UNIVERSITY OF CAPE TOWN
COMPUTATIONAL BIOLOGY DIVISION

Bioinformatics Support Request

Please provide us with more information on your request for support. Complete the form as comprehensively as possible, and please indicate where there is still uncertainty.

Please note, the earlier we are involved the better – for example, it would be better for us to be involved during the study design and even grant application stage.

CONTACT DETAILS	
Date of request	**25th May 2018**
Name	**Jenna Bleloch**
Email address	**blljen010@myuct.ac.za**
Research Group/Department	**Prince Laboratory, Division of Cell Biology, Department of Human Biology**
Faculty	**Health Sciences**
IF student, name & email of supervisor	**Professor Sharon Prince sharon.prince@uct.ac.za**

PROJECT DETAILS

1. What is the scientific question?

Background:

Various animal species can regenerate injured limbs, tails and organs. A unique feature of this process is the blastema, a mass of undifferentiated cells that forms at the site of injury. These primitive cells multiply and then differentiate into specialised tissues of the missing part, like muscle and bone. Little is known of the factors that control this remarkable event and each stage may have its own regulators. Malignancy, like the blastema, may represent a form of cell-growth that could respond to one or several of the regenerative factors. In 1948, Rose[1] implanted a frog kidney-tumour into the forelimbs of salamanders, a species noted for its powers of regeneration. The tumours spread widely and killed the animals. However, when a leg was amputated through or below the malignancy, the tumour cells re-differentiated as did those of the blastema. And as the limb regenerated, muscle-cell islands of frog origin could be distinguished from those of the salamander by their smaller nuclei. Similar results occurred when Seilern-Aspang and Kratochwil (1962)[2] induced malignancies in salamanders by painting the skin with carcinogenic agents. In the triclad, *Dendrocoelum lacteum,* they demonstrated that reversion of malignancy is not confined to the regenerating part only[3].

The differentiation factor(s) may be identical in all regenerates.

Research Question:

This study describes the effect of an agent, isolated from tadpole-tail blastemas, on the pediatric cancer, rhabdomyosarcoma.

Preliminary Data and Current Experiments:

We have generated some promising preliminary data (see attached) showing anti-cancer activity of tadpole tail extracts. We have gone on to do some MALDI to try and decipher what the effective agent could be (we have the data, but have done no analyses). We have recently prepared untreated and treated rhabdomyosarcoma cells (n=5) for mass spectrometry and if all goes well we should be receiving the data soon.

2. Who are the partners on the project?

Professor Vincent Harrison (retired pediatric surgeon who began this research 20 years ago and approached Sharon to continue the research), Professor Sharon Prince with PhD student Jenna Bleloch who is carrying out the work, and Blackburn group members Dr. Nelson Soares and Dr. Bridget Calder who have been assisting and advising on the mass spectrometry experiments.

3. What type of collaboration with CBIO is expected? For a project that is done as collaboration or for a fee, we will put the agreement in writing.

We would like to collaborate with CBIO for bioinformatic analyses of MALDI mass spec results (identify what could be responsible for anti-cancer activity within the tadpole extracts) and mass spec results of untreated and treated cancer cells (specifically the pediatric cancer rhabdomyosarcoma) to determine how this extract is exerting its anti-cancer activity.

4. Are there any ethical issues we should be aware of?

No. Ethics was acquired 20 years ago when the tadpole tail samples were extracted.

5. How much work is expected from CBIO and when?

We would require CBIO to advise on and do the bioinformatic analyses of the MALD and mass spec results in collaboration with Jenna Bleloch (who would love to learn)

6. What type of data will be generated (e.g. sequencing, genotyping, expression, etc.) and what technology platform will be used?

- Identification of peptides/proteins in efficacious tadpole samples that could be responsible for mediating anti-cancer activity with pathway analyses.
- Proteomic signature of treated cells and identification of pathways and signaling involved in mediating an anti-cancer response.

