



Proposals must be submitted as a pdf file in Anubis "Call for larger CBCS project support".
Please go to the CBCS homepage (www.cbcs.se) for further details.
Deadline for submission: xxxxxxxxxxxx

Project proposal with Chemical Biology Consortium Sweden

Instructions: Project proposals are uploaded directly as a **pdf file** into Anubis in the open call for larger CBCS project support. All material is considered confidential, and access is restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research.

The scope of the collaborative CBCS projects varies significantly and the different project types require different competencies from CBCS. To ensure that we can deliver support to prioritized projects, we categorize the project proposals as either of the types 1-5 below.

(1) Assay Development project

An example may include assay design, plate formatting, and assay optimization. This project type is applicable when:

- no suitable compounds are available for biological studies and an assay that meets the general screening requirements is not yet ready
- compounds are available but need optimization for biological/preclinical studies and an assay that meets the general optimization requirements is not yet ready

For assay development projects approved by PRC and that run successfully according to a pre-agreed project plan between CBCS and the PI, the following screening campaign (project type 2) or chemistry activities (project type 5) will not require a new PRC proposal.

(2) Screening project

A typical example includes assay transfer efforts followed by the completion of a screening campaign, including hit confirmation and the first follow-up studies to characterize the value of identified hits. This activity can also include virtual screening campaigns based on primary screening data or other ligand-based virtual screening approaches, structure-based screening, as well as fragment-based screening techniques. For studies further investigating the identified hits including the synthesis of hits and new analogs, or establishment of structure-activity relationship a new PRC proposal will be required. This project type is applicable when:

- no suitable compounds are available for the described biological studies and an assay that meets general screening requirements as listed in the technical feasibility section is available
- a crystal structure of the target protein exists and there are no suitable compounds for the described biological studies
- no suitable compounds are available for the described biological studies but ligands for the protein target are known

If the general screening requirements are not met, you should also apply for an assay development project (project type 1).

Appendix 1: Work in progress STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

(3) Target identification/mode of action project

This type of project typically follows a phenotypic screening campaign, and the main goal is to understand the molecular targets or mode of action of a compound or hit series. Techniques used for these projects are often multidisciplinary and can include Cell Painting, CRISPR screening, and proteomics-based approaches. This type of project is applicable when:

- a hit compound has been sufficiently validated, e.g. demonstrates efficacy in dose-response studies, and is not ruled out counter screens
- the compound is sufficiently cell penetrable for downstream studies
- in the ideal scenario an inactive close analogue of the hit compound has been identified

In some cases, it may be desirable to establish a structure-activity relationship for the series of compounds before the identification of the target/MOA. Choose an Enabling Chemistry (type 5 project) as well.

(4) Compound or disease profiling project

This type of project includes the profiling of disease models against libraries of compounds in functional precision medicine studies, as well as Cell Painting studies. This type of project is applicable when:

- Characterized disease models exist
- Relevant compound libraries exist

If the general optimization/SAR requirements are not met, you should also apply for an assay development project (project type 1).

(5) Enabling chemistry project

A typical example includes optimization activities post a screening campaign or otherwise identified small molecule modulators. More specifically, the request to optimize or modify compounds to suit the needs of the investigator may require activities such as structure-activity relationship (SAR) studies, *in silico* and/or *in vitro* physicochemical or pharmacokinetic characterization, and the various *in vitro* pharmaceutical profiling tests available. This can also include *in silico* hit expansion and lead optimization studies. This project type is applicable when:

- no suitable compounds are available for the described biological or preclinical studies
- an assay that meets the general optimization/SAR requirements as listed in the technical feasibility section is available
- if desired, data for *in silico* hit expansion can be delivered

If the general optimization/SAR requirements are not met, you should also apply for an assay development project (project type 1).

Appendix 1: Work in progress
STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

Date of Submission	On time!
Principal Investigator	XX
Primary Affiliation	Swedish University
Application for project type (1-5)	2

Title of proposal

A phenotypic screen to identify new tool compounds for low grade endometrial stromal sarcoma

Abstract (max 750 characters)

Low grade endometrial stromal sarcoma (ESS) is a rare malignant tumor and few cell lines for this disease are available today. We have generated unique patient-derived cells (PDCs) and patient-derived fibroblasts (PDFs) from a patient with low grade ESS. Importantly, the PDCs show good recapitulation of the genomic alterations found in the original tumor tissue. We have screened both the PDCs and PDFs separately against a library of 525 oncology drugs but uncovered few drugs that selectively kill the cancer cells. This suggests a need for new compounds to better understand the functional dependencies of this rare disease, as well as to provide new treatment options for patients. To address this, in this project we will screen for new compounds that selectively kill these cells.

