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PRINCIPAL INVESTIGATOR: **Alan Ashworth, MD**

CONTRACTING ORGANIZATION: **The Regents of the University of California, San Francisco**

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Ashworth, Alan
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University of California, San Francisco
1450 Third St. 271 Box 0128
San Francisco, CA 94158

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14. ABSTRACT
The goal of this project was to identify genes that drive resistance or sensitivity to PARP and/or ATR inhibition. Toward this end, we performed a pooled CRISPRi screen in the gastric cancer cell line AGS in the presence and absence of ATRi treatment. Our screen identified 57 potential genes whose knockdown induced ATRi resistance, including CDK2, which has an established role in ATRi resistance as well as multiple genes involved in RNA processing and stability. Top candidates were validated using an RNP mini-screen pipeline, and single cell clones of AGS cells lacking expression of candidate targets were generated for additional analysis including proteomic and transcriptomic studies. We used CRISPR-editing to knock out these genes in additional gastric cancer models to confirm if target loss is associated with ATRi sensitivity in different genetic contexts. To further evaluate these lead targets, we tested the sensitivity of these clones to a panel of approved oncology drugs. Additional experiments to uncover how RNA stability affects cellular response to ATRi are ongoing.

15. SUBJECT TERMS
Gastric cancer, BRCAness, DNA damage, DNA repair, ATR inhibitor, PARPi, targeted cancer therapy

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1. Introduction

Inactivating germline and somatic mutations affecting genes involved in DNA damage repair are features of upper gastrointestinal malignancies, but we do not know how common these lesions are. Genes encoding for proteins important for mismatch, base-excision, and homologous recombination (HR) repair are affected in subsets of these tumors. For example, mutations in the HR genes *BRCA1* and *BRCA2* have been found in some gastric cancers. Loss of BRCA1 protein expression has been found in 21% of gastric cancers and was associated with diffuse-type histology and poor survival. PARP1 (polyADP ribose polymerase 1) is an enzyme essential for base-excision repair, a complementary DNA repair pathway to the HR repair pathway inactivated by mutations in *BRCA1* and *BRCA2*. Inactivation of PARP1 enzymatic activity, and thereby base-excision repair, can produce a synthetic lethality in cells lacking HR function. Clinical activity of single agent PARP inhibitors has been observed in patients with germ-line *BRCA1/2* mutations as well as tumors displaying "BRCAness", which is characterized by genomic instability and susceptibility to PARP inhibitors in the absence of *BRCA1/2* mutations. Mutations conferring BRCAness have been identified in a number of genes involved in the DNA damage response, including *RAD51C, ATM, ATR, MDC1, MRE11A, PALB2, CHK1/2, RAD50*, and components of the Fanconi's anemia repair pathway but the disease-specific relevance of these mutations is not known. Oncogenic signal transduction pathways, such as PI3K as well as RAF-MEK-ERK pathways may be involved in the regulation of the DNA repair machinery.

The purpose of this research is to elucidate a) whether GI malignancies with mutations in genes conferring BRCAness will be sensitive to PARP inhibition, in particular in combination with inhibitors of oncogenic signal transduction pathways (MEK, PI3K, TGFb, WNT, Notch, Hedghog, JAK-STAT) or with chemotherapy; b) whether mutations conferring BRCAness provoke an immune response that could be enhanced pharmacologically; c) whether there is a DNA signature predictive of PARP inhibitor sensitivity or combinatorial therapies. Addressing these questions will set the stage for development of increasingly efficient treatment strategies for GI cancers involving PARP inhibitors.

2. Keywords.

Gastric cancer, BRCAness, DNA repair, DNA damage, PARP inhibitor, MEK inhibitor.

3. Accomplishments

What were the major goals of the project?

1. Obtain clinical samples from PARP inhibitor treated gastric cancers.
2. Apply genomic signatures of "BRCAness" to gastric cancer clinical samples.
3. Identify genes that drive resistance or sensitivity to PARP and ATR inhibition in gastric cancer cells.
4. Validation studies and analysis of archival tissue.

What was accomplished under these goals?

Goal 1. Obtain clinical samples from PARP inhibitor treated gastric cancers.

In the original proposal, we were to perform TCR sequencing on samples derived from Dr. Korn's Study. Unfortunately, the drug company that Dr. Korn was working with withdrew support for the clinical trial. Dr. Collisson has Dr. Mike Cecchin at Yale, who can provided the needed samples from his clinical trial on which we can now perform TCR sequencing.

Goal 2. Apply genomic signatures of "BRCAness" to gastric cancer clinical samples.

Previously our preliminary analysis indicated that gastric tumors do not appear to manifest a mutational signature consistent with HRD, at least in the cohort analyzed here. We are making efforts to obtain samples of patients with HR-deficient gastric cancer to assess them for presence of a genomic scar in the coming year.

Goal 3: Identify genes that drive resistance or sensitivity to PARP and ATR inhibition in gastric cancer cells.

From the last report we generated a list of 57 potential genes whose knockdown associated with ATRi resistance. CRISPRi-mediated knockdown of CDK2 was the strongest inducer of resistance to AZD6738, and promisingly, this hit was previously identified by our lab as an important mediator of ATRi resistance. CDK2 will be one of positive controls for induction of resistance to ATR inhibition. We have selected 7 additional candidate genes for validation, and have included these in the construction of a smaller targeted validation screen. Several additional control sgRNAs are also included in that group, and these are described in **Table 1**.