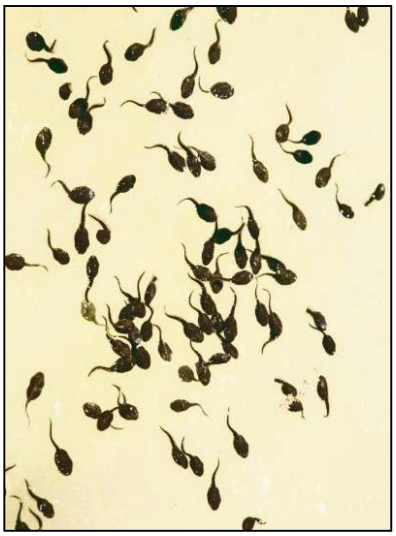
We will need to be advised on the technology platform to be used.

7. When do you expect the data? Does it need to be transferred from somewhere else?
We have the MALD results in an excel spreadsheet and should be receiving the mass pec results on treated cells soon. The data does not need to be transferred.
8. How large will the data be? How long does it need to stored for, and have you made arrangements for storage?
• 4 samples for MALDI • Mass spec on untreated and treated cells n=5 (vehicle and tadpole treated cells - 10 samples total)
9. What bioinformatics analysis needs to be done? Which tools are required?
We need to be advised on this.
10. If a collaborative model is being used, what papers are envisaged and who will the authors be?
We anticipate getting a publication out of this project and the authors will include Jenna Bleloch, Bridget Calder, Nelson Soares, Vincent Harrison, Sharon Prince and with your collaboration we will also include you (Katie Lennard and Nicola Mulder).
11. Can we add a short description and objective of the project to the CBIO website?
Yes.

PLEASE FORWARD THE COMPLETED FORM TO:

Nicola.mulder@uct.ac.za

Materials & Methods

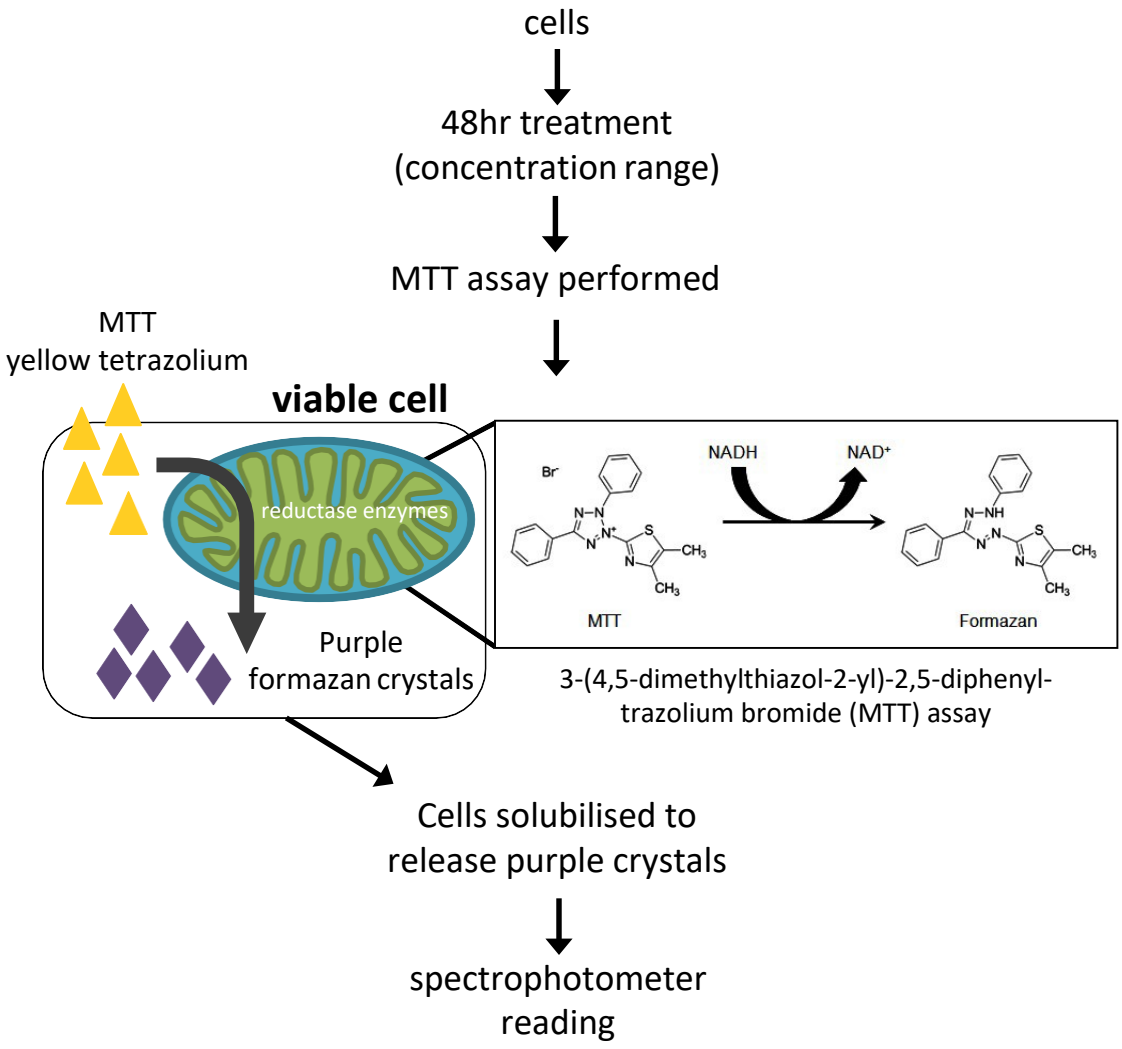


Tadpole Samples:
Samples were obtained from three separate tadpole specimens and evaporated to a crystaline deposit which was dissolved in 5ml PBS and filtered through 0.22um filter (this is more dilute than the samples from 2 decades ago which were dissolved in 1ml PBS before filtration).

Each dialysate was ultra-sonicated for 6 minutes, spun at 3000 rpm for 30 minutes. The deposit was discarded and the remaining solution was poured into a dialysis tube (6000 – 8000 daltons) and dialysed for 3 days in PBS.