Appendix 1: Work in progress STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

Background and expected scientific impact (max 3 pages)

(1) What scientific need does the proposed project address?

New strategies for drug discovery are urgently needed to bring treatments to patients with no further options. This is especially important for patients with rare diseases, where pharma industry has little incentive to invest in expensive development. Here, we propose to use cells isolated from a patient with a rare gynecological cancer in a phenotypic screen to either repurpose existing drugs outside of oncology indications or identify new tool compounds relevant to this rare disease. This study serves as in a pilot test of a new paradigm in drug discovery we call near-patient drug discovery.

(2) Concisely describe the scientific challenge and what makes your approach unique. Briefly explain the underlying biology of the proposed research. Include references.

We have generated patient-derived cell (PDC) cultures from disaggregated tumor tissue from a patient with relapsed low-grade endometrial stromal sarcoma (ESS). Importantly, the PDCs show good recapitulation of genomic features to the original tumor tissue (Figure 1A). This is a rare disease and only a handful of cell lines representing this disease are available to date. ESS cancers are often driven by fusion genes and transcriptomics of the tumor tissue and PDCs are ongoing to ideally uncover any fusion gene. We are also going to characterize these cell lines by proteomics. Importantly and unlike available cell lines, we have also isolated the corresponding patient-derived fibroblast (PDF) cultures.

