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TITLE: Identifying Therapeutics for Platinum-Resistant Ovarian Cancer by Next-Generation Mechanotyping

PRINCIPAL INVESTIGATOR: Amy Rowat

CONTRACTING ORGANIZATION: University of California, Los Angeles
Los Angeles, CA 90095

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

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14. ABSTRACT

Treatment of high grade serous ovarian is initially effective in reducing the growth of tumors, but cancer recurs in over 80% of ovarian cancer patients because cells become resistant to common, platinum-resistant chemotherapy drugs. There is a critical need for new drugs that target platinum-resistant cancer cells. We recently discovered that platinum-resistant ovarian cancer cells are more deformable than their drug-sensitive counterparts. We hypothesized that we could identify novel compounds that selectively target drug-resistant ovarian cancer cells by screening cells against libraries of small molecules using the novel Parallel Microfiltration (PMF) screening technology that we recently invented. In this second funding period, we have successfully conducted the first mechanotype screen, identifying top hits from the Library of Pharmacologically Active Compounds (LOPAC) that cause cisplatin-resistant ovarian cancer cells to be less deformable. Orthogonal studies across multiple human ovarian cancer cell lines reveal that top hits consistently cause ovarian cancer cells to be less invasive, suggesting that mechanotype screening may provide a surrogate to identify compounds that complement existing therapeutic strategies.

15. SUBJECT TERMS

ovarian cancer, cell mechanical properties, cell mechanotype, drug discovery

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1. INTRODUCTION:

Platinum resistance is the main cause of ovarian cancer-associated mortality. There is a critical need for new anti-cancer drugs to combat drug resistant and recurrent cancers. We propose that cancer-specific alterations in cell mechanical phenotype, or mechanotype, can be an alternative target for novel therapeutic agents. The altered mechanotype of cancer cells is an emerging biomarker that can enable more accurate diagnoses, which complement existing methods used by pathologists. We previously showed that cisplatin-resistant cells exhibit characteristics of mesenchymal-type cells (Qi et al, *Nat. Sci. Reports*); cytoskeleton reorganization and epithelial-to-mesenchymal transition (EMT) are features associated with cancer progression and metastasis, as observed in many *in vitro* models of drug resistance, as well as in patients. To enable screening based on cell mechanotype, we recently invented Parallel Microfiltration (PMF) that allows simultaneous measurements of cell mechanotype. **The goal of this project is to identify compounds that reverse the mechanotype of soft, platinum-resistant cells and are effective as anti-cancer agents against these drug resistant cells**. More broadly, this study will validate the use of mechanotyping as a complementary screening method to identify compounds with efficacy as anti-cancer agents that target platinum-resistant cells. Ultimately, identifying novel molecules that modulate mechanotype, reduce metastasis of platinum-resistant cancers, and could be administered to patients, would enable effective treatment strategies for patients that have resistant subtypes and improve patient survival.

2. KEYWORDS: ovarian cancer, cell mechanical properties, cell mechanotype, drug discovery

3. ACCOMPLISHMENTS:

- **What were the major goals of the project? [red font denotes updates since the 2018 progress report; yellow highlights illustrate tasks to be completed in the coming 6 months]**

Specific Aim 1: To identify molecules that reverse the softer mechanotype of platinum-resistant cells (Specified in proposal)	**Timeline**	**Site 1: UCLA**	**Site 2: Cedars-Sinai**	**Percent completed**
Subaim 1A: PMF-screen platinum-resistant ovarian cancer cells against small molecule libraries				
Major Task 1 - Demonstrate PMF function in the MSSR	Months			
Subtask 1 - Establish readout using plate reader	1	Rowat		100%
Subtask 2 - Fabricate PMF devices that interface with liquid handlers	1-6	Rowat		100%
Milestone #1 Achieved - Replicate filtration behavior of cisplatin-resistant OVCAR (CisR) cell lines plus positive control (taxol treatment)	6			100%
Subtask 3 - Establish standard deviation of PMF in MSSR: well to well, row to row, and plate to plate variability	7-9	Rowat		100%
Milestone #2 Achieved - Define threshold above which we define a 'hit'	9			100%
Major Task 2 - Conduct screen of LOPAC collection (1280 approved drugs)				
Subtask 1 - PMF validation screen	7-9	Rowat		100%
Subtask 2 - Validate hits from initial screen using orthogonal transwell migration assay	9		Lawrenson	100%
Milestone #3 Achieved - Verification of hits that are known cytoskeletal-targeting drugs	9			100%

Major Task 3 - Conduct screen of chemically diverse ChemBridge and Prestwick libraries (>30,000 compounds)				
Subtask 1 - PMF screen	10-12	Rowat		100%
Subtask 2 - Generate ranked list of hits	11-12	Rowat		100%
Milestone #4 Achieved - Identification and ranking of compounds for secondary tests	12			100%
Subaim 1B: Validation of lead compounds by PMF				
Major Task 4 - Validate lead hits				
Subtask 1 - Validation of hits using orthogonal (invasion) assay with the original cell lines used for the screen and independent (Kuramochi, Ince) cell lines	10-12		Lawrenson	100%
Subtask 2 - Perform dose response experiments using PMF with the original cell lines used for the screen and independent (Kuramochi, Ince) cell lines	10-12	Rowat		100%
Major Task 5 - Rank validated compounds based on specificity for platinum-resistant cells				
Subtask 1 - Determine IC_{50} values for platinum-sensitive (control) and -resistant (target) cells; calculate therapeutic index (TI)	12		Lawrenson	100%
Subtask 2 - Generate ranked list of validated compounds with specificity for platinum-resistant cells, TI < 5	12	Rowat	Lawrenson	100%
Milestone #5 Achieved - Identification of compounds that target platinum-resistant cells; 10 lead compounds will advance to functional studies	12			100%
Specific Aim 2: To characterize the anti-cancer potential of lead compounds using functional assays (Specified in proposal)				
Major Task 6 – Perform functional assays to investigate effect of lead compounds				
Subtask 1 - Cell cycle analysis	13-16		Lawrenson	100%
Subtask 2 - Cytotoxicity assays	13-16		Lawrenson	100%
Subtask 3 - Validate lead compounds using shRNA knockdown and overexpression studies		Rowat		0%
Subtask 4 - Conduct studies on primary patient-derived cells			Lawrenson	100%
Subtask 5 - Conduct studies on clinically relevant mouse model			Lawrenson	0%
Milestone #6 Achieved - Identification of anti-cancer compounds	19			0%
Major task 7 - Prepare and publish manuscript on high throughput mechanotype screening to identify novel anti-cancer compounds that target platinum-resistant cells				
Subtask 1 - Prepare figures and write results section		Rowat	Lawrenson	85%
Subtask 2 - Write Introduction and Discussion		Rowat	Lawrenson	75%