Samples to be Tested:
A1, A2, B1, B2, 1, 2, 3, 4, 5, 6, 7, 8

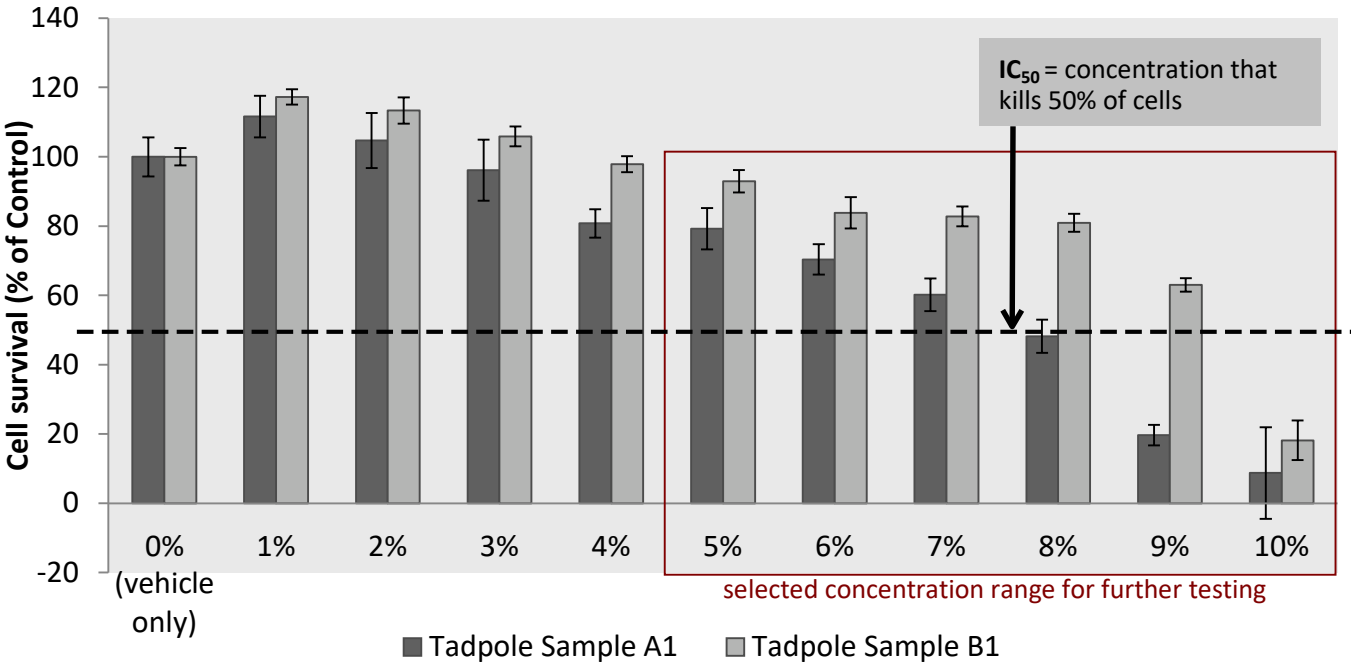
Method: Cytotoxicity Assay



Results: Initial screening to identify 'concentration' range

Concentration of tadpole samples is unknown so percentage of sample diluted in cell culture medium was used (e.g. 10% is 100ul sample in 1ml medium). Osteosarcoma cells were available and used to determine 'concentration' range with anti-cancer effects. Experiment done in quadruplicate.

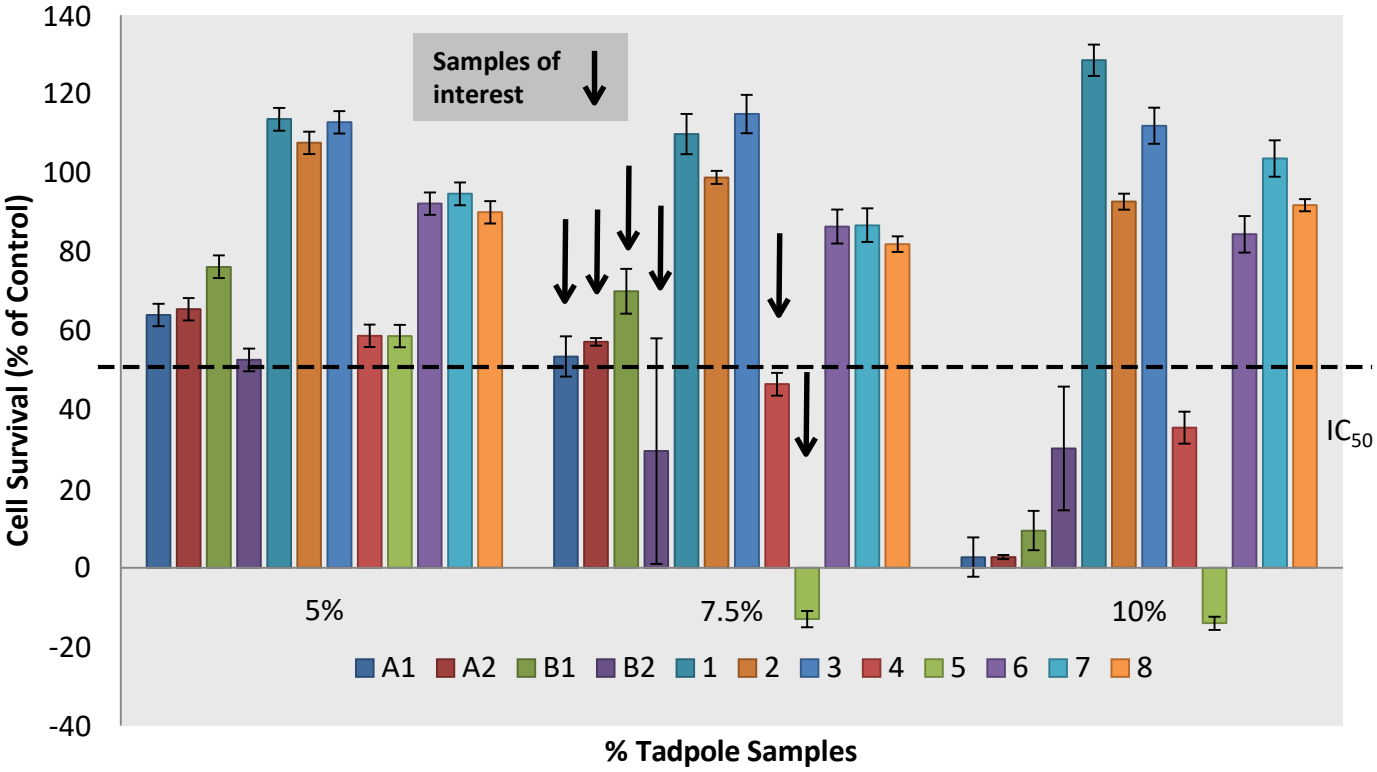
Fig. 1. Osteosarcoma sells (MG63) treated with tadpole samples A1 & B1 48h



Results: Screening of all samples for anti-cancer activity

Three 'concentrations' spanning the range of interest indicated in Fig. 1. were used to test the anti-cancer activity of tadpole samples A1-2, B1-2 and 1-8 in embryonal rhabdomyosarcoma cells (RD cell line).

