Targeting Chemotherapeutic Resistance through Bcl-2

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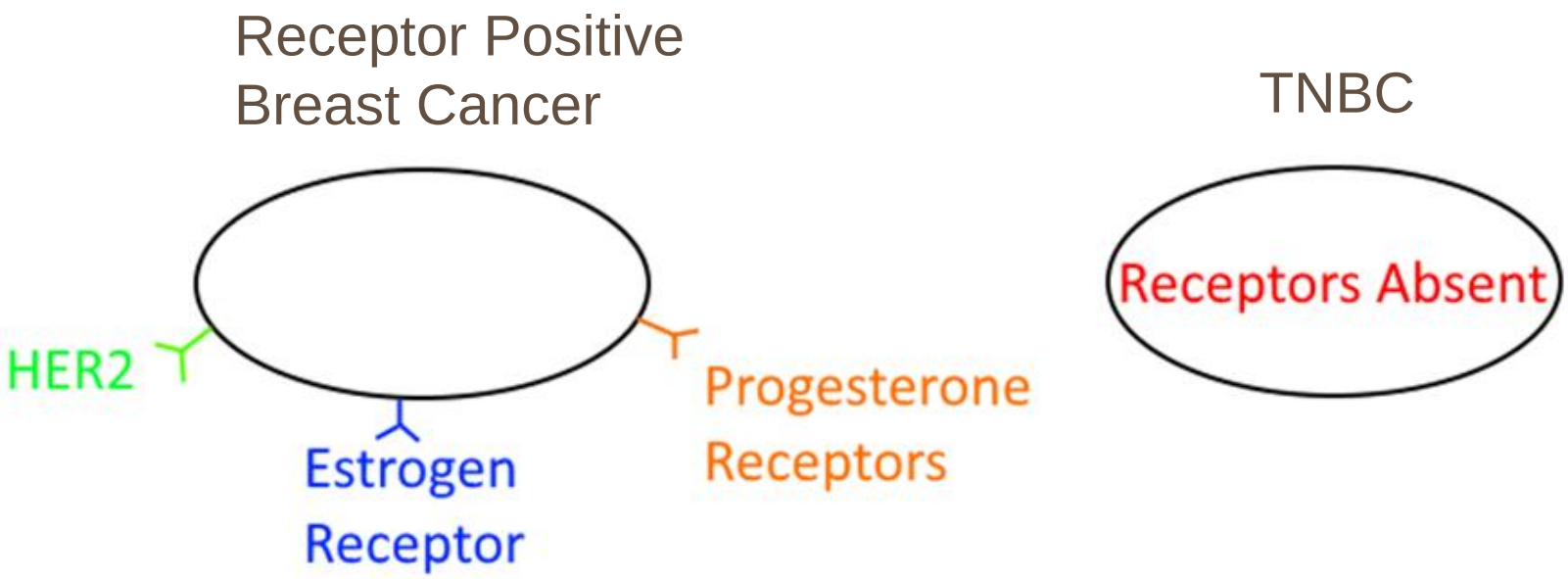
15-20% of all Breast Cancers are Triple Negative Breast Cancer (TNBC)