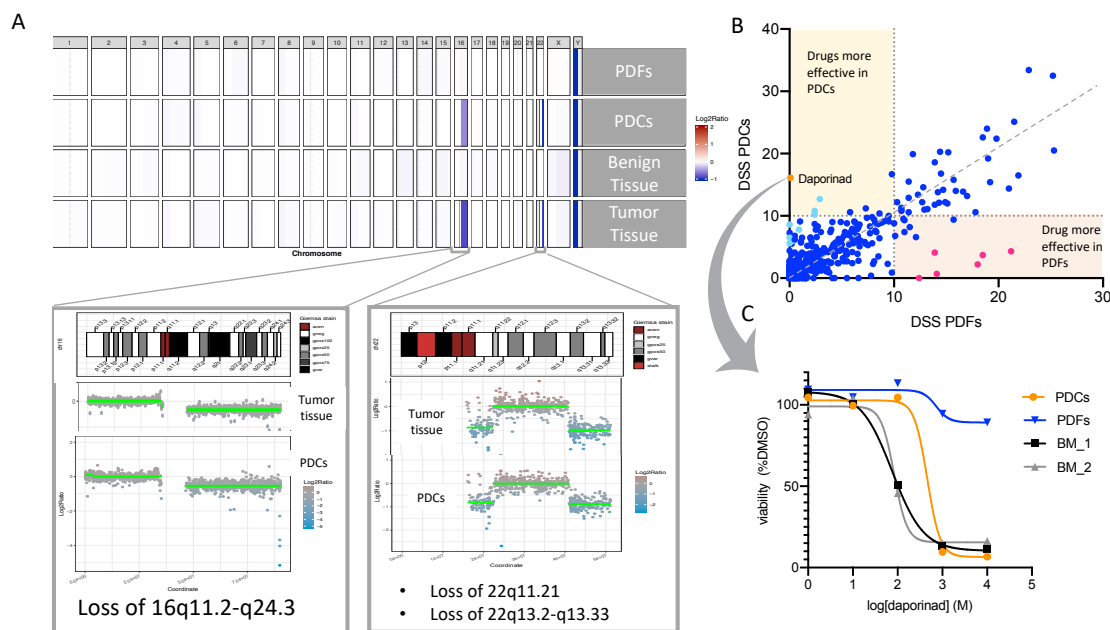


Figure 1. Near-patient drug testing in low-grade ESS reveals urgent need for new compounds. A) Copy number alterations found in PDCs, PDFs, tumor tissue and benign tissue from a patient diagnosed with low-grade ESS. The tumor tissue and PDCs share identical losses on chromosomes 16 and 22, demonstrating the relevance of the model, as shown in insets. B) Comparison of drug sensitivity scores (DSS) from PDCs (y-axis) and PDFs (x-axis) across 525 drugs and 3 combinations (dark blue points, larger DSS means more cell killing). There are several compounds with high response in PDFs and little response in PDCs (red-orange quadrant in bottom right, pink points). There are few compounds with high DSS in PDCs and low DSS in PDFs (yellow-orange quadrant top left, light blue points are HDAC inhibitors and the orange point is Daporinad.) C) Concentration-response curves from two healthy bone-marrow donors (BM_1, BM_2), PDCs and PDFs for Daporinad. Combined Figures B and C clearly demonstrate that novel compounds are needed for this disease, as there are few drugs giving PDC-selective responses and Daporinad demonstrates high toxicity in healthy bone marrow.

Appendix 1: Work in progress
STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

(3) Describe the relevance of the proposed study (biological/chemical novelty and applications of potential discoveries) and include any emerging data supporting this. Add relevant literature references, figures, and tables.

We have performed drug testing using 525 drugs plus several combinations on these cultures separately using CellTiterGlo. Comparing the drug sensitivity scores (DSS, higher number means more cell killing) between PDCs and PDFs demonstrate that there are few drugs that are more effective at killing the PDCs than PDFs (Figure 1B, upper left quadrant). Daporinad, a NAMPT inhibitor, which shows the most promising selectivity for the PDCs (Figure 1B orange dot). However, daporinad kills cells from healthy bone marrow samples from two donors more effectively (lower IC_{50}) than the PDCs, suggesting high toxicity of this drug and lack of therapeutic window for treatment. (Figure 1C). These data reveal an urgent need for new drugs and drug candidates in this space. Ideally, this could come from drugs outside of oncology indications screened here that could be rapidly translated to a new clinical space or new compounds.

(4) Are small molecule tools already available for the current purpose? Ensure you have made a detailed search. Add relevant literature references.

Please see above.

(5) Describe how new or optimized compounds can be used to increase the basic understanding of the biological system and why existing compounds, when available, cannot be used for this research purpose. (Note: This section may not be relevant for functional precision medicine and Cell Painting projects).

Optimized compounds have the potential to reveal novel cancer dependencies for patients with low grade endometrial stromal sarcoma.

(6) If the project concerns the study of a specific target or pathway for which there are currently no small molecule tools available, has this target or pathway been previously modulated by other means than small molecules (e.g. genetic methods)?

No.

(7) Are there already disease models in place for functional precision medicine studies or Cell Painting projects? Please describe their disease relevance and characterization.

NA

(8) Does your project require an ethical permit, an addendum to the existing ethical permit, or a material transfer agreement?

Work to be carried out in PIs lab where ethical permit is in place

Appendix 1: Work in progress STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

Technical description and feasibility (max 3 pages)

Most chemical biology projects are based on an assay (a primary screening assay, a SAR driving assay, or an otherwise needed method) used to initially assess the value of provided small molecules. Information on the development and current status of this assay and planned follow-up activities for the project is needed to estimate the resources required to carry out and publish the proposed work. For chemistry proposals including SAR studies, the assay must have a dynamic range, *i.e.* is sensitive enough, to rank small molecules with potencies of different orders of magnitude.

Questions for project types 1-4.

Assay Development, Screening, Target ID/MoA, or Compound/disease profiling projects.

(9) Give a detailed technical description of the primary screening assay and outline the successive steps. Describe the format of the assay (96- or 384-well plate format or another non-plate-based format). Include information on available positive and negative controls and describe their biological relevance.

