ENZYMATIC INHIBITORS FOR COMBINED ANTITUMORAL THERAPY IN GYNAECOLOGICAL CANCERS

RESEARCH PROPOSAL

Final Degree Project
2020

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Bachelor's degree in Biotechnology

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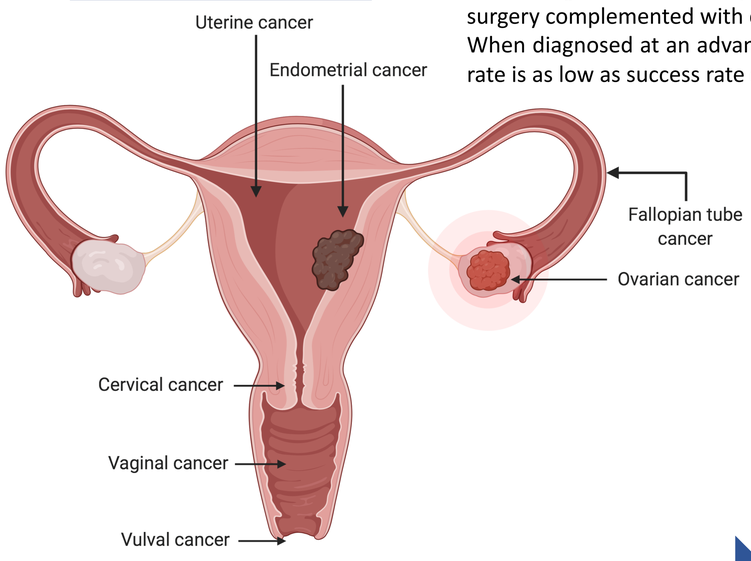
BACKGROUND

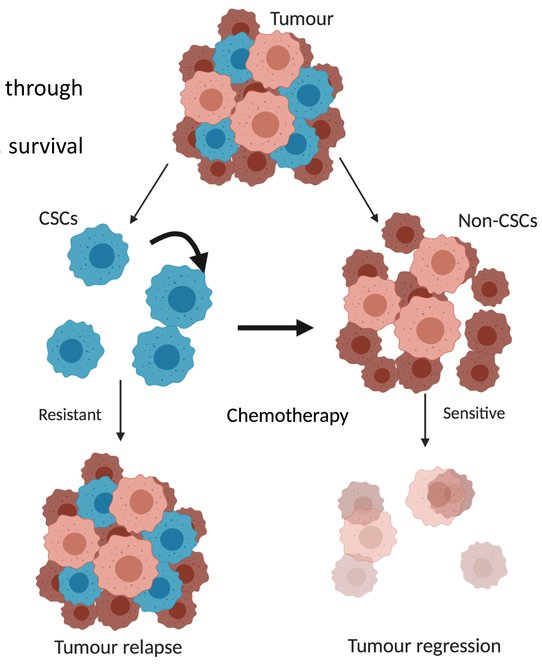


GYNAECOLOGICAL CANCERS

When diagnosed at an early stage, cancer can be treated through surgery complemented with chemotherapy.
When diagnosed at an advanced stage (75% of the cases), survival rate is as low as success rate of chemotherapy.

Cancer stem cells (CSCs)
Self-renewal
Differentiation
Tumorigenicity capabilities
Markers: CD44, CD133, CD117, ALDH, CD14.

Chemoresistance
Drug efflux
Necrosis: ALDH
Survival pathways: PI3K, PTEN, Akt



HYPOTHESIS AND OBJECTIVES

Chemotherapy combined with an inhibitor makes treatment more effective avoiding chemoresistance and making cells sensitive to it.

To identify and characterize CSCs.

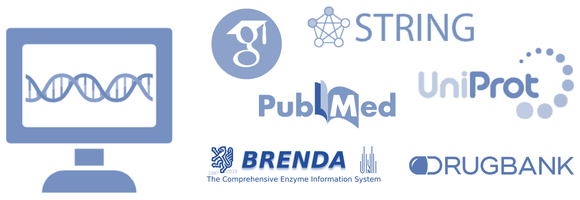
To analyse inhibitors and evaluate its potential as drugs comparing at different levels.

To investigate the role of inhibitors in CSCs targeting enzymes involved in chemoresistance.

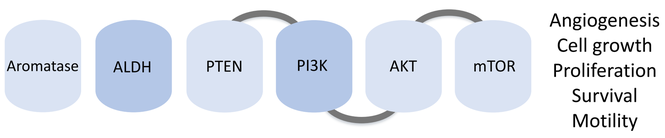
To implement HT assays to screen compounds.