Subtask 3 - Submit paper		Rowat	Lawrenson	0%
Subtask 4 - Respond to reviewer comments and resubmit		Rowat	Lawrenson	0%
Milestone #7 Achieved - Paper accepted for publication				0%

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

1) Major activities

Our activities focused on integrating the parallel microfiltration (PMF) system into the core high throughput screening facility at UCLA, the Molecular Shared Screening Resource (MSSR). We have now completed the screen of 1280 FDA-approved small molecules, orthogonal assays on additional ovarian cancer cell lines, and are currently validating effects of the top lead compounds using shRNA knockdown and cDNA-mediated overexpression, as well as testing in clinically-relevant animal models.

2) Specific objectives

We have fully optimized the PMF system in the MSSR high throughput screening facility. After performing the screen, lead compounds will be prioritized for follow up studies to determine their effects on invasion, proliferation, cytoskeletal structure and protein expression, using both established ovarian cancer cell lines as well as cells from patient ascites.

3) Significant results and key outcomes

PMF integrated into the Molecular Shared Screening Resource. The PMF device is now integrated into the high throughput screening facility at UCLA (**Fig 1**). An automated pipettor is used to deliver drugs to cells in multiwell plates; after the 24 h incubation period, the drug-treated cells are lifted off the substrate into suspension. The cell suspensions are then transferred into the PMF device, which is placed in the pressure chamber. Pressure is applied to drive the suspension of cells and media through the porous membrane. The resultant cell suspension that is retained in the top well is then transferred to a 96-well plate, and placed in a plate reader to determine absorbance. Standard plate reader software is used to determine absorbance readings; wells that have a high absorbance reading, thereby indicating increased retention, and identify 'hits'.

Figure 1. PMF setup in the Molecular Shared Screening Resource, UCLA's high throughput screening core facility. Photo shows 96-well pipette head loading samples into the PMF device top plate.

PDMS-PMF device for high throughput screening. To achieve high throughput screening, we developed a v2 PMF device that has an array of 96 filtration units that are fabricated in a polydimethylsiloxane (PDMS) membrane using soft lithography (**Fig 2C-E**). The filtration units each contain an array of pillars with a gap size of 5 – 10 μm; the prototype device uses polycarbonate membranes that have similar pore sizes (Qi et al, *Nat Sci Reports*, 2015). Similar to the prototype device, air pressure is applied to drive an array of 96 individual cell samples through the array of posts; the filtrate is collected in the bottom wells and the volume, which is measured using a plate reader, reflects the deformability of the cells. A higher filtrate volume indicates the cells are more deformable, while a lower filtrate volume reflects a sample of cells that are less deformable and tend to occlude the gaps between pillars. We proposed to use the

v2 PDMS-PMF device as these can be rapidly fabricated, enable precise control of the gap sizes, and can interface with existing high throughput screening equipment (Fig 2B), including plate readers that make the assay readout more efficient. Our collaborative manuscript describing the PDMS-PMF method was published in early 2019 (Gill et al, *A scalable filtration method for high throughput screening based on cell deformability*, Lab Chip).