To date we have expanded the PDCs such that we have sufficient cells to perform a primary screen on 15000 compounds, as well as hit validation and follow-up on 300 compounds. We also have viably frozen disaggregated tissue from this patient if needed for additional follow-up. The primary screen will be CellTiterGlo, using only the PDCs and applying the drugs at a single concentration, looking for compounds that kill the PDCs (Figure 2). CellTiterGlo is a luciferase based assay system that quantifies ATP as a surrogate for viability. We have good precedence for this screen as we have already tested the PDCs and PDFs using the FIMM Oncology library of 525 drugs as described above (Question 3) in 384 well plates (8 plates for each cell type). In these assays benzethonium chloride is used as cell death control and DMSO is used as the negative control. Cells are seeded into plates pre-spotted with compound using a Multidrop (25 μ L cell suspension) and incubated for 72 hours prior to readout with CellTiterGlo (30 μ L). CellTiterGlo signal is read using an EnSight Plate reader by luminescence and results are normalized per plate and passed into a curve-fitting algorithm. In the proposed project, the hit list will likely be large and hits ($n=200-300$) will be prioritized for further study by first looking for concentration response in PDCs. Then to remove overtly toxic compounds, we will counter-screen the hit list in bone marrow (BM) cells isolated from $n=2$ donors using CellTiterGlo in concentration response, looking for compounds where a reasonable difference in IC_{50} between PDCs and BM cells is observed. The remaining hit compounds will then be tested in concentration response in both the PDCs and PDFs using an in house multiparametric, time-lapse assay (see below) to prioritize compounds that induce cell death in the PDCs and spare the PDFs ($n\sim 20-40$). We will further characterize the hit compounds for toxicity across a panel of hepatocytes using this in-house assay as well. At the end of this screening campaign we hope to have 1-5 candidates that kill the PDCs selectively and will move forward with validation studies in other cellular model systems, such as Ba/F3 cells with the suspected fusion protein, or appropriate CRISPR-Cas9-engineered cell models. We will also follow-up in patient material from patients with ESS identified during the project.

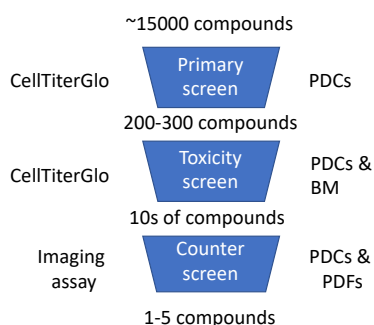


Figure 2. Proposed screening funnel.

(10) Do you have a suggestion on what compound library/libraries to screen?

We currently have enough PDCs to screen 15000 compounds at single dose and up to 300 compounds in dose. PDFs are slower growing and we currently have sufficient numbers to use for counter screening ca. 100 compounds in 10 point dose. Compounds will be selected from the *Primary Screening Set* of the Chemical Biology Consortium Sweden, which comprises compounds that are

chemically diverse and have lead- to drug-like properties, as well as selected screening sets with known drugs outside of oncology indications. We would prioritize compounds with known mechanism of action and then some of the diverse set.

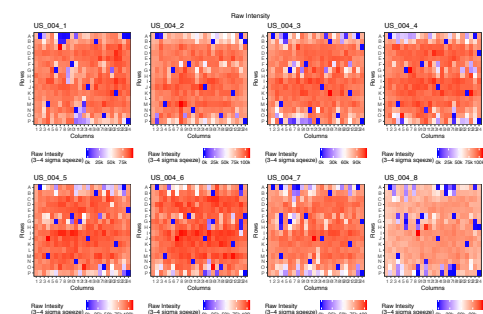
Appendix 1: Work in progress STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

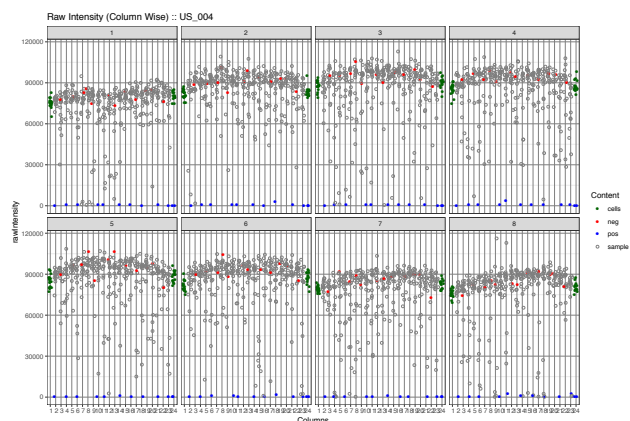
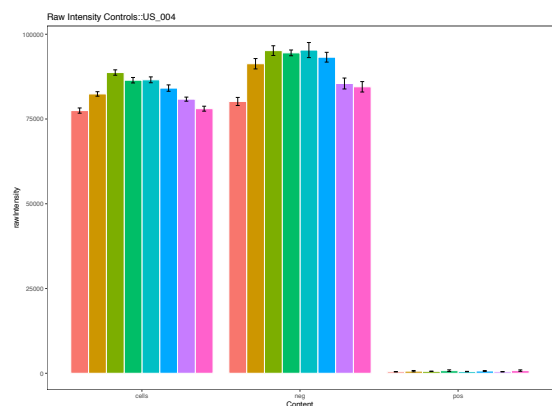
(11) List the availability, source (in-house production or commercial), and estimated costs of the reagents needed to perform a screening campaign of the number of compounds stated in *question (10)*.

