QIMR Berghofer

Biology Data Resource Pack

Complementing the Queensland Senior Biology Syllabus, Unit 1 – Cells and multicellular organisms

Dataset 1

A chemical called EBC-46, which was discovered from the fruit of the north Queensland rainforest tree, Blushwood Berry (*Fontainea picrosperma*), was proposed as a new chemotherapy drug. EBC-46 can be injected directly into skin tumours to kill the cancerous tissue. The effective dose of EBC-46 and the time taken for it to kill tumour cells were assessed using different cell lines of melanoma. Cell lines are populations of identical cells that are grown in the laboratory from a single originating cell and used as an experimental model. Cancerous cells differ between people, and even within a single tumour, so the scientists tested multiple cell lines to understand how varied the responses to a drug could be. The experiments to investigate how EBC-46 kills cancer cells were performed in three different ways:

1) using cancer cells grown in cultures in the laboratory ("*in vitro*"),

2) by monitoring the responses of tumours growing on live mice ("*in vivo*"), and

3) by extracting treated tumours from mice and studying them in cultures in the laboratory ("*ex vivo*").

Figure 1: A dose response showing how different concentrations of EBC-46 kills melanoma cells. The killing effect of the compound is shown for different lines of melanoma cells ("Cell line 1" and "Cell line 2"). Cells were grown in the laboratory with EBC-46 for 4 days and the percentage of surviving cells was recorded. **Note:** a log-scale is used on the x-axis.

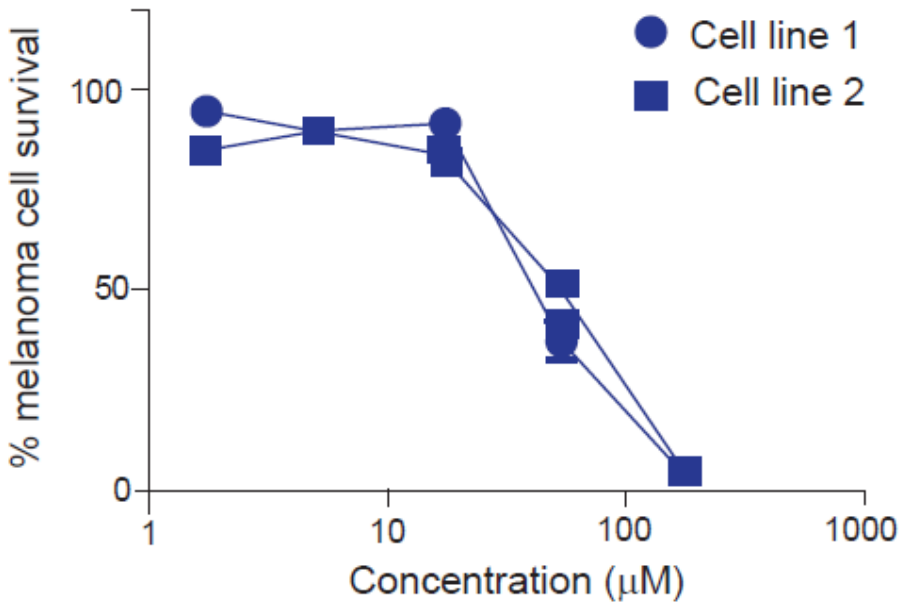


Figure 2: The percentage of melanoma tumours that remained below a 100 mm^3 size threshold in mice following a single treatment with 30 µg EBC-46 (black line) or a control solution alone (grey). Data was obtained from 5 mice per group, 2 tumours per mouse; n = 10. Control and EBC-46 treatments were given when the tumours reached approximately 50 mm^3.