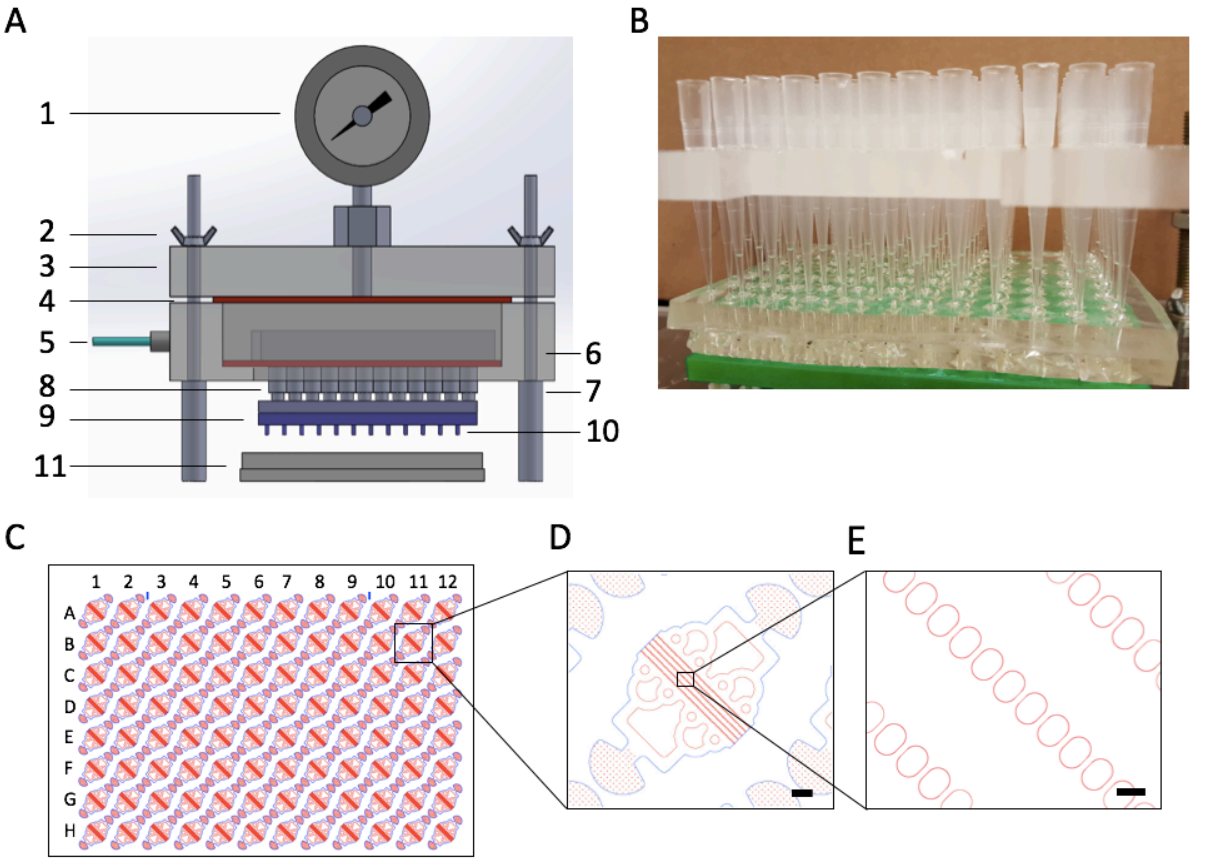

Figure 2. **PDMS-PMF device**. (A) Schematic of PDMS-PMF device. 1. Pressure gauge. 2. Wing nuts for assembling. 3. Pressure chamber. 4. Rubber sealing pad. 5. Tubing that connects to compressed air (pressure source). 6. Custom-fabricated plate holder. 7. Supporting pillars. 8. 96-well plate with tubing. 9. PDMS-membrane that contains array of 96 individual microfluidic devices. 10. Outlet tubing. 11. Collection (96-well) plate. (B) PDMS-PMF device aligns with a standard 96-well pipette array. (C) Plan view of array of 96 microfluidic devices in the PDMS membrane. (D) Single filtration device. Scale, 1 mm. (E) Micropillars with defined interpillar gap size through which cells are filtered. Scale, 100 μm.

Identifying hits. To perform the HT-PMF deformability-based screen of cells treated with LOPAC library compounds, we treat cells with compounds for 24 h prior to filtration. Hits are identified as compounds that result in largest increase in the % retention of the samples; less deformable cells cause occlusion of the pores in polycarbonate membrane resulting in increased retention volume. Hits are compounds of interest that result in reversal of the more deformable mechanotype of the drug-resistant ovarian cancer cells. We rank all the compounds in the library based on the % retention measurements and calculate the respective Z-factors as

$$Z = 1 - \frac{3\,SD_{Sample} + 3\,SD_{Control}}{|Mean_{Sample} - Mean_{Control}|}$$

Hits are defined as compounds with Z-factor > 0.538 (**Fig 3A**). Based on this criterion we identify 67 drugs as hits, ~5% of the total compounds in the library (**Fig 3B**). We discover a large percentage of 'Cytoskeletal & ECM'

targeting compounds as lead compounds; compounds targeting cytoskeleton as well as ECM are known modulators of cell mechanotype. Additionally, ~91% of hits identified are in other drug classes such as, 'neurotransmitters' and 'cell cycle'. We prioritize the top 0.05% of the total compounds (6) as lead compounds for follow up secondary validation assays.