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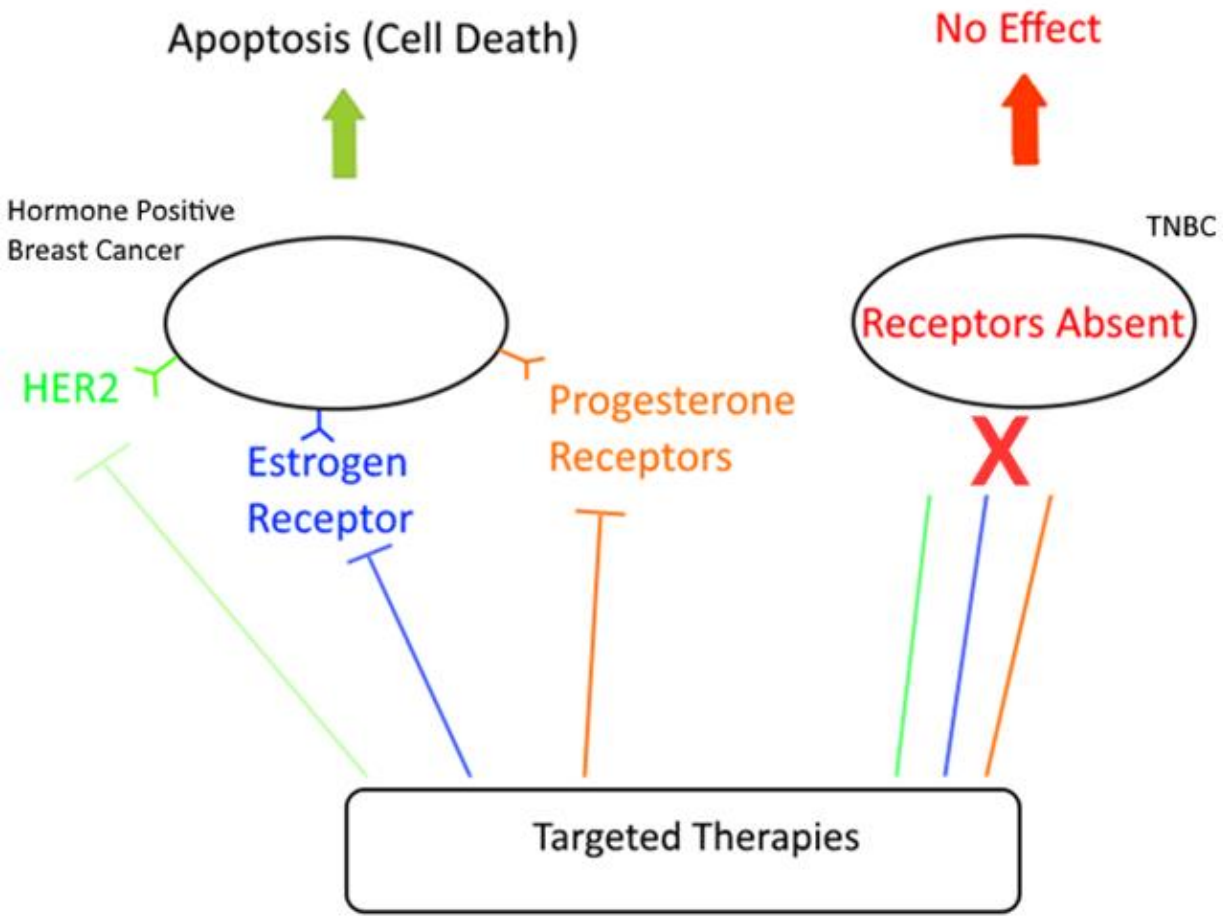
Targeted Therapies are ineffective in TNBC