Fig. 2. Embryonal rhabdomyosarcoma cells (RD) treated with tadpole samples for 48h



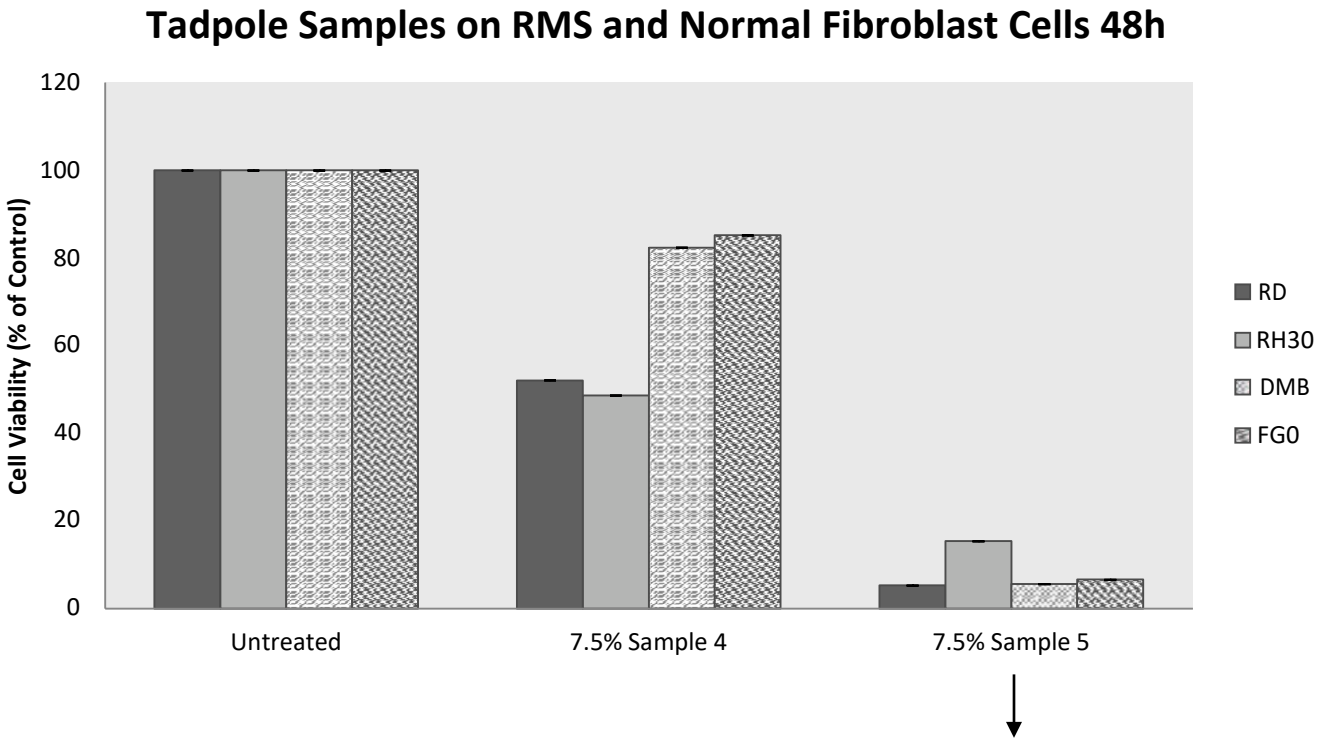
NOTE: one cannot really compare the efficacy of samples as the original concentrations are unknown and are most likely to be different.