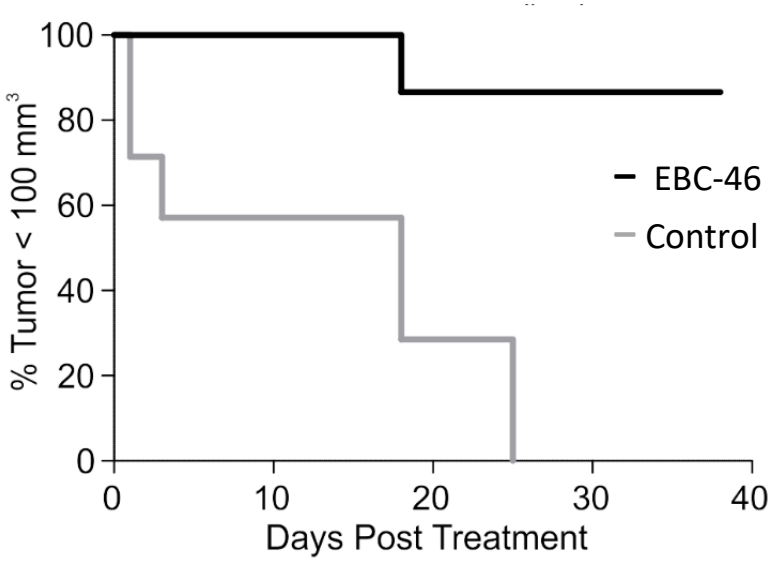
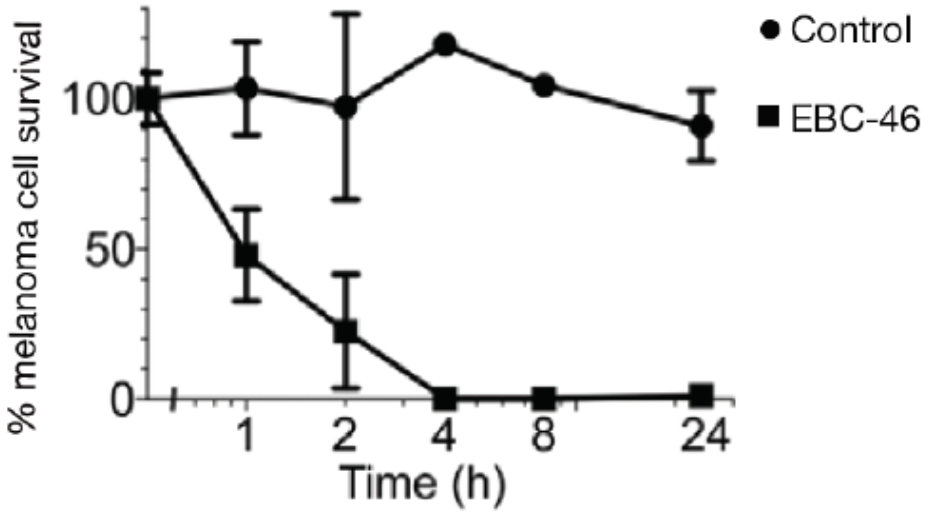


Figure 3: Survival of melanoma cells, removed from mice following treatment with a control solution or 30 µg EBC-46. Melanoma tumours on mice were injected with EBC-46 or a control solution, then the tumours were surgically removed from the mice at various intervals after injection. Cells from each of the tumours were then grown in the laboratory for 6 days, and the percentage of surviving tumour cells was recorded.



Source: Boyle GM, D'Souza MMA, Pierce CJ, Adams RA, Cantor AS, Johns JP, et al. (2014) Intra-Lesional Injection of the Novel PKC Activator EBC-46 Rapidly Ablates Tumors in Mouse Models. *PLoS ONE* 9(10): e108887. https://doi.org/10.1371/journal.pone.0108887

Questions

Apply understanding

1. Determine the concentration of EBC-46 that halved the survival of melanoma cells.

2. Determine how long it takes for EBC-46 to kill melanoma cells.

Analyse evidence

3. Contrast the responses of the two melanoma cell lines to EBC-46, as represented in Figure 1.

4. Categorise the type of experiment used to derive the data in each figure according to whether it was performed *in vitro*, *in vivo* or *ex vitro*.

Figure	**Type of experiment**
1	
2	
3	

5. Identify the characteristics of Figure 1 that contribute to it being called a “dose response” graph.

6. Identify a limitation of presenting the data in Figure 3 using the dependent variable % Tumor **<** 100 mm^3.

Interpret evidence

7. Deduce an optimal concentration of EBC-46 to use in future trials that compare the responses of a larger number of cell lines to this chemical.