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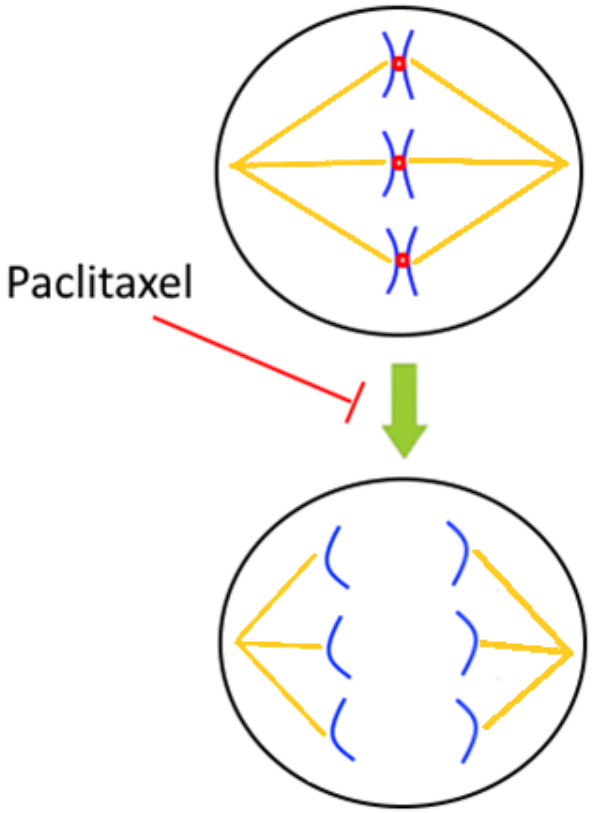
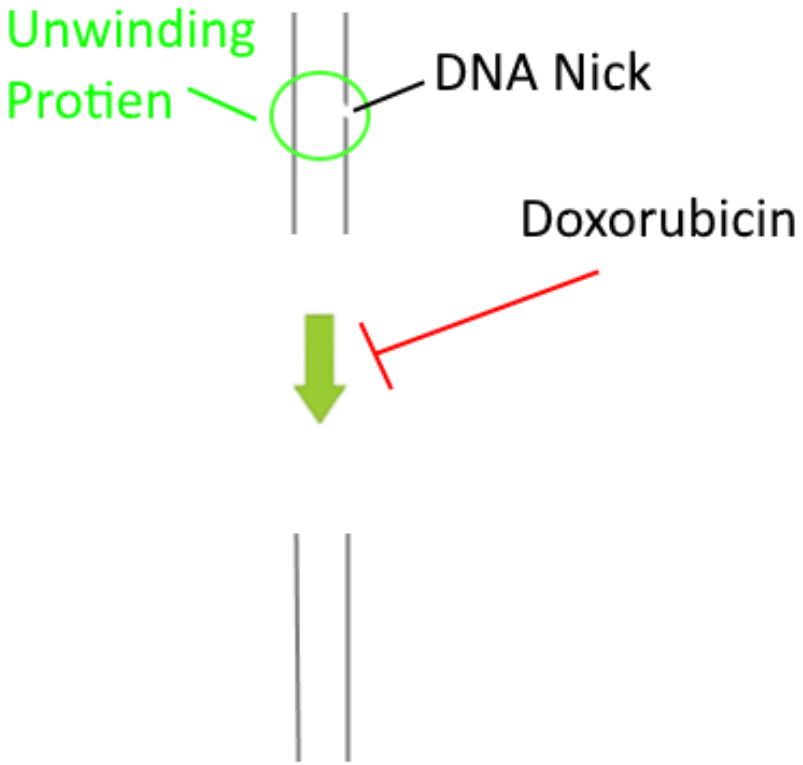
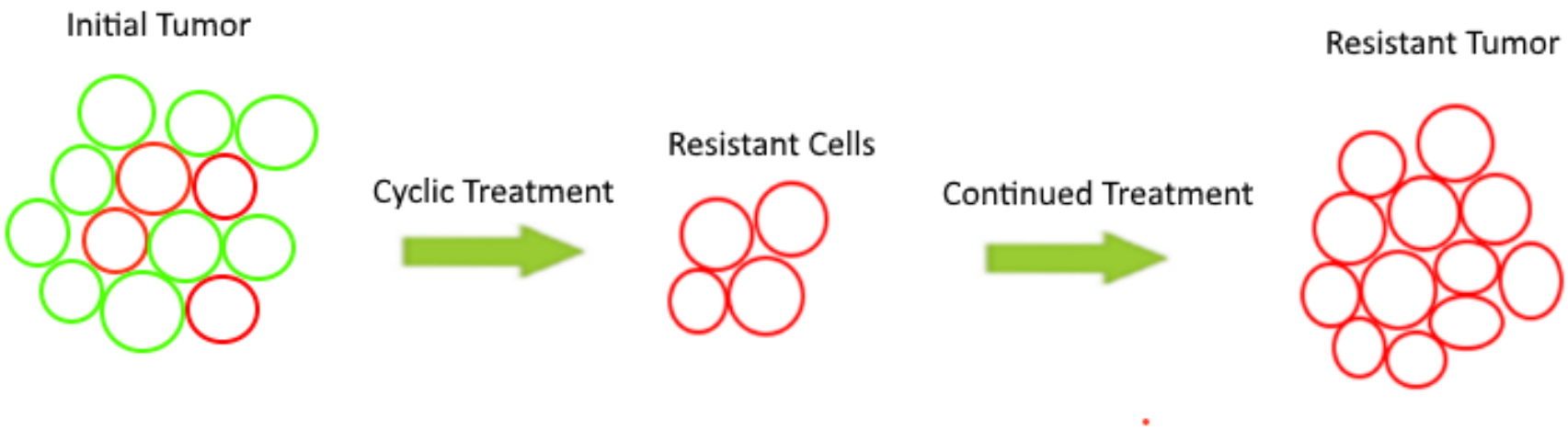
Standard Chemotherapeutics are used to treat TNBC