We currently use healthy bone marrow samples to rank compounds in our precision medicine pipeline in AML and can have access to these cells through our AML systems medicine project. CellTiterGlo is commercial. Estimated cost for full screen is 5 000 SEK for plates, 30 000 SEK for CTG, 60 000 for compound plating, and 30 hours from CBCS. The PI will take care of cell expansion and all cell culture reagents.

(12) Show all current experimental data illustrating the performance of the assay: relevant controls, Z' factor (for a screen project $Z' > 0.5$ is desirable), signal to background ratio, DMSO tolerance, reagent stability, and plate edge effects.¹ If your assay is at a stage where this is not applicable please explain what steps are lacking. For functional precision medicine assays characterization of disease relevance or plans for these studies are necessary.



We have done cell titration of the PDCs and PDFs in 384 well plates and completed an initial screen using CellTiterGlo of the FIMM Oncology Library (525 compounds, 3 combinations, 5-point concentration response) using 1500 cells/well. In these screens (8 X 384 well plates for each cell type) the Z' scores range from 0.76 to 0.88. The images to the left give plate overviews for all plates screened for PDCs to visualize if there are plate edge effects (none observed here). In the images blue is more cell killing (and dark blue is usually cell death control benzethonium chloride, which is scattered in the plate layouts.)



Above left: Average signal for each of 8 plates for PDCs with no treatment, PDCs treated with DMSO and PDCs treated with positive control, benzethonium chloride. Above right: Column-wise plot of signal for each well for each of the 8 plates to look for column trends (Blue is positive control: Benzethonium chloride, red is DMSO and green is PDCs alone (no DMSO)).

¹ If you are not familiar with these terms, please see e.g. the Assay guidelines manual [Internet], available from: <https://www.ncbi.nlm.nih.gov/books/NBK53196/> (Sittampalam GS, Coussens NP, Brimacombe K, et al., editors. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004-), or contact CBCS personnel for assistance.

Appendix 1: Work in progress STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

(13) Describe the availability and current status (up and running/under construction/at the planning stage) of an appropriate counter assay² and an assay with an orthogonal readout.³

To rule out overtly toxic compounds and compounds that interfere with luciferase based detection system in CellTiterGlo we will screen the hits against bone marrow cells from healthy donors (n=2). Compounds that kill the PDCs more effectively than the bone marrow cells will be prioritized. We use healthy bone marrow cells to validate our findings in our AML precision medicine pipeline, thus this assay is well established in our lab. (Pemovska, Cancer Discovery, 2013).

(14) Describe the availability and status of additional secondary assays such as selectivity assays, cellular assays, and in vivo models (if applicable to your project).

We currently receive fresh tissue, ascites (a buildup of fluid in peritoneum) and blood from patients with gynecological cancer in a project. Any additional cases could be used to validate these results or determine if the compounds uncovered in our screen have a broader clinical relevance. Furthermore, we have access to iPSC-derived hepatocytes from six donors that we can use to examine liver toxicity across a panel of individuals.

