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

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O'Leary, Patrick

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13. SUPPLEMENTARY NOTES

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

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.

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

Gastric cancer, BRCAness, DNA damage, DNA repair, ATR inhibitor, PARPi, targeted cancer therapy

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.

1. PI to seek regulatory approval from DoD HRPO office. (Korn)
2. PI to seek regulatory approval from IACUC and DoD ACURO office. (Janjigian)
3. **Define a genomic signature of BRCAness in gastric cancer (Collisson, Ashworth)**
4. **Regulation of DNA repair activity by signal transduction pathways (Korn, Ashworth, Janjigian, Collisson)**
5. Define the T cell receptor diversity of gastric cancer patients (Fong, Janjigian)

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the

emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

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

Cell line identification and validation

The initial goal of this project was to identify genes that drives resistance or sensitivity to PARP or ATR inhibitors in gastric cancer. We decided to focus on ATR inhibitors as clinical trials testing ATRi are currently ongoing in gastric cancer. We initially amassed a collection of gastric cancer cell lines, and set about generating stable cell lines applicable to relevant screening formats used in the lab: dCas9-KRAB expressing stable cell lines for CRISPR interference (CRISPRi), and nuclear RFP-labelled cell lines for cell counting in large scale microplate assays. We successfully generated MKN7, AGS, KATO-III, SNU-1, SNU-16 and NCI-N87 cell lines that stably express dCas9-KRAB, a fused protein used for CRISPRi transcriptional repression (**Table 1**), and functionally validated five of these cell lines for CRISPRi knockdown. We confirmed knockdown efficacy in the dCas9-expressing AGS, MKN7, KATOIII and NCI-N87 cell lines using an sgRNA targeting CD59 (**Figure 2**). These validated cell lines would be suitable for large scale CRISPRi pooled sgRNA screening. We also generated cell lines expressing nucRFP, that would be used in drug screens and validation assays on the Incucyte Live-Cell Analysis System (**Figure 1**).

Table 1: Phenotypic and genetic characteristics of gastric cancer cell lines studied from our panel, along with whether a stable cell line has been generated

Cell line	Growth type	MSI Status	Tissue	NucRFP	dCas9-KRAB	Validated for CRISPRi
AGS	Adherent	Stable	Gastric adenocarcinoma	+	+	+
MKN7	Adherent	Low	Metastatic site; Lymph node	+	+	+
KATO-III	Adherent/ Suspension	Stable	Metastatic site; Pleural effusion	+	+	+
NCI-N87	Adherent	Stable	Metastatic site; Liver	+	+	+
SNU-1	Suspension	High	Gastric carcinoma	+	+	+
SNU-5	Suspension	Stable	Metastatic site; Ascites	+	-	-
SNU-16	Suspension	Stable	Metastatic site; Ascites	+	+	-

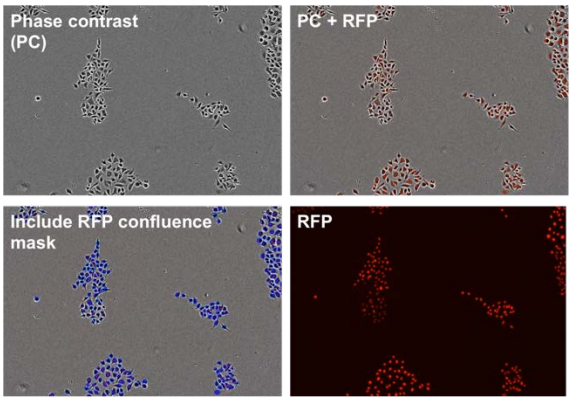


Figure 1: Cells were tagged with a nuclear-tagged RFP transgene to allow quantification of cell number with the Incucyte imaging platform

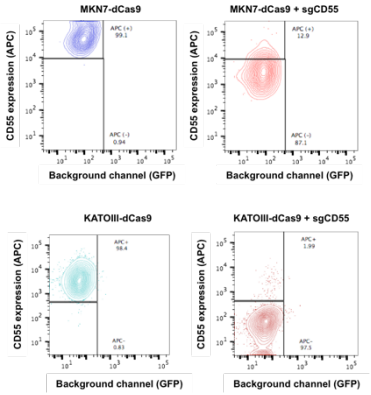


Figure 2: Validation of CRISPRi transcriptional repression efficiency using a sgRNA targeting CD59