Develop a combination of drugs for gynaecological cancers treatment

TARGET IDENTIFICATION



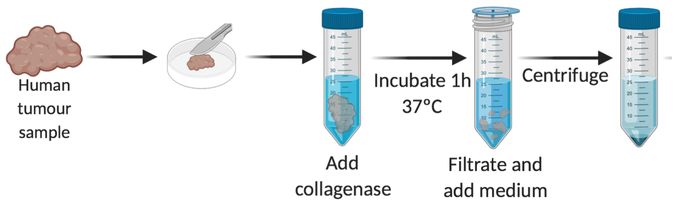
Keywords: chemoresistance, enzymes, drug discovery, inhibitors, gynaecological cancers, combined therapy, cancer stem cells



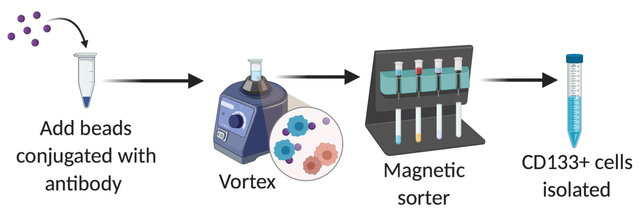
Angiogenesis
Cell growth
Proliferation
Survival
Motility

Cancer Stem Cells culture

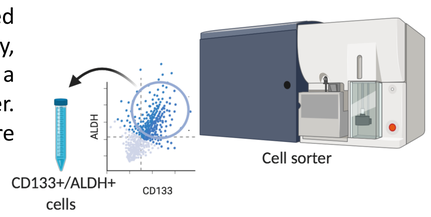
Digestion: Human tumour sample is obtained surgically from a biopsy. Mechanically and enzymatically disaggregated using collagenase. Then, filtration and centrifugation are performed to recuperate cells.

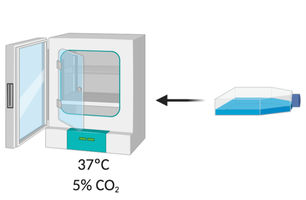


First selection: CD133+ are selected by MACS method. Beads conjugated with specific antibody against CD133 are added to the sample. CD133+ cells are recovered using a magnetic sorter.



Second selection: Based on ALDEFLUOR™ assay, cells are selected with a cell sorter cytometer. CD133+/ALDH+ cells are obtained and seeded.



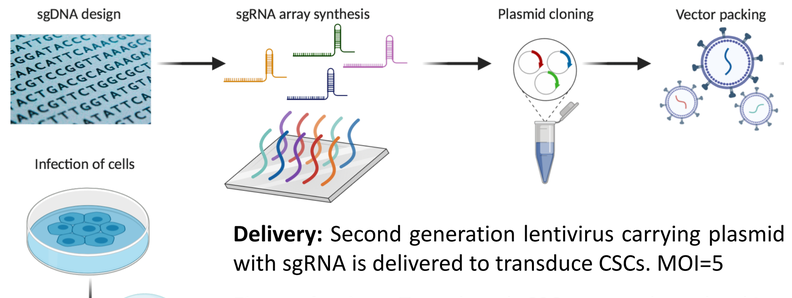


Culture conditions:
37ºC, 5% CO_2
DMEM/F12 medium supplemented with growth factors (hEGF, bFGF, B27)

TARGET VALIDATION

Knockout – CRISPR/Cas9 system

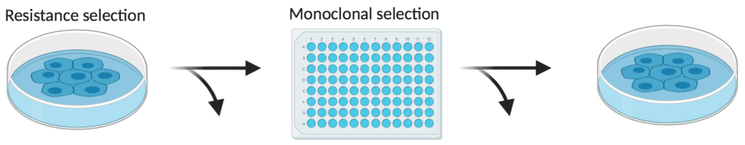
System construction: sgDNA is designed and constructed for PI3K and ALDH. Selection is essential to ensure high yields. Then, cloned into a plasmid with puromycin resistance. Packed in lentiCRISPR vector that encodes for Cas9.



Delivery: Second generation lentivirus carrying plasmid with sgRNA is delivered to transduce CSCs. MOI=5

First selection: Transduced CSCs are selected taking advantage of the resistance included in the plasmid.

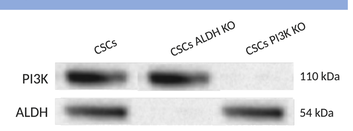
Second selection: Clones are serially diluted in a 96 well and correctly transduced clone is amplified, after analysis.



DNA and protein samples are obtained from the culture before editing and after each selection.

Knockout validation

Protein expression
Western Blot using antibodies against PI3K and ALDH and protein quantification with BCA assay.