We are currently validating a live-cell imaging assay to quantify cell death with single-cell resolution. *Preliminary results.* To move away from bulk measurements of relative cell viability, we are focusing on time-lapse imaging of drug-treated cells in the presence of live-cell staining reagents. Specifically, we use a high-content imaging platform that gives single-cell resolution and where multiple measurements characterizing cellular response to drug treatment can be taken simultaneously over time (5 lasers for excitation + brightfield or digital phase contrast). We hypothesize that data from this platform will allow us to characterize single-cell response to drug treatment over time, creating fingerprints of drug sensitivity for each compound and cell system, which may have better predictive value than the current state-of-the-art. We have already assembled a set of live-cell dyes reporting on cell health or different stages of cell death (Figure 3). We have created reproducible protocols for cell seeding, dye addition and drug treatment. We have preliminary data from treatment of CaOv3 cells and PBMCs with BzCl (cell death control used in our routine CellTiterGlo assays), staurosporine (multikinase inhibitor) and FCCP (mitochondrial phosphorylation uncoupler) over 24 hours further strengthening the feasibility of the workflow. Initial image analysis of these results show that we are able to effectively segment the cells and even trace a majority of single cells across each time point (Figure 3B-D). Importantly, this image analysis shows expected trends, including loss of mitochondrial membrane potential with all three compounds, plasma membrane permeabilization by BzCl in 2-3 hours, and caspase 3/7 activation starting around 12 hours with staurosporine treatment (Figure 3B,C). Furthermore, individual cell displacement and accumulated distance in representative wells for each condition are distinct both between treatments and timepoints. Interestingly, distinct morphology changes are observed due to drug treatment (e.g. rounding, changes in cell area), and we plan to quantify these during the proposed project.

² The purpose of a counter assay is to identify compounds that interfere with the read-out in the primary assay by e.g. auto-fluorescence. A counter assay can also be used to identify compounds with undesirable properties such as cytotoxicity.

³ An orthogonal assay is generally performed using a different read-out than that applied in the primary assay to further eliminate false positive compounds from the screen.

Appendix 1: Work in progress
STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