More information on samples of interest:
A1, A2, B1, B2 and 4 are dialysates (i.e. not put on the column)
A2 was heated to 100°C
Sample 5 is the 10 minute post-column eluent from the sample 4 dialysate

Sample 4 and 5 selected for further analysis

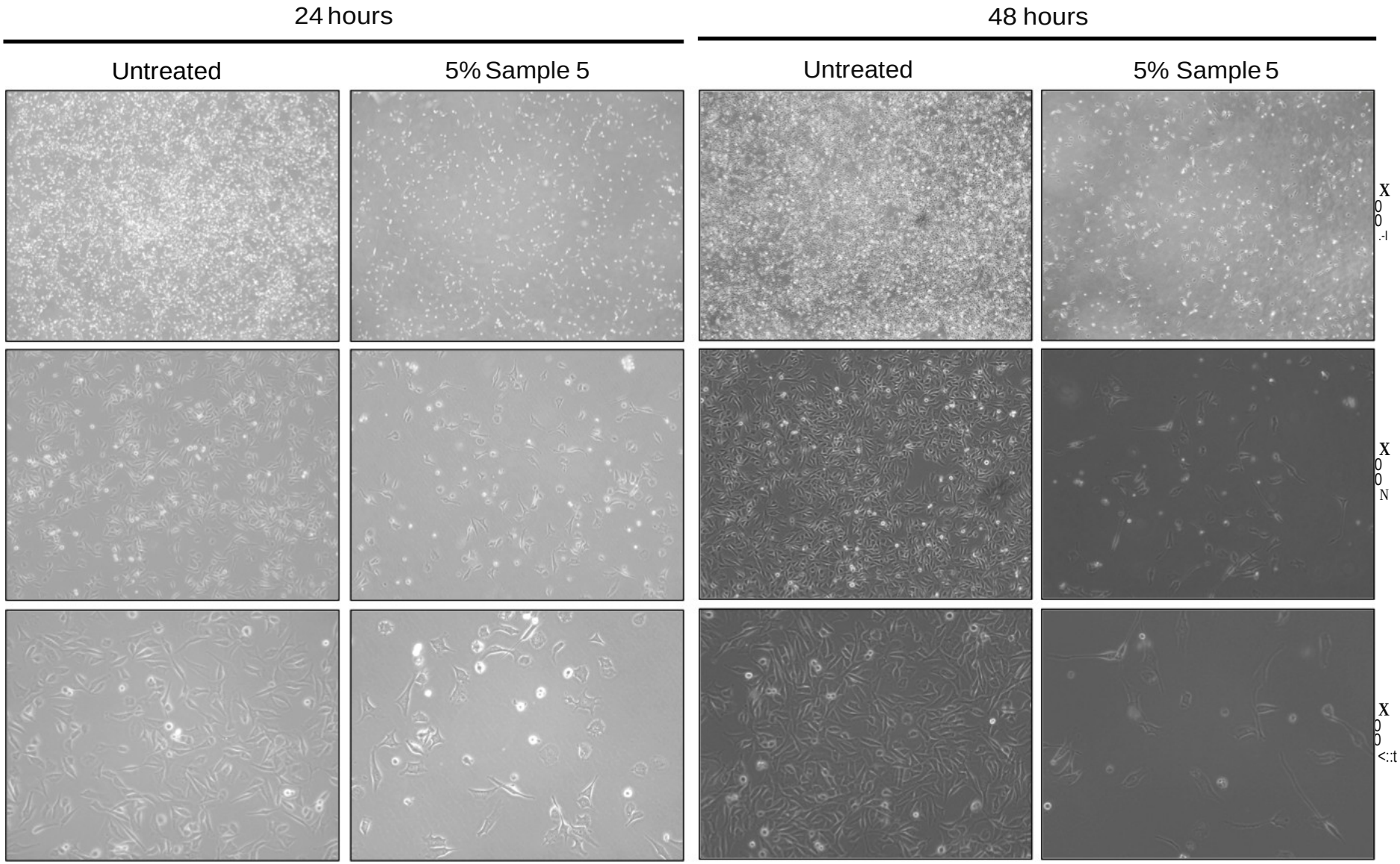
Results: Differential activity of samples in cancer vs normal cells

Sample 4, a dialysate, and it's 10 minute post-column eluent, Sample 5, at a 'concentration' of 7.5% (75ul sample diluted in 1ml medium) were used to investigate the effect of tadpole tail extracts on normal human fibroblast cell lines (FG0 and DMB) and both embryonal (RD) and alveolar rhabdomyosarcoma (RH30) cells