Over time the TNBC can become resistance to treatment



Currently, 90% of treatment failure in advanced cancer is due to acquired resistance (Cleere 2010).

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How Does Chemotherapeutic Resistance Happen?

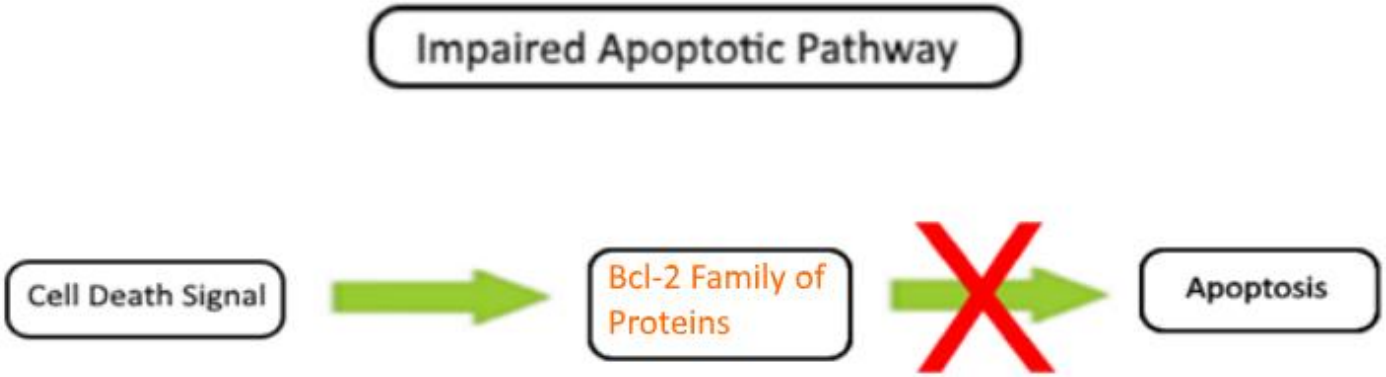
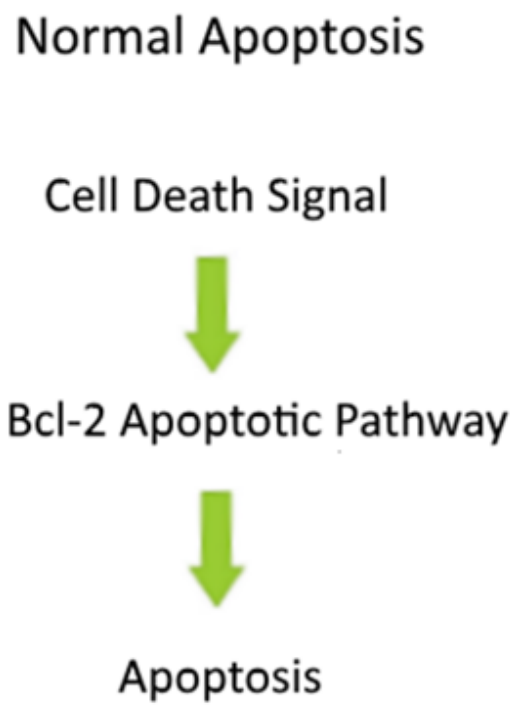
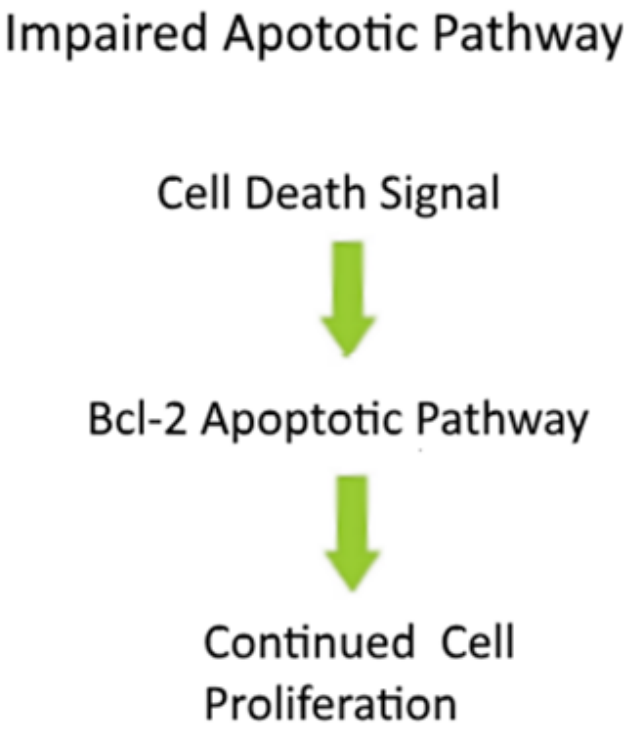