Development and implementation of CRISPRi resistance screen

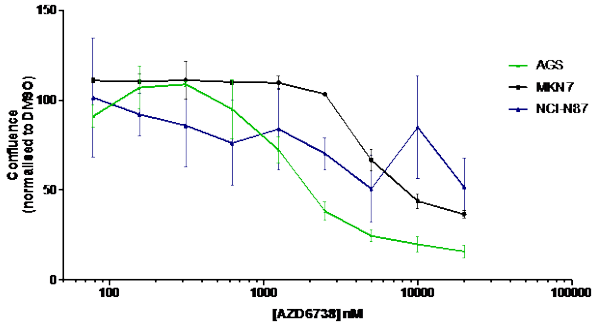


Figure 3: Response of gastric cancer cell lines to ATR inhibition by AZD6738

In previous reports, we described our drug sensitivity screening studies across various gastric cancer cell lines (AGS, KATOIII, MKN7 and NCI-N87) to determine their drug response to PARP inhibitors and the ATR inhibitor, AZD6738. From the adherent cell lines, AGS cell lines were sensitive to ATR inhibition, while MKN7 cells were resistant (**Figure 3**). Due to their sensitivity to ATRi, we selected AGS cells for our proposed CRISPRi screen to identify genes involved in resistance to ATR inhibition.

In order to choose a concentration of drug that would be suitable for a large scale sensitivity or resistance screen, we carried out pilot screens in T25 flasks where we treat cells with a couple of concentrations and split/retreat the cells every 3-4 days over a 3 week period. This would mimic how we would run a larger pooled screen in T175s or 15cm plates. The data supported the results we see from the shorter micro-plate dose response experiments. MKN7 are more resistant than AGS and KATOIII cell lines to ATR inhibition (**Figure 4A**).

Due to their sensitivity to ATRi, we selected AGS cells for our proposed CRISPRi screen to identify genes involved in resistance to ATR inhibition. We had previously confirmed knockdown efficacy in the dCas9-expressing AGS cell lines using an sgRNA targeting CD59 (**Figure 4B**). From the pilot screen, we selected a highly toxic concentration (0.5uM) of the ATR inhibitor, AZD6738. The screen had two arms, treating the cells with a DMSO control or 0.5uM AZD6738 **(Figure 4C)**. The libraries

we transduced contain 5 sgRNAs targeting each of 8000 genes; those selected are potential drug targets, kinases, phosphatases, apoptosis and cancer-related genes **(Figure 4D)**. Our screen lasted three weeks and cells were passaged every three days. At each split, we confirmed BFP expression, counted cells and reseeded 45 million cells per arm in fresh drug or vehicle. BFP coverage in each arm remained above 90% throughout the experiment (data not shown). Extrapolating the growth rate using the cell counts (and seeding rate) and compared the DMSO to AZD6738 arms, we showed that after 21 days of treatment, 0.134% of the cells had survived in the AZD6738 arm (**Figure 4E).** To ensure adequate sgRNA coverage, we maintained a minimum of $45x10^6$ cells in each arm and froze down aliquots of approximately 30 million cells from T0, $T21^{AZD}$ and $T21^{DMSO}$. After collecting DNA from these samples, we carried out PCR to enrich the sgRNA amplicon and add ligation adaptors, SPRI-purification for PCR product cleanup, QC analysis using the Bioanlayzer, and used the HiSeq4000 to sequence the sgRNAs to sufficient depth.

When we dissect the essential hits from the 'gamma' comparison (growth phenotype – untreated/T0),