8. Draw a conclusion about the effective dose of EBC-46 for the treatment of melanoma.

Extension questions

1. Imagine you are a researcher working on this project and are collecting the data for Figure 3. There are 10 mice in your experiment, each with two tumours on their backs; five mice had EBC-46 injected into their tumours and the other five mice were injected with a control solution. Create a table to record your data on Day 0 (the start of the experiment) through to Day 5 of the experiment. What would you measure or count?
2. Formulate two follow-up questions that this researcher could investigate based on one or more of the above graphs.
3. Look at Figures 2A and 2B in the original research article, which reports data for responses to another chemical called PMA (phorbol 12-myristate 13-acetate), which is structurally similar to EBC-46. PMA activates a larger, and less-specific set of Protein Kinase C (PKC) enzymes than does EBC-46. Infer why PMA was found to be more effective than EBC-46 at inhibiting cell growth *in vitro*, yet EBC-46 was more effective than PMA at curing tumours *in vivo*.
4. Draw on your knowledge of cell, tissue and organ structure, and the data and summary above, to infer the mechanism by which EBC-46 treats cancer tumours. Reference the results to support your explanation. You may like to read parts of the original research article to aid your understanding.

Researcher Profile

Professor Glen Boyle

Professor Glen Boyle is a research scientist and group leader at QIMR Berghofer Medical Research Institute, where he studies the development of melanoma and head and neck cancers. Melanoma is a type of skin cancer and is the fourth most commonly occurring cancer in Australia, while head and neck cancers are the seventh most commonly occurring form of cancer nationwide. Taken together, these cancers are expected to contribute 20 000 new cases to Australia's health burden in 2020. Glen's work dually focusses on how drugs work to treat cancers and assessing the potential of different naturally-derived chemicals to treat tumours. Glen has a particular interest in working with plant-derived drugs from native Queensland species, and one such drug called "EBC-46" is the focus of this data resource. Already, EBC-46 has been tested in clinical trials, and has saved the lives of hundreds of companion animals (mainly dogs – see this ABC news article).

Dataset 2

Researchers grew human heart organoids to imitate the structural and functional properties of mature heart tissue. The method involved three stages: (1) incubating human stem cells so that they would differentiate into heart muscle cells (called cardiomyocytes), (2) incubating the cardiomyocytes with a second cell type (called fibroblasts, which form connective tissue), then (3) maturing the heart organoid by exercising it using a small machine that stretches the organoid then allows it to contract naturally. This significant advance in tissue engineering occurred through testing different stem cell lines and culture conditions.

Plate 1: The 3-stage process of growing a heart organoid: *[left to right]* (stage 1) stem cell differentiation, (stage 2) organoid tissue consolidation, and (stage 3) organoid maturation.

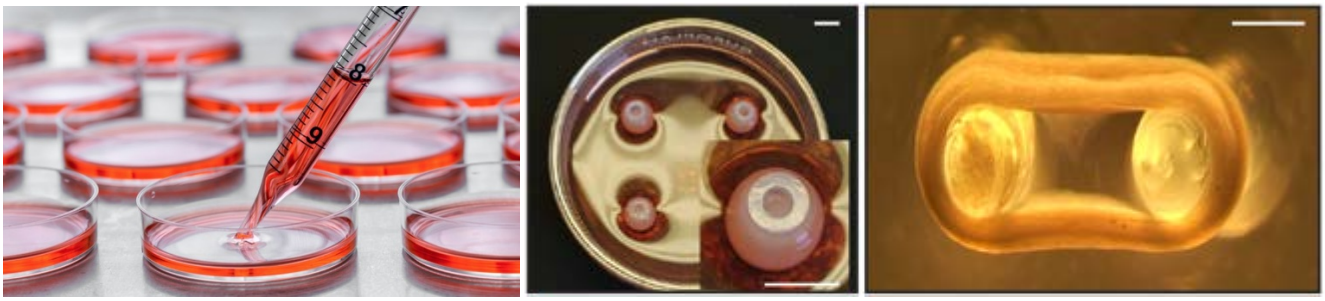


Figure 1: The relationship between the cardiomyocyte concentration (as a percentage of all cells in the culture) and the contractive force of heart organoids created from three different stem cell lines.