Photo Credit: Daniel Weber

When the apoptotic pathway is impaired the cells will not die when the death signal is received





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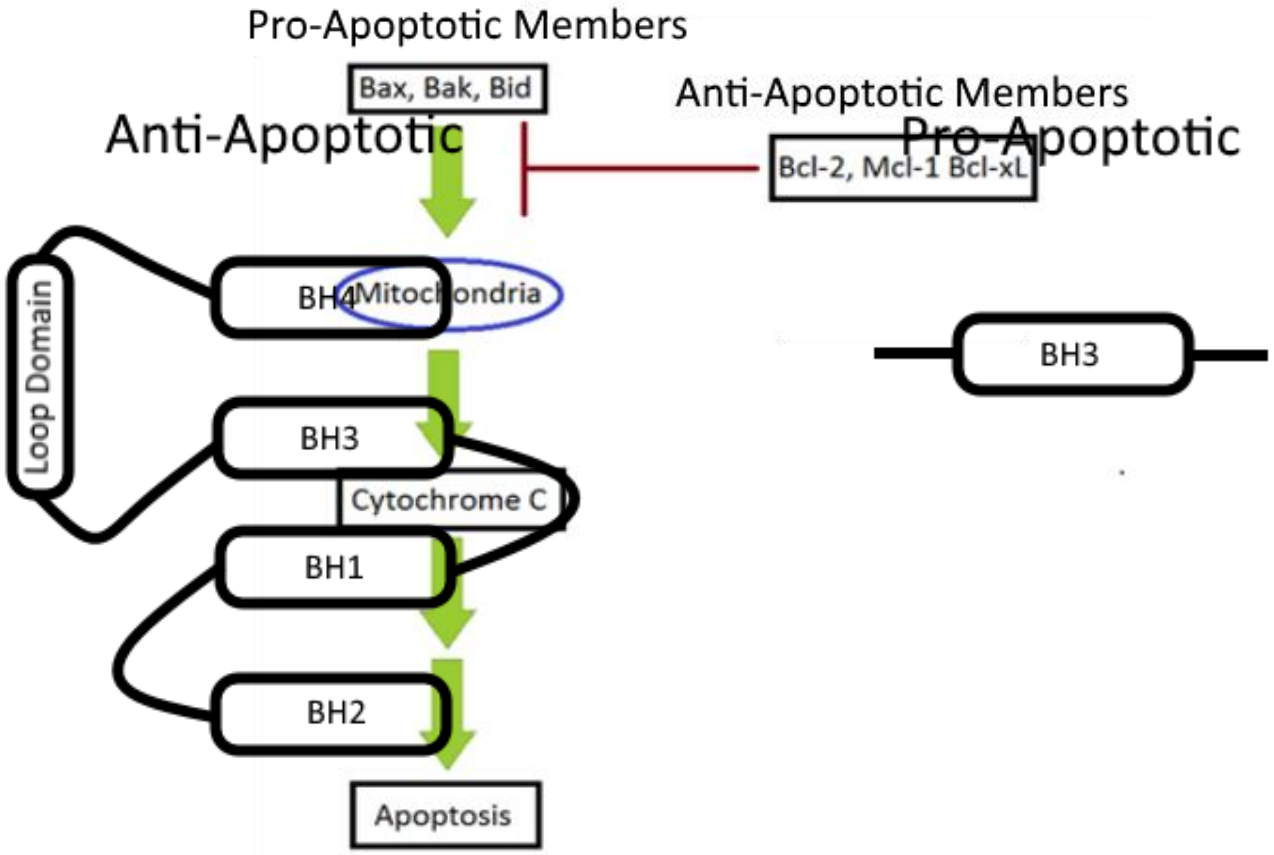
The Bcl-2 Family of Proteins contains both anti-apoptotic and pro-apoptotic proteins.