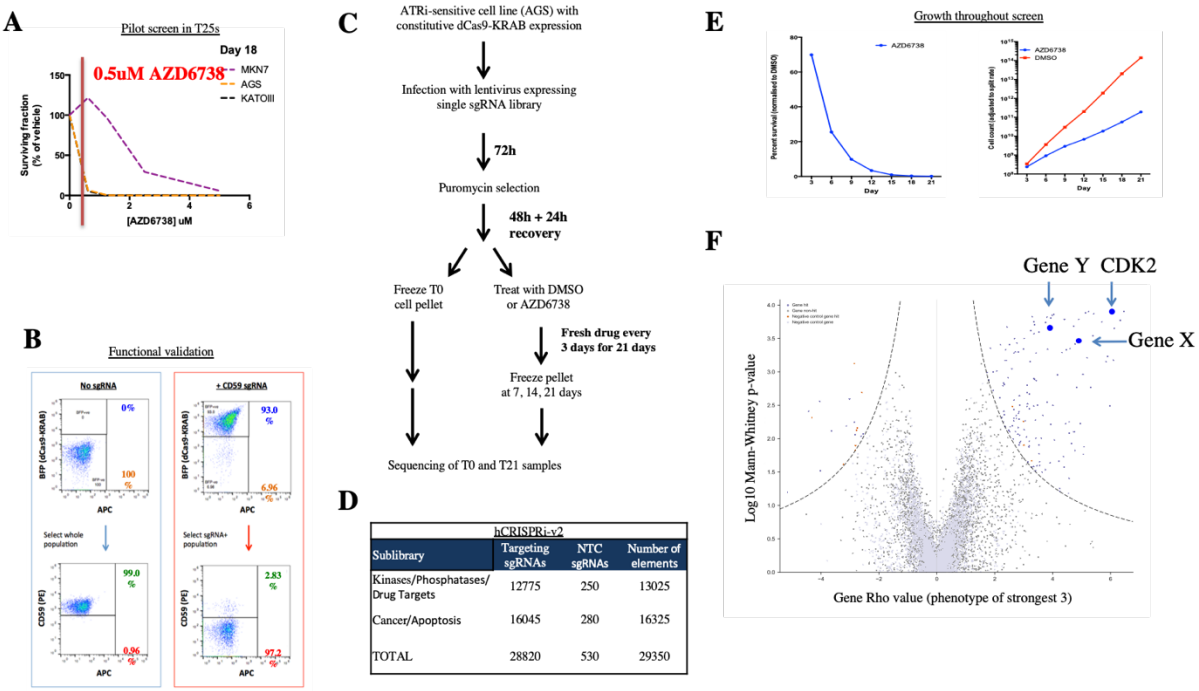


Figure 4: CRISPR interference screen to identify mediators of resistance to ATR inhibition

we see many of the expected genes on our list, such as TERT and ABCE1 (volcano plot figure not shown). Perhaps unsurprisingly, ATR itself also come up as an essential hit in this ATR inhibitor-sensitive cell line. To select resistance hits, we used the following criteria (resistance phenotype – treated/untreated):

- Focus primarily on the **resistance** hits, as we treated with a toxic concentration of inhibitor on a sensitive cell line
- **Impact** on drug response: select genes with average 'rho' phenotype values above or below 2.0/-2.0 and 'rho' p-values below 0.05
- **Essential** genes: avoid genes with a 'gamma' phenotypes below -2.0

Validation of potential resistance hits

Our criteria generated a list of 57 potential genes whose knockdown associated with ATRi resistance **(Figure 4F)**. CRISPRi-mediated knockdown of CDK2 was the strongest inducer of resistance to AZD6738, and promisingly, our lab previously identified CDK2 as an important mediator of ATRi resistance. CDK2 was used as one of positive controls for induction of resistance to ATR inhibition. Interestingly, two genes from the same family of regulators of RNA stability (labeled as Gene X and Gene Y in report) came up among the top 10 strongest resistance hits. We selected these two genes and five additional candidate genes for validation, and 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 2**.

Table 2: 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 inhibitor
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 used the complementary gene-editing tool, Cas9 RNPs, in a multi-well high throughput format **(Figure 5)**. Three unique crRNAs targeting each gene along with non-targeting controls were complexed with a tracrRNA and Cas9 protein to form a RNP. Using the Lonza Amaxa 96-well shuttle electroporator, we electroporated these RNPs into nuclear-tagged AGS cells, which were then seeded into multiple 384 well plates. We extracted genomic DNA from each of these samples too for later PCR, sequencing and knockout analysis. 24 hours later, the 384 well plates of cells were treated with DMSO control or AZD6784 or an additional ATR inhibitor, VX970 (concentrations = SF50, SF25, SF5). We assessed the growth of these cells twice daily for 7 days using the Incucyte Live-Cell Analysis System.

