. Inhibition of activated DDR1 by RO887 further increased the sensitivity of Trametinib-sensitive ASPC-1 and BXPC-3 cells but not the Trametinib-resistant CFPAC-1 cells, under the conditions tested.

5. DDR1 is an important signaling factor during pancreatic injury, tumor development, and tumor progression because loss of DDR1 inhibited PDAC progression.

4) Other achievements:

Although not directly supported by this award, in a series of related studies using MiaPaCa-2 cells expressing DDR1b aimed at elucidating how DDR1 promotes malignancy and eventually drug resistance, we found that the pro-tumorigenic effects of DDR1b are potentiated by the presence of collagen I. These studies further supports the notion that DDR1 + collagen cooperate to promote malignancy. We conducted in *vitro* studies analyses of genes regulated by DDR1b in the presence of collagen by performing RNAseq analyses and phosphoproteomics. These ongoing studies are revealing promising targets of DDR1 in pancreatic tumors. We are currently following these results by validating some of the promising targets of DDR1 action. We believe these studies will shed light into the action of DDR1 in PDAC.

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

Nothing to report.

- **How were the results disseminated to communities of interest?**

Nothing to report.

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

We are currently in a non-cost extension period until July 2021. With the remaining funds, we attempt to perform the following studies:

We will continue to examine the effects DDR1 expression and activation on Trametinib sensitivity in human and mouse PDAC cell lines.

We will attempt to use organoids to determine the effect of DDR1 and MEK inhibitor combinations on cell viability and proliferation.

We will continue our analyses of DDR1 downstream effectors in PDAC xenografts.

4. IMPACT

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

Nothing to report.

- **What was the impact on other disciplines?**

Nothing to report.

- **What was the impact on technology transfer?**

Nothing to report.

- **What was the impact on society beyond science and technology?**

Nothing to report.

5. CHANGES/PROBLEMS

We are currently under a non-cost extension period. Therefore, we are compelled to adapt our experiments to the limits of the remaining budget.

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**

Ruggeri JM, Franco-Barraza J, Sohail A, Zhang Y, Long D, Pasca di Magliano M, Cukierman E, Fridman R, Crawford HC. Discoidin Domain Receptor 1 (DDR1) is Necessary for Tissue Homeostasis in Pancreatic Injury and Pathogenesis of Pancreatic Ductal Adenocarcinoma. Am J Pathol. 2020 Aug;190(8):1735-1751. PMID: 32339496

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

Nothing to report.

- **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? See Note below Table**

Name	Project Role	Nearest Person Months Worked	Contribution to the Project	Funding Support
Rafael Fridman	PI	0.24	Design of experiments and data analyses	This grant
Anjum Sohail	Research Scientist	3		This grant
Howard Crawford (University of Michigan)	Co-I	0.24	Design of experiments and data analyses	Subcontract
Daniel Paglia (University of Michigan)	Research Technician	3	Establishment and maintenance of organoid cultures	Subcontract

- **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?**

Organization Name: Hoffmann-La Roche
Location of organization: Basel, Switzerland
Partner's contribution to the project: Supplied antibodies for DDR1 and a small molecule inhibitor for DDR1.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES

Nothing to report.