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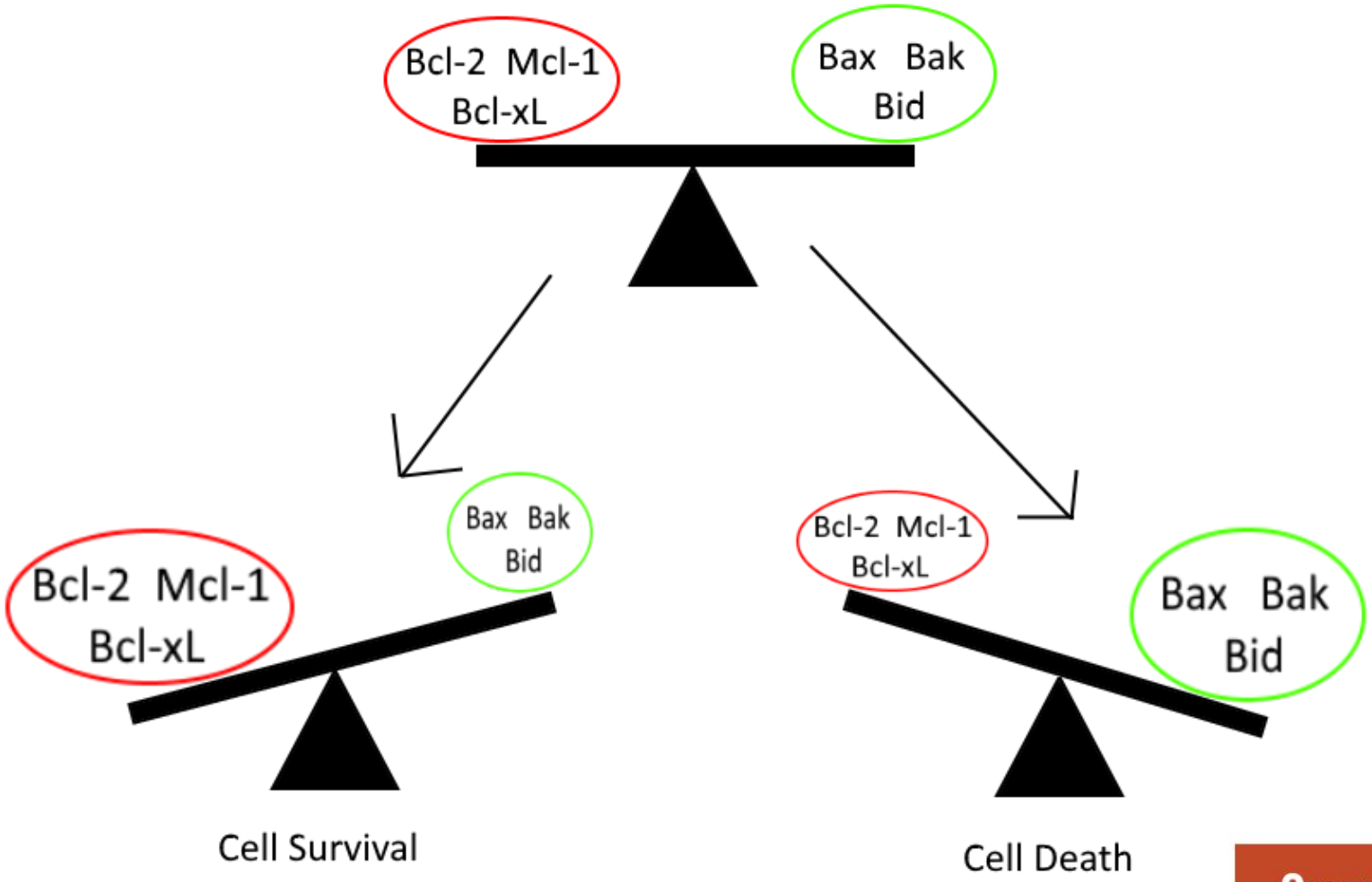
The relative ratio of anti- and pro-apoptotic proteins at the mitochondrial membrane determines the cells fate.