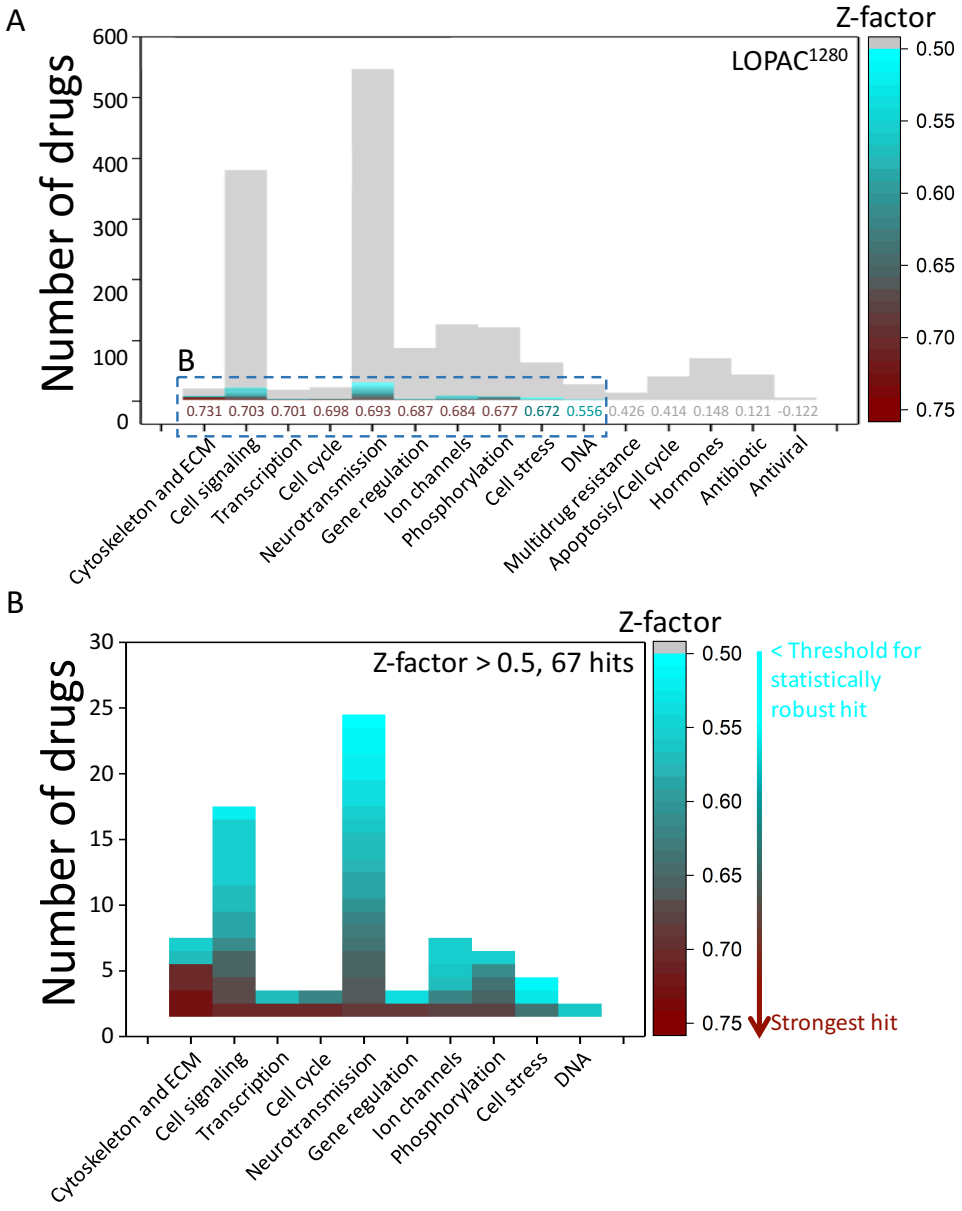

Figure 3. HTS and lead compounds identified by the screen (**A**) % Retention measurements obtained using HT-PMF are used to calculate Z-factors for all the compounds in the library. Compounds are then ranked based on their Z-factor within their respective drug classes. Drug classes are ranked (left to right) based on the highest observed Z-factor in the class. (**B**) 67 lead compounds (Z-factor > 0.5) are identified from the HTS in the indicated drug classes.

Validating hits. We prioritize the top 6 hits (0.5% of compounds) for in vitro validation (**Fig 4A**). To confirm compounds alter cellular mechanotype, we measure the effects of increasing concentrations of drug on cellular mechanotype using HT-PMF; this confirms that the identified top 6 hit compounds reduce deformability of OVCAR5 Cis-R. We observe similar decreased deformability in additional high-grade serous ovarian cancer (HGSOC) cell lines, FUOV1 (**Fig 4B**).

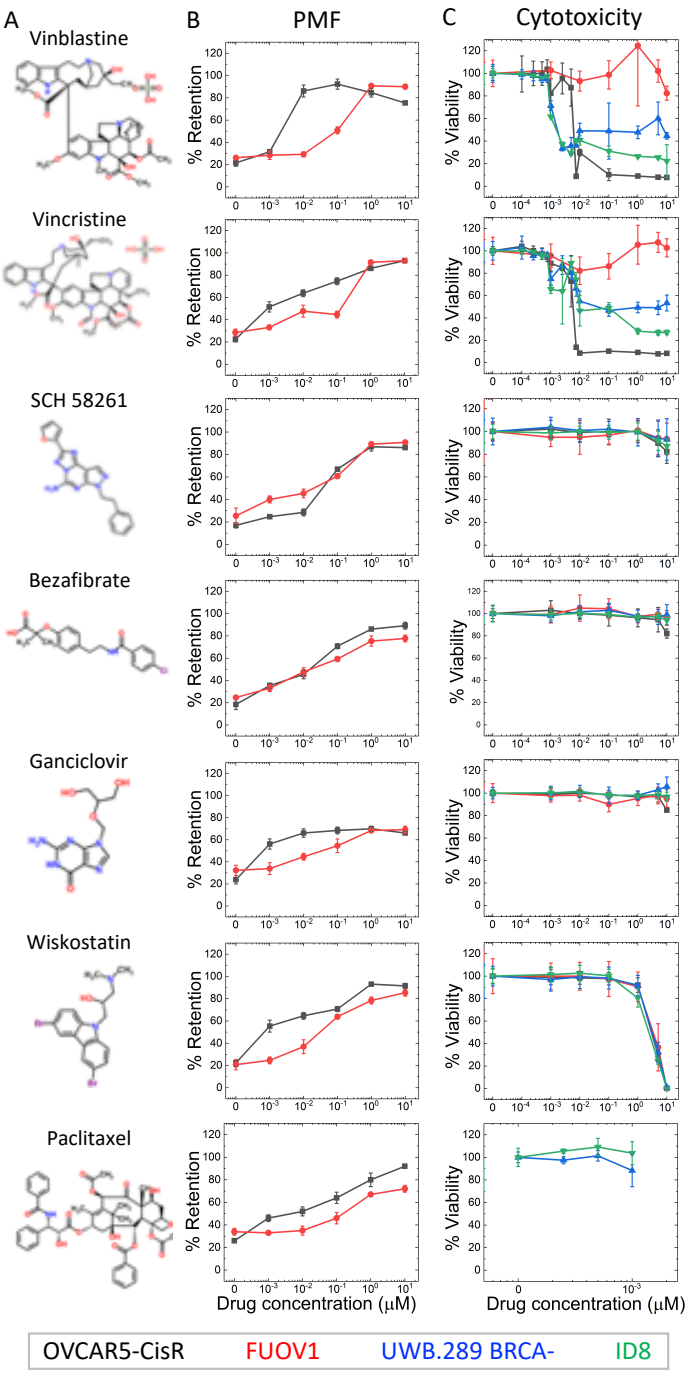

Figure 4. Dose response assays for effect of top hits on cell deformability and cytotoxicity. (**A**) Top 6 lead compounds identified from the HT deformability-based screen. (**B**) Cells are treated with a range of concentrations of lead compounds for 24 h before PMF through 10 μm membrane at 2.1 kPa for 50 s. (**C**) Cells are treated with a range of concentrations of lead compounds for 48 h before quantification of the number of viable cells using CellTiterGlo to determine sublethal dose. Data obtained from three independent experiments. Each data point represents mean ± S.D.