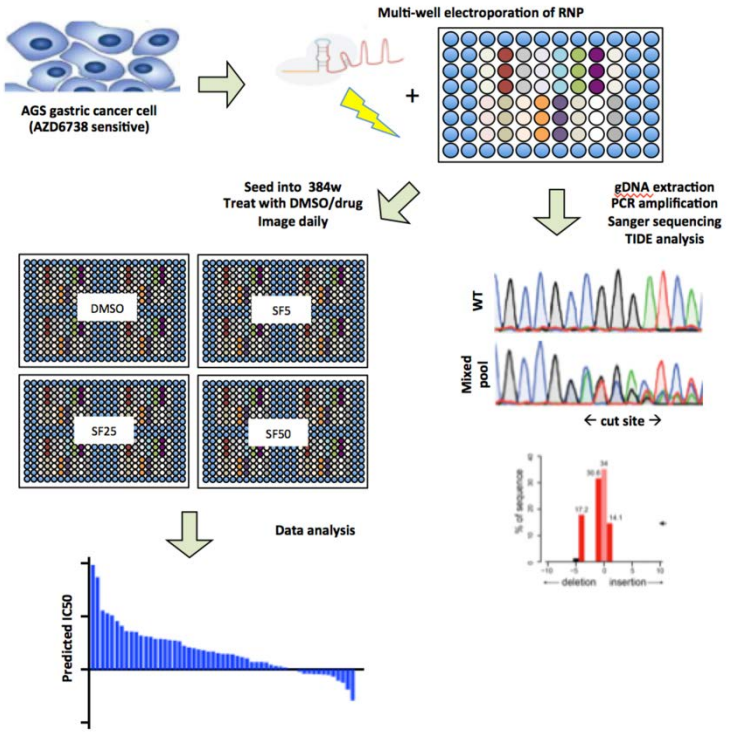


Figure 5: Workflow for RNP mini-screen to validate candidate ATRi resistance genes in AGS cells.

As expected, knockdown of CDK2 induced resistance to both ATR inhibitors, which was noticeable across all concentrations of either drug (**Figure 6; left panels**). Several of our novel hits also validated in the validation screen, with sgRNAs targeting the RNA stability regulation genes, Gene X and Gene Y, showing consistent resistance to ATR inhibition (**Figure 6; right panels**). It is further convincing that perturbation of these genes identifies as inducers of ATRi resistance across two independent platforms: CRISPR interference, which represses transcription, and Cas9 nuclease, which generates an indel edit.

As this mini-screen produces pools of cells with potentially variable knockout of the gene of interest (GOI), we generated single cell clones of AGS cells lacking expression of those two lead novel targets. We generated two candidate clones with deletion of Gene Y, and seven candidate clones with deletion of Gene X. We show the decrease in protein expression across these clones by Western Blotting in **Figure 7**. Presence of indels was confirmed by exon sequencing. Similar to both previous screens, these clones were more resistant to ATR inhibition using two different inhibitors (**Figure 8**). Similar to the pooled knockout experiment, clonal knockout of Gene X shows a stronger phenotype for inducing resistance to ATR inhibition than knockout of Gene Y.

Figure 6: Effect of RNP-based target knockout of AGS cells followed by treatment with a DMSO control or various concentrations of the ATR inhibitors, AZD6738 or VX970. Data shown from three independent sgRNAs, control or targeted. Data shown from 96-hour timepoint.