Cells much more sensitive to Sample 5 – concentration used here is too high – both cancer and normal cells died

RH30 Cells


24 hours
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RD Cells

24 hours

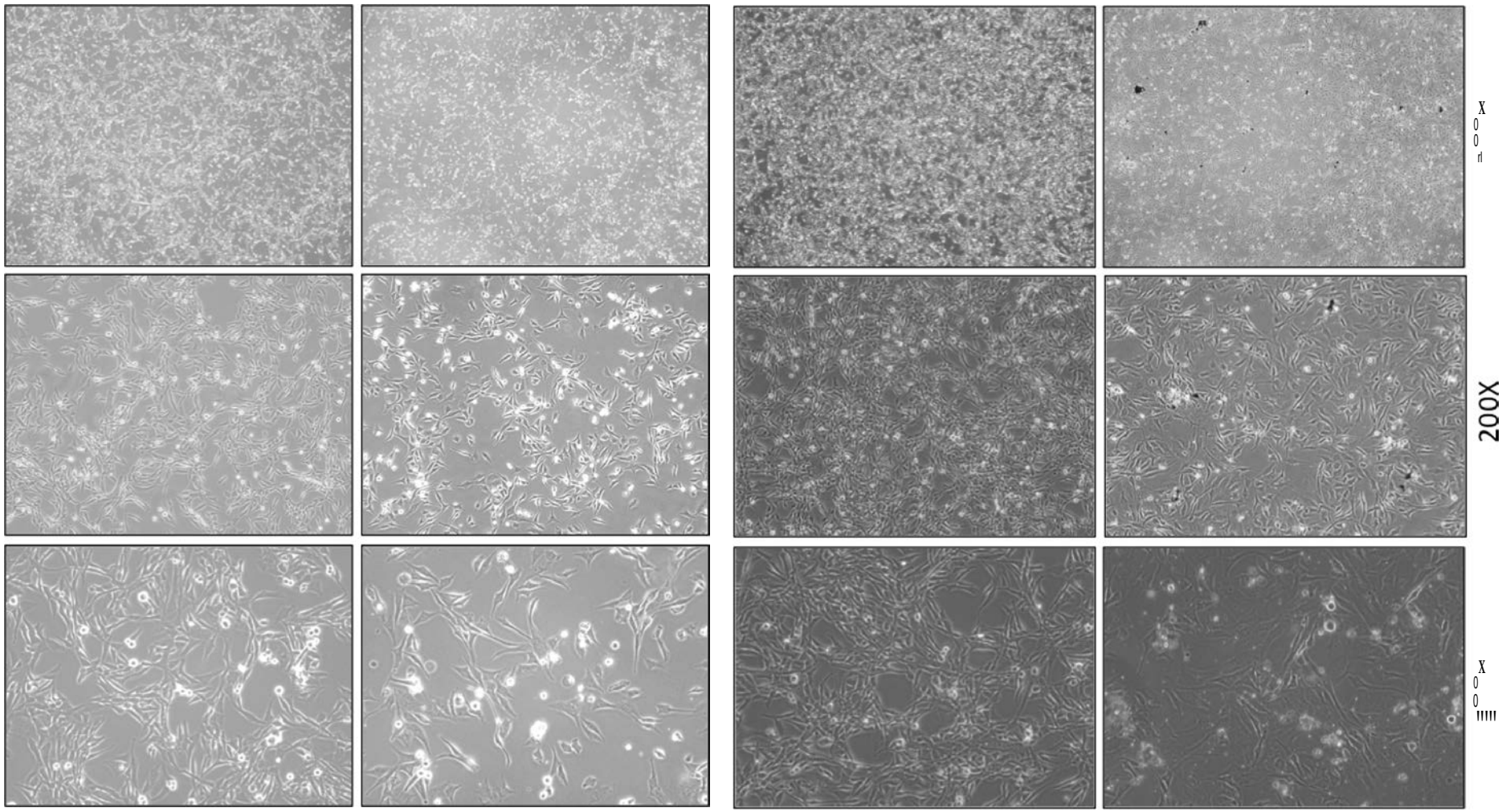
48 hours

Untreated

5%Sample S

Untreated

5%Sample S



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