Orthogonal assays: To elucidate the effects of the top 6 lead compounds on cellular functions, we perform additional secondary orthogonal assays. ***Cytotoxicity.*** We first performed cytotoxicity assays from 1 nM to 10 μM compound concentrations. These cytotoxicity data enabled us to determine the sublethal dose in 4 cell lines: human ovarian cancer OVCAR5 Cis-R (acquired cisplatin-resistance), FUOV1 and UWB.289 BRCA- (inherently chemoresistant), and murine ID8 (chemoresistant) cells (**Fig 4C**). These findings confirm that the top 6 compounds are not consistently killing the cells, and thereby making them stiffer. ***Cell Cycle.*** Since cell

deformability is sensitive to cell cycle stage, we also performed cell cycle analysis. While some compounds arrest cells in G2/M phase (vinblastine, vincristine), we find that the lead compounds do not consistently lead to change in cell cycle distribution (**Fig 5C**). ***Invasion/Migration.*** To assay cell invasion, we measure the number of cells that invade through basement membrane extract (BME) matrix in 8 μm pores using a transwell assay; we measure cell migration through uncoated transwell membranes. We find that all top 6 drugs consistently reduce the cell invasion and migration (**Fig 5A, B**). These findings are aligned with previous reports of how more invasive cancer cells are more deformable, and reflects how cellular deformability and motility are regulated by shared molecular mediators, suggesting that deformability can be used as a proxy for functional change for drug screening. Taken together, our data indicate that mechanotype screening can identify compounds that impair cancer cell motility, and thus may complement existing treatment strategies that are cytotoxic or induce cell cycle arrest. Future work will define the extent to which mechanotype screening can expand the feature space of drug discovery to identify compounds that improve the efficacy of existing treatments.