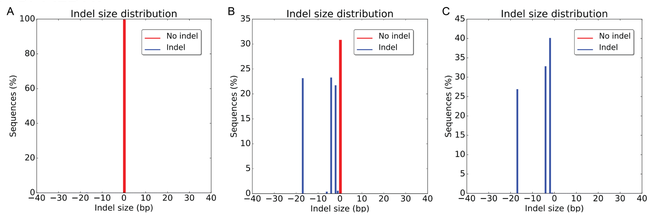


PI3K is not expressed in CSCs knocked out for this gene. The same results are shown for ALDH.

DNA Sequencing

Results presented after the second selection comparing to CSCs before editing.

Extraction, purification and amplification of DNA to sequence. PI3K is deleted from the sequence in CSCs knocked out for this gene. The same results are shown for ALDH.



HIT & LEAD IDENTIFICATION

In silico

To narrow compounds library creating an small subset of molecules using databases and bioinformatic tools.

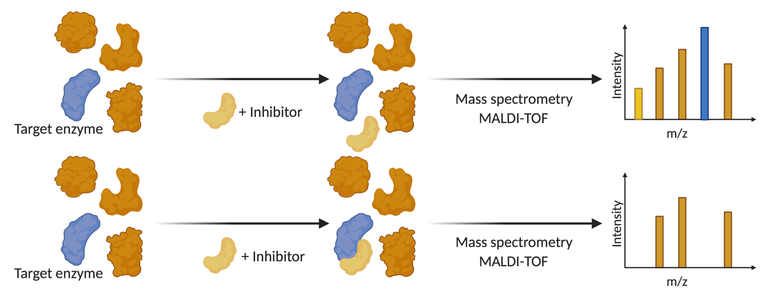
PI3K	ALDH
Buparlisib	Phenylglyoxal
Wortmannin	Disulfiram
PI103	CM10
LY294002	N-acetyl-N-acetoxy-4-chlorobenzene sulphonamide
GDC-0941	DEAB
BYL719	DIMATE
PF-04691502	673A

molinspiration cheminformatics

Lipinski parameters
Hydrogen bond donor count, Hydrogen bond acceptor count, LogP (Lipophilicity), Molecular weight, Volume, O and N atoms, PSA (Polar Surface Area), Routable bonds.

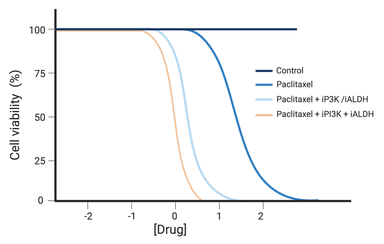
In vitro

SCREENING
Created library is used to screen inhibitors against PI3K and ALDH in order to analyse hits. Mass spectrometry intensity fading (IFMS) based on direct reaction with MALDI-TOF.

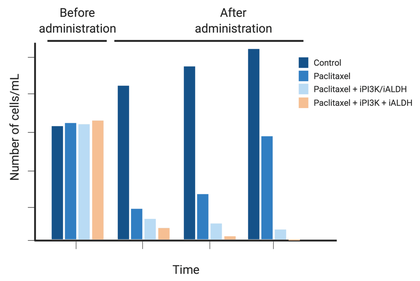


Inhibitors interacting with PI3K or ALDH disappear from the graph alongside with the enzyme.

CELL ANALYSIS



Cell viability characterizes each drug to calculate IC50 and compare these concentrations. Conditions that require a low concentration are considered promising.



Cell proliferation in different conditions is tested in order to see its efficiency in CSCs eradication. Combined therapy shows successful results.

HIT TO LEAD

LEADS SELECTION

LEADS VALIDATION

LEADS OPTIMIZATION

DRUG DEVELOPMENT

CONCLUSIONS

1. The use of CD133 marker and ALDH enzymatic activity is more efficient than a unique method when isolating cancer stem cells.
2. Either PI3K or ALDH gene knock out slows down cell proliferation and makes cells sensitive to paclitaxel treatment.
3. PF-04691502 and Disulfiram, a PI3K inhibitor and an ALDH inhibitor, respectively, combined with paclitaxel chemotherapy achieves a lower IC50 in cancer stem cells.

Combined administration of PF-04691502 and Disulfiram with paclitaxel chemotherapy makes cancer stem cells sensitive to paclitaxel administration ensuring effectiveness.

DISSEMINATION PLAN

2 or more publications

Cancer Cell
Oncogene

- Cancer Stem Cells characterization
- Relevant enzymes validation in chemoresistance
- Promising inhibitors that reduce resistance to chemotherapy

Results presentation at national and international relevant conferences in cancer area

Dissemination in seminars to state holders

Patent

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