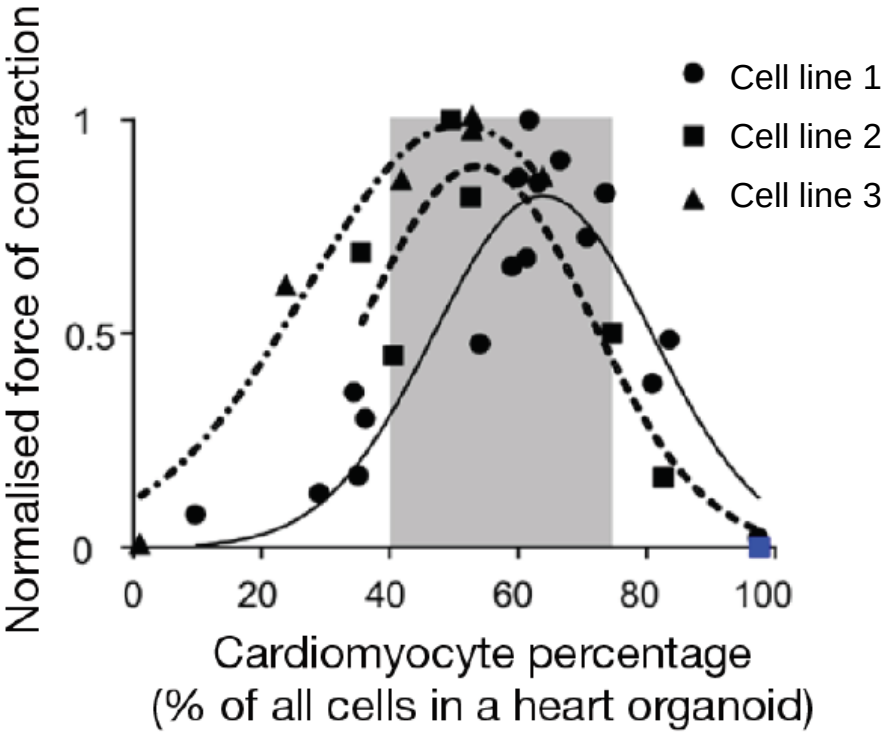


Figure 2: The effect of changing the proportions of cardiomyocytes and connective cells (fibroblasts) at stage 2 of the growing process on the contractive force of cardiomyocytes in mature 14-day-old heart organoids. Open circles indicate the mean ± standard error for each treatment group.

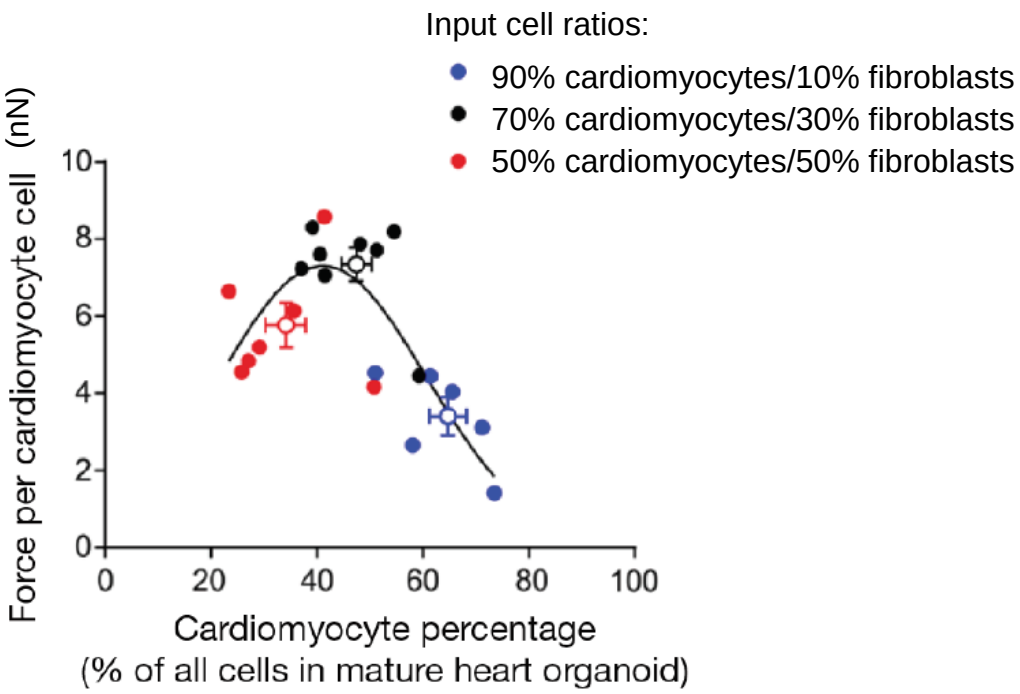
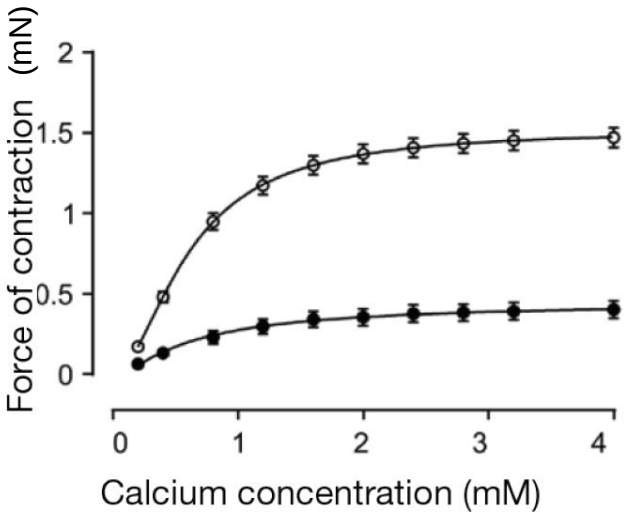