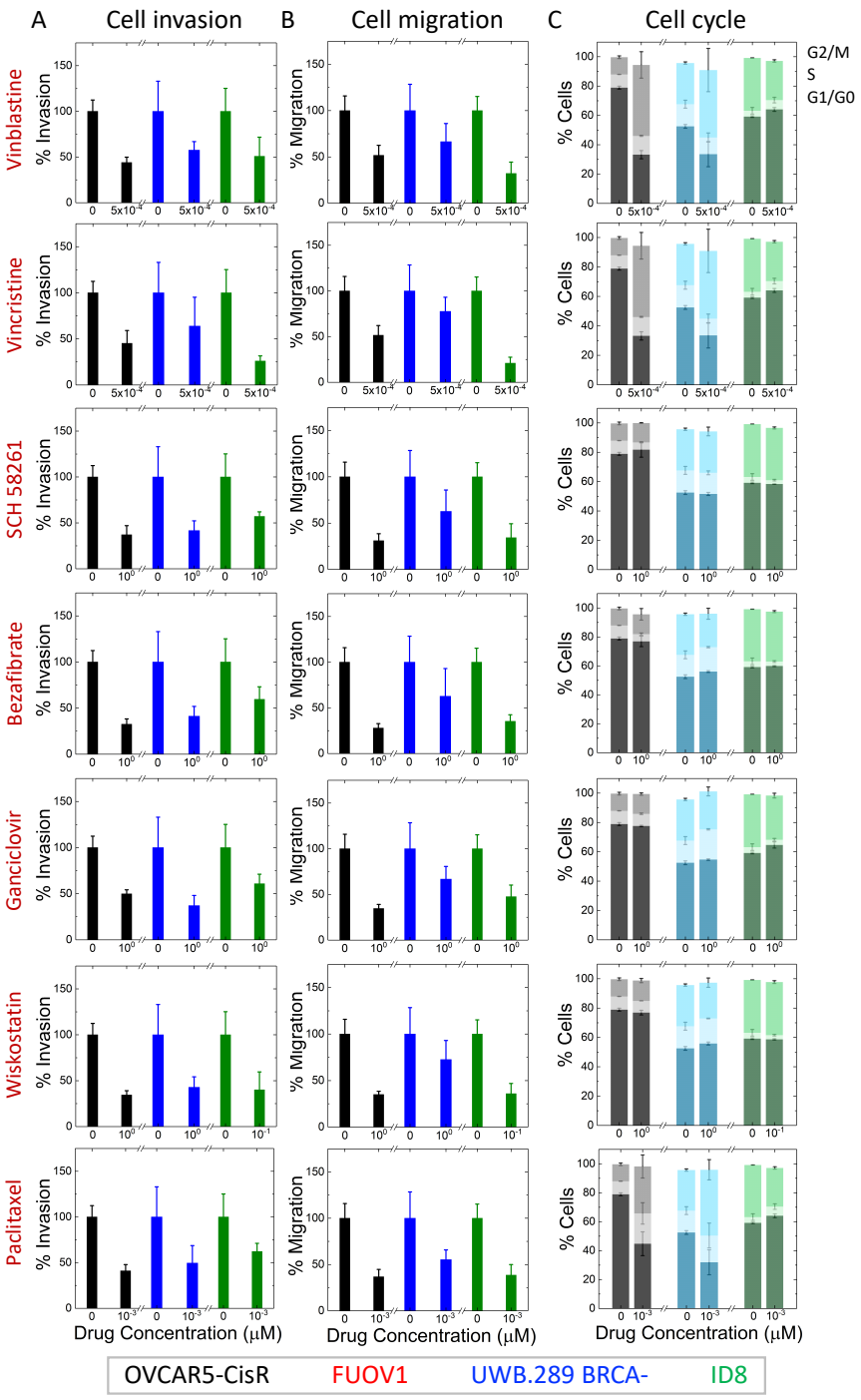

Figure 5. Functional assays to determine the effect of hits on cell invasion, migration and cell cycle. Cells are treated with the indicated sublethal concentrations of drugs for 24 h before and 24 h period during the transwell migration of cells through 8 μM transwell (A) coated with basement membrane extract (BME) matrix to quantify invasion, and (B) uncoated membrane to quantify cell migration. (C) Cell cycle distributions of cells with and without the drug treatments. All data obtained from three independent experiments. Each data point represents mean ± S.D.

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

The project is providing an excellent training opportunity for graduate student researcher in Molecular, Cellular, and Integrative Physiology (MCIP), Navjot Kaur Gill. Working on this challenging multidisciplinary project, Ms. Gill has developed her unique skill set in cancer biology and biotechnology in establishing this novel mechanotyping assay in the Molecular Shared Screening Resource (MSSR). She has worked closely with PI Rowat to troubleshoot the integration and optimization of the PMF technology in the MSSR. Ms. Gill also works closely with Dr. Robert Damoiseaux, Scientific Director of the MSSR to integrate PMF into the MSSR and implement the mechanotype screen. She has therefore gained valuable skills in assay development and laboratory automation. In addition, Ms. Gill is gaining valuable knowledge in ovarian cancer biology through the collaboration with Dr. Rao and Cedars Sinai. She is involved in the design of orthogonal experiments to test the effects of lead compounds on the invasion and proliferation of ovarian cancer cells, including patient cells.

Ms. Gill is the first author on a manuscript on the PDMS-PMF platform (Lab Chip 2019). She is also the first author on the protocol describing PMF methodology that is published on the Nature Protocol Exchange. Ms. Gill has also presented her work in a seminar for the MCIP graduate program on 07/11/17, entitled "*Cell mechanotype in tumor progression and metastasis*". She additionally authored the manuscript, '*DYT1 dystonia patient-derived fibroblasts have increased deformability and susceptibility to damage by mechanical forces'* (Gill et al, Frontiers Develop Cell Biol, https://doi.org/10.3389/fcell.2019.00103), and contributed to the published papers that use the PMF technology to measure cancer cell physical phenotypes:

Sobreiro MR, Chen JF, Novitskya T, You S, Morley S, Steadman K, Gill NK, Eskaros A, Rotinen M, Chu CY, Chung LWK, Tanaka H, Yang W, Knudsen BS, Tseng HR, Rowat AC, Posadas EM, Zijlstra A, Di Vizio D, Freeman MR (2018) Emerin deregulation links nuclear shape instability to metastatic potential. Cancer Research, 78: 6086-6097.

Nyberg KD, Bruce SL, Nguyen AV, Chan CK, Gill NK, Kim TH, Sloan EK, Rowat AC# (2018) Predicting cancer cell invasion by single-cell physical phenotyping. Integrative Biology 10: 218-231.

Lawrenson K, Segato F, Lee J, Pejovic T, Karlan BY, Freedman M, Gayther S, Vavra K, Lin X, Lin Y, Mhawech-Fauceglia P, Rowat AC, Gill NK, Drapkin R, Noushmehr, Hazelett D, Fonseca M, Liu A, Corona R, Dinh H, and Abbasi F. A Dualistic Model for High-Grade Serous Ovarian Cancer Origins. Under review.

Dr. Gill defended her thesis on Nov 28, 2018, and worked in the Rowat lab until February 2019. Dr. Gill is currently securing a postdoctoral researcher position.

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

PI Rowat delivered the following talks describing the project and acknowledging DoD funding:

• Department of Molecular Physiology & Biophysics and the Holden Comprehensive Cancer Center, University of Iowa, Colloquium

• Jonsson Comprehensive Cancer Center, UCLA, Colloquium (November 2018)

• Institute for Biomedical Engineering, Science and Technology (iBEST) Visiting Lecturer Series, Keenan Research Centre, St. Michael's Hospital, Toronto, Canada

• Society for Laboratory Automation and Screening (SLAS) Annual Conference, San Diego, USA [SLAS 2018 Innovation Award Top Candidate]

Co-I Lawrenson gave the following invited seminar:

• October 2018 - UPenn, invited seminar, invited by Dr. R Drapkin and Dr. George L. Gerton. Title: “Decoding the Noncoding Genome of Ovarian Cancers”

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

In the next reporting period, we will characterize effects of lead compounds on clinically-relevant mouse models. We will also validate the compounds using shRNA knockdown and cDNA-mediated overexpression.

4. IMPACT: *Describe 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?**

PMF-mechanotype screening provides a new paradigm for high throughput screening. Existing methods for measuring cell deformability rely on sequential measurements of cells. The ability to simultaneously measure the deformability of cell samples in a multiwell plate format enables scale-up of deformability assays. This is a key step towards advancing the use of mechanotype in clinical and research applications that require high throughput studies, such as in the context of small compound screening.

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

The ability to screen cells based on mechanotype opens up possibilities for screens that are relevant to other fields from cell biology to cancer. For example, a shRNA-screen could identify the origins of nuclear shape stability and mechanotranduction. We also recently completed a study on ‘*DYT1 dystonia patient-derived fibroblasts have increased deformability and susceptibility to damage by mechanical forces’* (Gill et al, Frontiers Develop Cell Biol) demonstrating how the PMF technology can provide insights into physical phenotypes of other disease models.