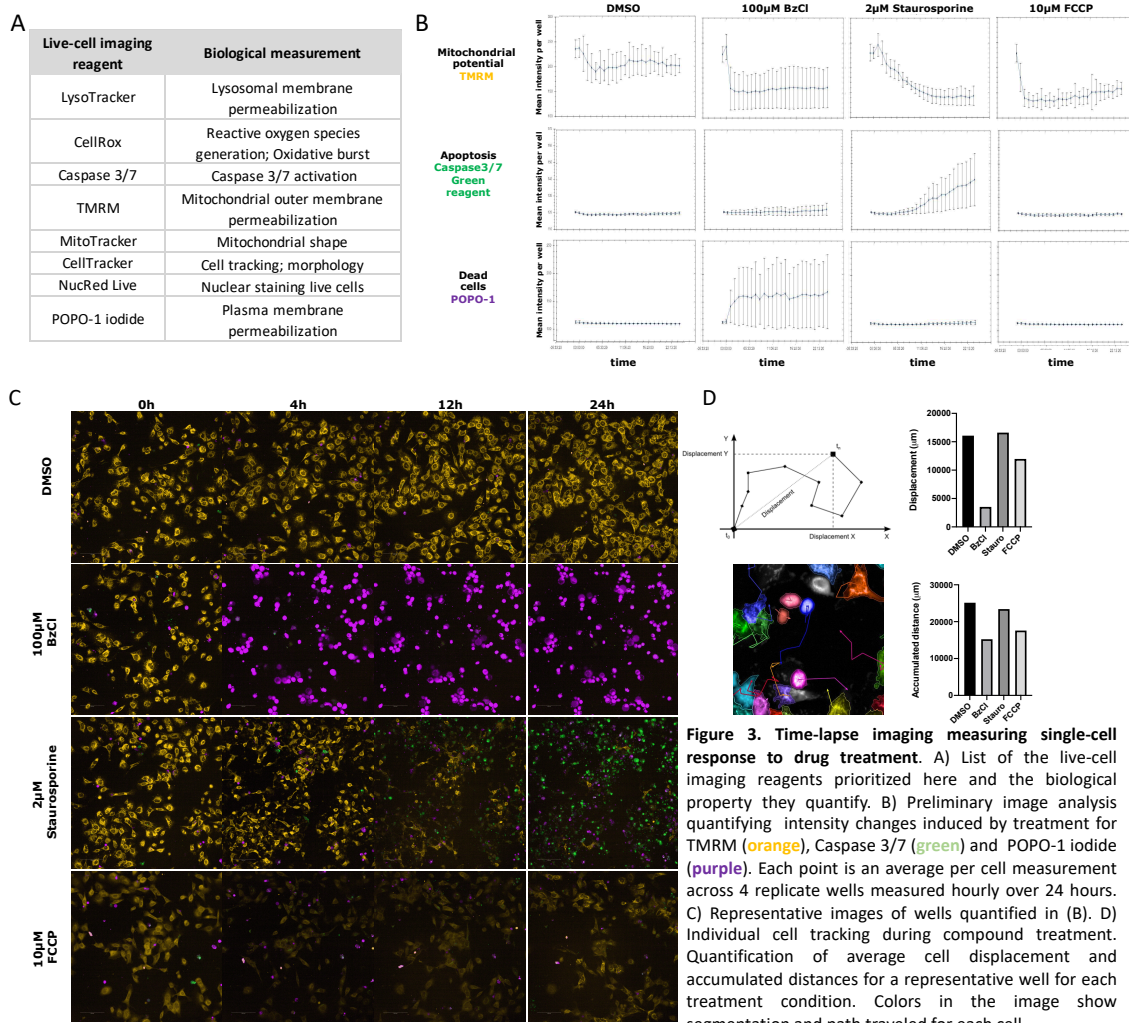


Figure 3. Time-lapse imaging measuring single-cell response to drug treatment. A) List of the live-cell imaging reagents prioritized here and the biological property they quantify. B) Preliminary image analysis quantifying intensity changes induced by treatment for TMRM (orange), Caspase 3/7 (green) and POPO-1 iodide (purple). Each point is an average per cell measurement across 4 replicate wells measured hourly over 24 hours. C) Representative images of wells quantified in (B). D) Individual cell tracking during compound treatment. Quantification of average cell displacement and accumulated distances for a representative well for each treatment condition. Colors in the image show segmentation and path traveled for each cell.

(15) If in silico studies like virtual screening are planned describe steps and available data (e.g. crystal structure).

NA

Questions for project type 5

Enabling chemistry (must include a description of the SAR-driven assay)

(16) Describe the chemical series including identification, validation studies, and intended sites for modification.

[Click here to enter text.](#)

Appendix 1: Work in progress
STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

(17) Give a detailed technical description of the intended SAR driving assay or alternative method for compound testing. Outline the successive steps, and describe the assay format. Include information on available controls/reference compounds and describe their biological relevance.

[Click here to enter text.](#)

(18) Present experimental data with the intended reference compound demonstrating a dose-response experiment (n=3 with a minimum of 7 points) tested at a minimum of two independent occasions.

[Click here to enter text.](#)

(19) Have several compounds with different potencies been tested in the SAR driving assay?

[Click here to enter text.](#)

(20) Suggest secondary assays such as selectivity assays, cellular assays, and *in vivo* models (if applicable to your project) and describe the current status (up and running/under construction/at the planning stage). For assays not available in your lab, please describe plans for accessing them (via other research infrastructures or else).

[Click here to enter text.](#)

(21) Describe any available plans (if applicable) for pharmaceutical property profiling?

[Click here to enter text.](#)

(22) If *in silico* studies are planned describe steps and available data (eg crystal structure).

[Click here to enter text.](#)

Appendix 1: Work in progress
STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

Project plan (max 2 pages)

(23) What are the short and long-term goals of the proposed project?

It takes the pharma industry up to 15 years and an average of €2B to develop a new drug. One key reason for the poor translation to clinical efficacy is the inability of existing model systems to accurately report on disease biology in and between patients. Model systems traditionally used in drug discovery campaigns are often selected based on ease of handling rather than physiological relevance. Through our molecular precision medicine pipeline we have validated patient-derived cells, including more than one cell type, e.g. fibroblasts, and cancer cells from a patient with low grade endometrial stromal sarcoma. In this project we will apply these primary cell cultures to phenotypic drug screening to uncover new opportunities for drug repurposing and drug development.

Overall, using this strategy, we hope to identify new candidate compounds for patients with endometrial stromal sarcomas, and potentially repurpose drugs outside of oncology indications into this space. The short-term goals are to complete the screen and hit validation. The longer-term goals are to bring new treatments to patients with rare gynecological cancers. This study serves as a proof-of-concept test for our near-patient drug discovery pipeline and we hypothesize that leads identified through this pipeline will be more relevant than hits coming from traditional drug screening approaches on isolated proteins.

(24) Please provide information on the proposed research project plan by listing activities and potential milestones in the table below (Table 1). Add all activities relevant to reaching publication. For each activity, disclose the tentative time required, which aspects of the project require input/resources from CBCS, and the current availability of different types of resources (human, financial, and instrumentation). All technical/scientific details relating to each activity and milestone should be provided in the section on technical feasibility (see above).