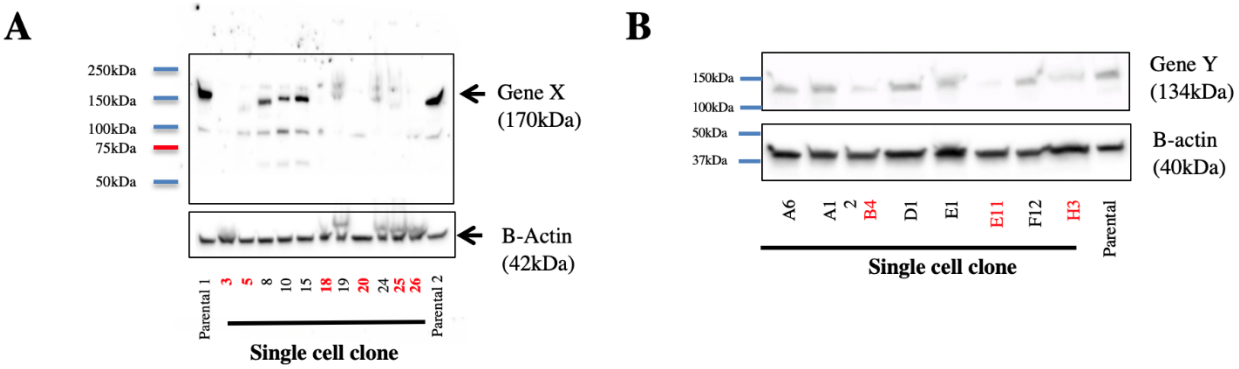


Figure 7: Western Blotting showing AGS single cell clones generated with knockout of Gene X or Gene Y. B-actin was used as a loading control. Candidate clones used for further validation are highlighted in red.

These clones were used for further validation, as we started to elucidate the role of this specific family of regulators of RNA stability in the mechanism of ATR inhibitor resistance in these cells. For further -omic characterization, we used two knockout clones of Gene X, two knockout clones of Gene Y, and parental AGS cells. The target assays discussed later in the report include drug library screening, RNA sequencing, global proteomics and phospho-proteomics, and the data will focus on Gene X as the gene that had the strongest association with ATRi resistance.

Validation in additional cancer contexts

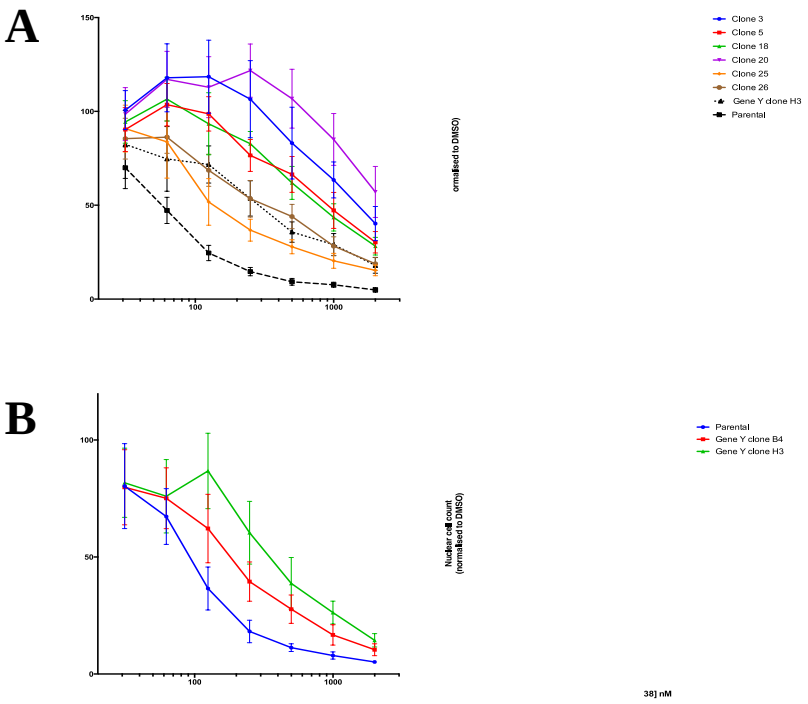
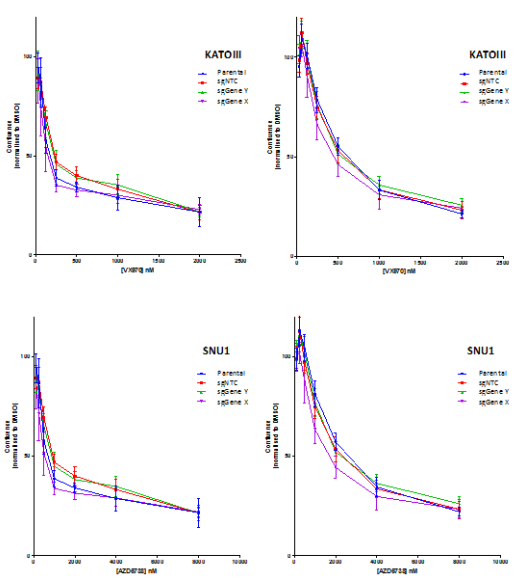


Figure 8: Dose response curves showing knockout clones derived from AGS cells followed by treatment with a DMSO control or various concentrations of the ATR inhibitors, AZD6738 or VX970. Data shown from 96-hour timepoint.