Figure 3: The contractive force of mature heart organoids in response to increasing calcium concentrations.



Source: Tibucy M, Hudson JE, Balfanz P et al. (2017) Defined Engineered Human Myocardium With Advanced Maturation for Applications in Heart Failure Modeling and Repair. *Circulation* 135:1832–1847.
https://doi.org/10.1161/CIRCULATIONAHA.116.024145

Questions

Apply understanding

1. Identify which cell line and cardiomyocyte concentration achieved the highest force of contraction in Figure 1.

2. Determine the range of results for force of contraction in response to increasing calcium concentrations in Figure 3.

Analyse evidence

3. Contrast the three cell lines presented in Figure 1 with respect to the force of contraction resulting from increasing concentrations of cardiomyocytes in cell culture.

4. Sequence the “input cell ratios” shown in Figure 2 according to increasing force produced by each cardiomyocyte in cell culture.

5. Identify the relationship between calcium and heart cell function, based on results in Figure 3.

Interpret evidence

6. Deduce the change in cardiomyocyte and fibroblast concentrations that occur from the start of stage 2 of a cell culture experiment through to maturity of a heart organoid, with reference to Figure 2.

7. Infer why the scale used for the dependent variable in Figures 2 and Figure 3 differ by an order of magnitude.

8. Draw a conclusion about the cell culture conditions that will result in the strongest heart muscle.

Extension questions

1. Propose a reason for stem cell lines reacting differently in this study.
2. Drawing on your knowledge of cell, tissue and organ structure, and the data above, propose a role for calcium in heart function. Reference the results to support your suggestion.
3. Using your background knowledge of the structure and function of the mammalian heart, evaluate the authors' use of the "Force of contraction" for measuring heart organoid function. Why might this measure be used, and what limitations might there be to its use and interpretation?
4. Figures 1 and 3 both use Force of Contraction as the dependent variable, yet the axes titles indicate that the data is presented in two different formats. Compare the two formats and propose a reason for presenting the data in this way.
5. Formulate two follow-up questions that this researcher could investigate based on one or more of the above graphs.

Researcher profile

Associate Professor James Hudson

Associate Professor James Hudson is a research scientist and group leader at QIMR Berghofer, where he studies the development of organoids. Organoids are small versions of organs, such as hearts, that are made in the laboratory from stem cells. Adult stem cells are harvested and are directed to develop into the various cell types needed to make a particular organ by manipulating the media in which the stem cells grow. These constituent cell types can then be combined and exercised to create the model tissue, or organoid, which in turn can be used for understanding the biology of disease and testing new drugs.

Last edited on 11/06/2020