Table 1. Summary of all project activities to reach publication

Project activity <i>E.g., assay development, assay transfer, assay optimization, assay validation, screening, hit confirmation, enabling chemistry, efficacy models, in vivo studies, etc.</i>	Time (months) ^a	Responsible ^b	Resources ^c		
			Human <i>(e.g., postdoc or Ph.D. student)</i>	Financial <i>(Secured or unsecured)</i>	Instrument <i>(Available in-house, yes or no)</i>
Screen 15000 compounds PDCs	1	CBCS	PI will grow cells for screening	Funding available	yes
Data analysis and hit selection	1	CBCS/PI	Technician	Funding available	
Dose-response PDCs	1	CBCS/PI	Technician	Funding available	
Dose-response BM	2	CBCS/PI	Technician	Funding available	
Selection of compounds for further follow-up	1	CBCS/PI	Technician	Funding available	
Multi-parametric imaging assay	3	PI	Technician	Funding available	Yes, microscope
Mechanism of action validation (if known compound)	4	PI	Technician	Funding available	

Appendix 1: Work in progress
STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

Target id (CETSA MS, CRISPR-Cas9 genome-wide)	6-12	PI	Technician	Need funding
Follow-up and validation (Ba/F3, CRISPR-Cas9) models	6-12	PI	Technician	Need funding
(a) Estimated time for a full-time employee to complete these activities; (b) Indicate who will be primarily responsible for resourcing the activity (CBCS, PI, co-PI, CRO, etc.); (c) Please specify PI resources as human : e.g. postdoc or Ph.D. student/ financial : secured or unsecured/ instrument : available in-house yes or no.				

(25) Propose at least one stop/go decision point relating to any uncertainties concerning the technical feasibility as described above.

If no selective compounds are identified, known compounds could be used to characterize the functional dependencies of these cells as an outcome. If we cannot access healthy bone marrow, peripheral blood mononuclear cells (PBMCs) from 2 donors will be used. If the live-cell imaging assay does not work adequately for this project we will use Cell Painting to validate the cells or other fixed cell staining assays that are available in-house.

(26) Have you contacted CBCS personnel and discussed the proposal and project plan in detail (required for submission)? Specify CBCS node and person.

Yes, Hanna Axelsson.

Publication strategy (max 1 page)

(27) Chemical biology projects are complex and often require significant validation steps including multiple model systems and detailed mechanism of action studies. Please detail the steps to gather this information for your project to ensure the identification of a robust small-molecule tool. This includes a publication strategy and potential intellectual property interests if applicable. Suggest a tentative journal of publication for a successful project and disclose which aspects of a draft manuscript that require input/resources from CBCS and comment on the importance of these efforts. The publication plan will often determine the requirements for validation of tool compounds, eg if animal models are necessary.

Depending on the results we will implement different publication strategies. If a class of compounds with known target/mechanism-of-action is identified, these will immediately be selected for follow up and validation studies. These results will be published after validation, resulting potentially in 1 medium-high impact paper. Further clinical studies will be initiated based on these results. If the compounds identified have no known target or mechanism of action we will then try several methods for target identification before publication (ie CETSA MS, pooled CRISPR-Cas9 screening or shRNA libraries). This will probably result in a need for new funding application and based on these results we may also initiate chemistry efforts to identify better lead compounds with CBCS (new PRC application). Together the screen and target-identification studies will result in 1 medium-high impact paper. Any additional chemistry efforts will also result in communication on the chemistry efforts used to drive the SAR as well. If no compounds are identified that have selectivity for the PDCs we will use the known drugs and annotated compounds that were screened to describe the functional landscape of these cells resulting in 1 low-medium impact paper.

Appendix 1: Work in progress STRICTLY CONFIDENTIAL

Information within this appendix is provided to and restricted to a limited group of CBCS Project Review Committee members and CBCS personnel under conditions of confidentiality, solely for the purpose of evaluating the proposed research. The nature of the material is such that it is considered as unconfirmed work in progress until the proposed research is completed and the resulting data are published in peer reviewed scientific journals. All material herein and any related information provided during the review process are strictly confidential and will be treated as such by reviewers and CBCS personnel.

Additional Comments

(Please enter any additional comments you see fit to clarify your proposal)