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

The PMF mechanotype-screening is now established in the UCLA Molecular Shared Screening Resource (MSSR). This core facility is available to UCLA and external researchers, who will now have the possibility to conduct mechanotype screen. Unexpectedly we discovered that the PMF system can enable washing of cells that are larger than the pores of the membrane in a high throughput setting as it interfaces with the automated liquid handling platform.

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

Nothing to Report.

5. CHANGES/PROBLEMS:

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

Changes that had a significant impact on expenditures – N/A

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

Significant changes in use or care of human subjects – N/A

Significant changes in use or care of vertebrate animals – N/A

Significant changes in use of biohazards and/or select agents – N/A

6. **PRODUCTS:**
   a. **Publications, conference papers, and presentations**

      i. **<u>Journal publications (since award start; red denote changes since last review period)</u>**

      Nyberg KD, Bruce SL, Nguyen AV, Chan CK, Gill NK, Kim TH, Sloan EK, **Rowat AC**[#] (2018) Predicting cancer cell invasion by single-cell physical phenotyping. *Integrative Biology* 10: 218-231. *Acknowledgement of federal support: Yes.*

      Sobreiro MR, Chen JF, Novitskya T, You S, Morley S, Steadman K, **Gill NK**, Eskaros A, Rotinen M, Chu CY, Chung LWK, Tanaka H, Yang W, Knudsen BS, Tseng HR, **Rowat AC**, Posadas EM, Zijlstra A, Di Vizio D, Freeman MR (2018) Emerin deregulation links nuclear shape instability to metastatic potential. *Cancer Research*, 78: 6086-6097. *Acknowledgement of federal support: Yes.*

      Gill NK, Ly C, Nyberg KD, Lee L, Qi D, Tofig B, Sobreiro MR, Dorigo O, Rao JY, Wiedemeyer R, Karlan B, **Lawrenson K**, Freeman MR, Damoiseaux R, **Rowat AC**. (2019) A scalable filtration method for high throughput screening based on cell deformability. *Lab Chip.* 19: 343 – 357. *Acknowledgement of federal support: Yes.*

      Gill NK, Ly C, Kim P, Fong LG, Young SG, Saunders CA, Luxton GWG, **Rowat AC** (2019) DYT1 dystonia patient-derived fibroblasts have increased deformability and susceptibility to damage by mechanical forces. *Frontiers Develop Cell Biol*, in press. *Acknowledgement of federal support: Yes.*

      Under review:

      Lawrenson K, Fonseca MAS, Liu AY, Segato F, Lee JM, Lin X, Corona RI, Abbasi F, Vavra KC, Dinh H, Gill NK, Seo JH, Coetzee S, Lin YG, Pejovic T, Mhawech-Fauceglia P, Rowat AC, Drapkin R, Karlan BY, Hazelett DJ, Freedman ML, Gayther SA, Noushmehr H. Dualistic Modeling of High-Grade Serous Ovarian Cancer Origins Identifies SOX18 as a Master Transcription Factor in Tumor Development . *Cell Reports, revision under review. Acknowledgement of federal support: Yes.*

      Manuscripts in preparation:

      Gill NK, Kim TH, Abbasi F, Karlan BY, Yang X, Lawrenson K, Rowat AC. High throughput filtration screening identifies novel anti-cancer compounds. (90% of data collected, draft of manuscript in progress) *Acknowledgement of federal support: Yes.*

      ii. **<u>Other publications, conference papers, and presentations</u>.**

      Other publications:

      Gill NK, Qi D, Kim TH, Chan CK, Nguyen AV, Nyberg KD, Rowat AC. A protocol for screening cells based on deformability using parallel microfiltration. Nature Protocol Exchange. *Acknowledgement of federal support: Yes.*

Presentations:

Jonsson Comprehensive Cancer Center, UCLA, Colloquium (November 2018)

Department of Molecular Physiology & Biophysics and the Holden Comprehensive Cancer Center, University of Iowa, Colloquium (November 2018)

Institute for Biomedical Engineering, Science and Technology (iBEST) Visiting Lecturer Series, Keenan Research Centre, St. Michael's Hospital, Toronto, Canada (October 2018)

Society for Laboratory Automation and Screening (SLAS) Annual Conference, San Diego, USA [SLAS 2018 Innovation Award Top Candidate] (February 2018)

National Taiwan University College of Medicine, Taipei, Taiwan, Workshop on '*Interdisciplinary Cell Culture and Analysis Technologies*' (September 2017)

b. **Technologies or techniques**
The mechanotype-screening platform is now installed in the UCLA core facility, the Molecular Shared Screening Resource.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Amy Rowat (UCLA)
Project Role:	PI
Nearest person month worked:	2.00
Contribution to Project:	Dr. Rowat oversaw all aspects of the project, including experimental design, execution, and data analysis and interpretation. She wrote the manuscripts (Gill et al, Lab Chip 2019 and Gill et al Frontiers Develop Cell Biol 2019) and prepared presentations as well as the progress report.

Name:	Dr. Robert Damoiseaux (UCLA)
Project Role:	Co-Investigator
Nearest person month worked:	0.5
Contribution to Project:	Dr. Damoiseaux oversaw the integration of Parallel Microfiltration (PMF) into the Molecular Shared Screening Resource at UCLA. He also oversaw the design of the mechanotype-screen and contributed to software developments to ensure seamless integration of PMF into the high throughput facilities.

Name:	Dr. Jianyu Rao (UCLA)

Project Role:	Co-Investigator
Nearest person month worked:	0.5
Contribution to Project:	Dr. Rao contributed expertise on the use of platinum-sensitive versus -resistant carcinomas in the mechanotype-screen.