As our screen and target validation was all carried out on the AGS gastric cancer cell line, we decided to expand to additional gastric cancer cell lines and cell lines from other cancer models. RNP-mediated knockout of Gene X and Y in the gastric cancer cell lines, SNU1 and KATOIII, had no effect on resistance to the ATR inhibitor VX-970 (**Figure 10**). We carried out similar experiments in the gastric cancer cell lines, SNU5, SNU16, YCC6 and NCI-N87, and the breast cancer cell lines, MDA-MB-231 and SUM149. Upon knockout of gene X and gene Y, we also saw no correlation with protein knockout and relative sensitivity to ATR inhibitor (data not shown).

Further -omic characterization



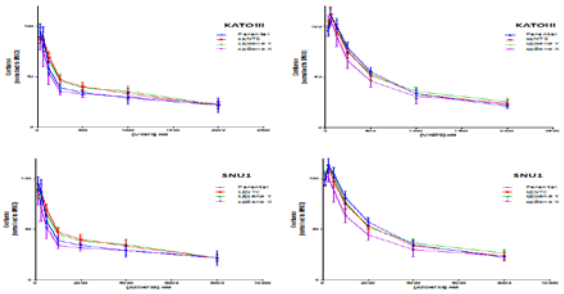


Figure 9: Dose response curves showing Gene X and Gene Y knockout clones derived from additional gastric cancer cell lines followed by treatment with a DMSO control or various concentrations of the ATR inhibitors, AZD6738 or VX970. Data shown from 96-hour timepoint. The right panel confirms by Western blotting the knockout of Gene X in both of these cell lines.

As our two GOIs are critical regulators of RNA stability, we set about assessing the impact of Gene X on protein abundance using global protein abundance analysis. Using phosphoproteomics, we assessed what signaling pathways are activated or impaired in the absence of these genes. Global proteomics identified 201 proteins with significant alteration (-2 < FC < 2) across both KO clones. Several proteins involved in RNA processing, RNA stability, chromatin remodeling were identified as being downregulated in the absence of Gene X. DAVID pathway analysis identified a cluster of GO protein clusters related to RNA processing that were altered upon knockout of Gene X (mRNA 3'-end procressing, RNA export from the nucleus, termination of RNA polymerase II transcription).

The phospho-proteomic data also yielded similar results. Of the 1353 phosphorylation sites with significant alteration (-2 < FC < 2) across both KO clones, we identified multiple individual sites on proteins critical to RNA degradation. Interestingly, we also identified altered phosphorylation sites in the CDK2-cyclin complexes. Several cell cycle genes were also lead candidates selected on the CRISPRi screen (CDK2, CCNA2, CUL1, CDC23A, CCNE1). This suggests that more research needs to be done to assess this RNA stability/cell cycle/ATRi interplay. We are currently validating several of these proteomic and phosphoproteomic changes using Western blotting.

To see if knockout of our gene targets is also involved in resistance to other commonly used oncology drugs, we carried out a multiplex screen using the Approved Oncology Drugs Set, which contains 128 drugs. The parental AGS cells and both Gene X^{KO} clones were treated with 3 concentrations of each compound (0.2uM, 1uM, 5uM) or a DMSO control. The cells were treated with the drugs, and imaging on the Incucyte system for 7 days. Figure 10 shows some of the more promising drug families after 96 hours of treatment. Nucleoside analogs and mTor inhibitors all showed a trend towards resistance when Gene X was knocked out.