Table 1: Control sgRNAs included on our validation screen

Type	Gene	Purpose
Test	CDK2	sgRNA leads to resistance in screen previously published by our lab
Control	ATR	Target gene
Control	PLK1	Essential gene
Control	*ARID1A*	Synthetically lethal partner with ATR inhibtior
Control	*TP53*	ATR inhibition is synthetically lethal in *TP53* mutant CLL cells
Control	*SLFN11*	Schlafen-11 inhibits RNA synthesis. Involved in PARPi resistance
Control	*CDC25A*	CDC25A a major determinant of sensitivity to ATR inhibition

For our validation screen, we will use the complementary gene-editing tool, Cas9 RNPs, in a multi-well high throughput format **(Figure 1)**. Three unique crRNAs targeting each gene along with non-targeting controls will be complexed with a tracer RNA and Cas9 protein to form a RNP. Using the Lonza Amaxa 96-well shuttle electroporator, we will electroporate these RNPs into nuclear-tagged AGS cells, which are then seeded into multiple 384 well plates. We will extract genomic DNA from each of these samples too for later PCR, sequencing and knockout analysis. 24 hours later, these cells are treated with DMSO control or AZD6784 (concentrations = SF50, SF25, SF5). We will assess the growth of these cells twice daily for 7 days using the Incucyte Live-Cell Analysis System.

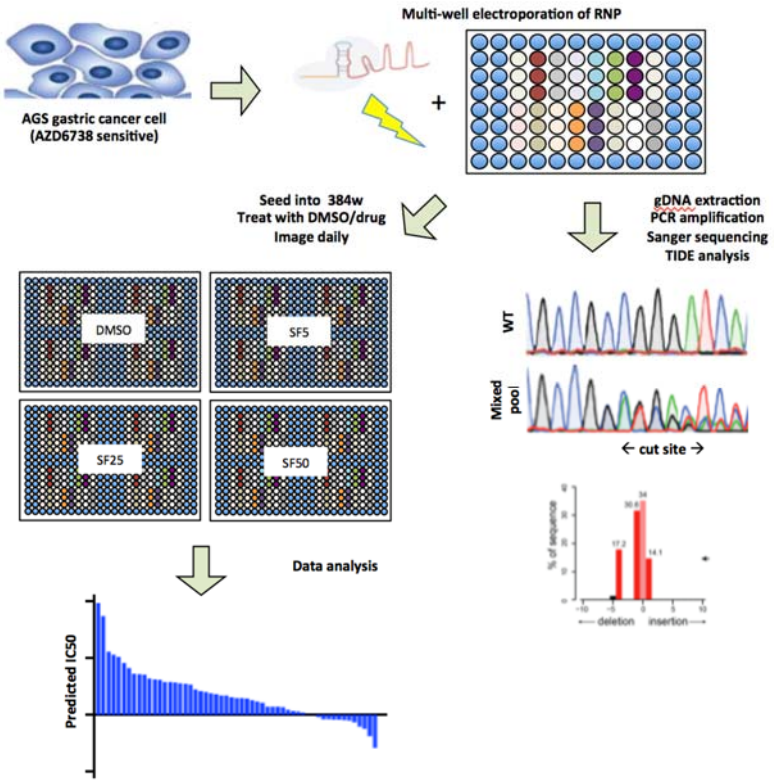

Figure 1: Workflow for RNP mini-screen to validate candidate ATRi resistance genes in AGS cells.

Goal 5. Validation studies and analysis of archival tissue (Janjigian et al Cancer Discovery 2018)

We observed no single gene in DNA repair pathways, such as BRCA1/2, were significantly associated with treatment response when we correlated the genomic findings with treatment response and patient outcomes in the 187 patients with HER2-negative disease treated with first-line fluoropyrimidine/platinum.

IMPACT

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

Reflecting her academic and research achievements, in 2018, Dr. Janjigian was appointed Chief of MSK's Gastrointestinal Oncology Service. This service is one of the largest at MSKCC and Dr. Janjigian's selection as its leader after an international search is a testament to her remarkable accomplishments.

How were the results disseminated to communities of interest?
National meetings and manuscript published in Cancer Discovery 2018 Jan;8(1):49-58. doi: 10.1158/2159-8290.CD-17-0787. Epub 2017 Nov 9. PMID: 29122777

A second manuscript outlining the rapid autopsy data is in re-submitted for second review to Cancer Discovery

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

We will keep expanding the cohort of PDX to better reflect our diverse patient population.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

CHANGES/PROBLEMS
Changes in approach and reasons for change

None

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

PRODUCTS

• Publications, conference papers, and presentations

published in Cancer Discovery 2018 Jan;8(1):49-58. doi: 10.1158/2159-8290.CD-17-0787. Epub 2017 Nov 9.PMID: 29122777

A second manuscript outlining the rapid autopsy data is in re-submitted for second review to Cancer Discovery

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers, and presentations
Nothing to report

• Website(s) or other Internet site(s)
Nothing to report

• Technologies or techniques

Improved efficacy of tumor implantation to immune deficient mice
1. Dissect human tumor to 100mm3 pieces
2. With blunt edge of scissors, scrape off soft/whitish necrotic tissue
3. Cut the fragment to 20-30mm3 pieces by removing non-viable tissue
4. Implant these 20-30mm3 pieces in bilateral flank of immune deficient mice as described anywhere

• **Inventions, patent applications, and/or licenses**
Nothing to report

• **Other Products**
Nothing to report