Name:	Dr. Beth Karlan (Cedars Sinai)
Project Role:	Co-Investigator
Nearest person month worked:	0.12
Contribution to Project:	Dr. Karlan advised on the translational aspects of the proposal and participated in experimental design.

Name:	Dr. Kate Lawrenson (Cedars Sinai)
Project Role:	Co-Investigator
Nearest person month worked:	0.12
Contribution to Project:	Dr. Lawrenson advised on the translational aspects of the proposal and participated in experimental design.

Name:	Navjot Kaur Gill (UCLA)
Project Role:	Graduate Student Researcher
Nearest person month worked:	12.00
Contribution to Project:	Ms. Navjot Kaur Gill has conducted all aspects of the project, including developments in the hardware and software for the mechanotype screen, cell culture and drug treatment optimizations, fabrication of PMF devices, as well as experimental design, execution, and data analysis and interpretation. She has written manuscripts describing her work and prepared presentations.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

• **Dr. Rowat**:

Title: *Plant-based scaffolds for marbled clean beef*
Agency: The Good Food Institute
Agency Contact: Erin Rees Clayton, ErinC@gfi.org
Role: Principal Investigator
Performance Period: 02/15/2019 – 02/14/2021

Funding: $227,273 (Total Direct Costs)
Effort: 1.0 summer month, 100% effort
Goal: The goal of this project is to engineer marbled clean beef to have similar texture as conventional beef by engineering myocyte-adipocyte co-cultures.
Specific Aims: (1) Establish a structured scaffold to promote myotube formation. (2) Produce marbled clean meat with myotubes and adipocytes.

Title: *Re-purposing beta-blockers to improve chemotherapy response*
Agency: UCLA Jonsson Comprehensive Cancer Center
Agency Contact: Nancy Presseau, NPresseau@mednet.ucla.edu
Role: Principal Investigator
Performance Period: 01/15/2019 – 01/14/2020
Funding: $50,000 (Total Direct Costs)
Effort: 0.1 months
Goal: The goal of this proposal is to test the hypothesis that β-blockers increase response to chemotherapy by decreasing tumor stiffness.
Specific Aims: (1) Test the hypothesis that β-blockade increases the penetration of chemotherapy drugs to kill tumor cells. (2) Define molecular mechanisms of how β-blockers modulate tumor cell response to chemotherapy.

Title: *Understanding how stress hormone signaling impacts cellular mechanotype*
Agency: National Science Foundation
Agency Contact: Director of Biomechanics and Mechanobiology, Laurel Kuxhaus, lkuxhaus@nsf.gov
Role: Principal Investigator; co-PI Dr. Parag Katira
Performance Period: 05/15/2019 – 04/30/2022
Funding: $323,357 (Total Direct Costs)
Effort: 1 calendar months
Goal: The goal of this proposal is to understand the molecular pathways through which soluble stress hormone cues regulate cellular mechanotype.
Specific Aims: (1) Test the hypothesis that stress hormones alter the mechanotype of epithelial cells through a βAR-RhoA-ROCK-NMII axis. (2) Define how stress hormone signaling impacts cell-matrix interactions.

Dr. Lawrenson:

Title: *Genomic and Transcriptomic Analysis of Breast and Ovarian Cancers*
Agency: National Institutes of Health (1R01-CA211574-01A1) / University of Virginia
Agency Officer: Leslie Hickman - (301) 631-3009
BG 9609 MSC 9760, 9609 Medical Center Drive, Bethesda, MD 20892-9760
Period: 02/01/2018 – 01/31/2023 **Funding:** $186,474 Annual Direct
Role: Co-Investigator **Effort:** 10% - 1.20 Calendar Months
Project Goals: This study will identify common pathways underlying susceptibility to ovarian and/or breast cancer, through transcription-wide analysis of gene expression associated with risk variants identified by GWAS, and to use functional assays to validate target genes and pathways identified by transcriptomics.
Specific Aims: (1) Identify Candidate Susceptibility Genes Associated with Ovarian and Breast Cancer PrediXcan Expression Quantitative Trait Locus Analysis; (2) Validate the Functional Role of Candidate Genes in Experimental Models of Breast and Ovarian Normal and Cancer Tissues; (3) Perform Genome Wide Functional Screens of Breast/Ovarian Cancer Experimental Models of Breast Ovarian Cancer Susceptibility to Common Biological Networks; (4) Perform Chromosome Conformation Capture Based on Confirmed Breast/Ovarian Cancer Genes and Functional Evaluation of Linked Interactive SNP-enhancers.

Title: *Single Cell Analyses of Epigenomes and Transcriptomes to Characterize the Biological Links Between Endometriosis and Ovarian Cancer*
Agency: Cedars-Sinai Leon Fine Award in Translation Science

Agency Contact: Martin Saavedra – (310) 423-0406
8700 Beverly Boulevard, Davis 5903, Los Angeles, CA 90048
Period: 09/01/2018 – 08/31/2023 **Funding:** $99,935 Annual Direct
Role: Principal Investigator **Effort:** 1% - 0.12 Calendar Months
Project Goals: To use single cell profiling to map cellular and transcriptional heterogeneity in endometriosis.
Specific Aims: (1) Mapping inter-patient heterogeneity in endometriosis; (2) Generating novel models of endometriosis.

What other organizations were involved as partners?

Organization Name: Cedars-Sinai Medical Center

Location of Organization: Los Angeles, CA

Partner's contribution to the project:

Collaboration: During the reporting period PI Rowat met with Co-Investigator, Dr. Kate Lawrenson, at Cedars-Sinai Medical Center to discuss research directions.

SPECIAL REPORTING REQUIREMENTS

a. **COLLABORATIVE AWARDS:** An independent report is being submitted by Collaborating PI, Dr. Lawrenson from Cedars Sinai Medical Center.