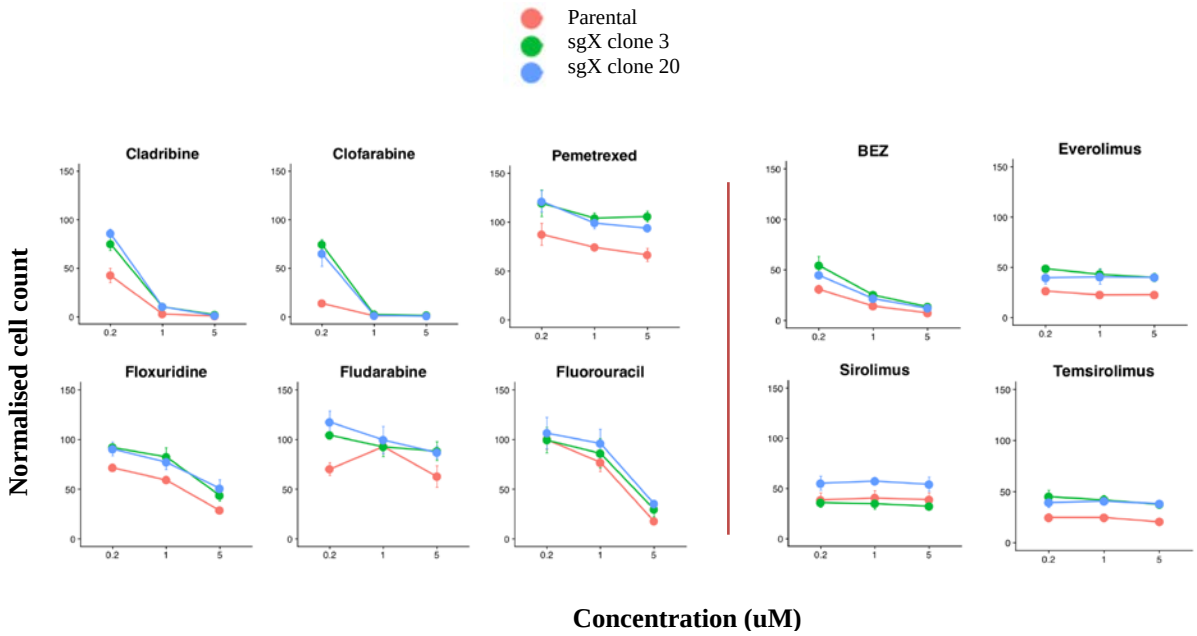


Figure 10: Dose response curves from selected drugs after treating parental AGS cells and two individual clones with knockout of Gene X.

Conclusions

In conclusion, we used pooled sgRNA CRISPRi screening to identify novel mediators of resistance to the ATR inhibitor, AZD6738. Using Cas9 editing, an orthogonal method of validation, we validated that several of these genes when suppressed induced resistance to two independent ATR inhibitors. As our main focus was on two genes involved in RNA stability (Gene X, Gene Y), we developed a series of single cell knockout clones, which we are now using to further understand the interplay between RNA stability and resistance to ATR inhibitors in gastric cancer. Our proteomic and phospho-proteomic data has also suggested that the mechanism of ATRi resistance is through control of RNA stability processes. However, our future plansis to tease out this mechanism to a greater extent. One of the pitfalls of our observation so far is that it is limited to one cell line so far, the gastric cancer cell line, AGS. The next stage of this project will be to work through our collection of gastric cancer cell lines to find additional models that show our resistance phenotype.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This award provided salary and research support for Patrick O'Leary during his postdoctoral training. It also provided financial support for Junior Specialist, Tess Woods, and the project provided them both with a great basis in large scale CRISPR screening and validation. Drs. Ashworth and Diolaiti helped Dr. O'Leary define the scope of the project, but allowed Dr. O'Leary to develop independence as a project lead and gain experience mentoring junior researchers, all key skills for a future career as a senior scientist.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Patrick O'Leary has presented this work at departmental and inter-campus symposia.

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.

Nothing to report

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

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

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."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

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."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*

- *improving social, economic, civic, or environmental conditions.*

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
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.

Nothing to report

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

none

Significant changes in use or care of vertebrate animals

none

Significant changes in use of biohazards and/or select agents

none

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. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Patrick O'Leary presented his work at the 2018 and 2020 EAC Meeting of the Cancer Cell Mapping Initiative (San Diego 2018, San Francisco 2020). Patrick also presented this data at regular UCSF Research-in-Progress Seminars.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  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

- **Inventions, patent applications, and/or licenses**
  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**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*

- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

We have assembled an extensive panel of gastro-esophageal cell lines that are a great resource for ongoing screening and validation studies.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Alan Ashworth, PhD, FRS* *No change*
Name:	*Patrick O'Leary, PhD* *No change*
Name:	*Morgan Diolaiti, PhD* *No change*
Name:	*Tess "Jefferson" Woods* *No change*

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."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No change

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

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

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.*

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

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.