Bcl-2 overexpression has been implicated in Resistance in ovarian cancer

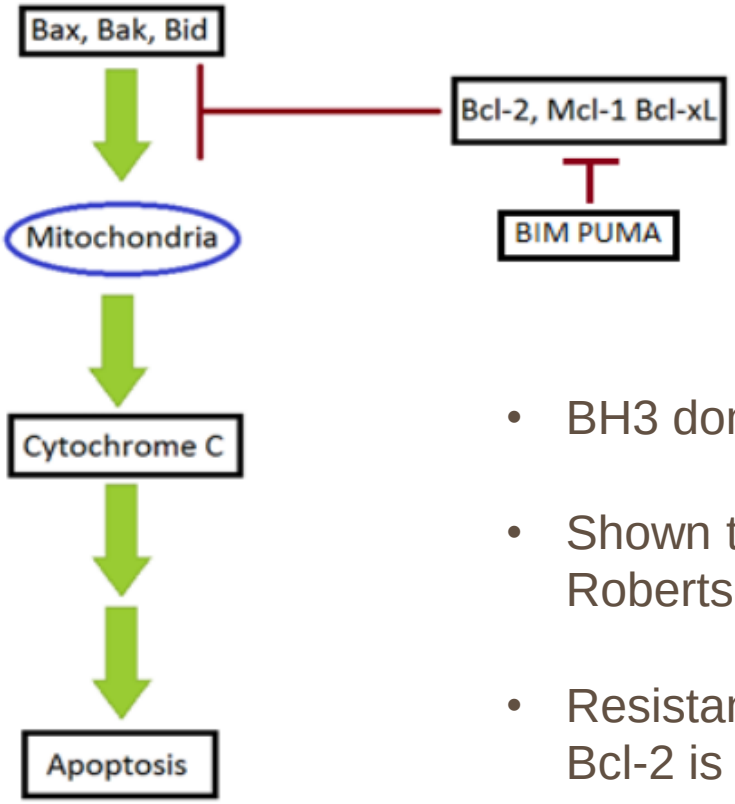
Parameter	Low Bcl-2	High Bcl-2
	<63.8 (n = 6)	≥63.8 (n = 20)
Primary resistance		
No	100%	50%
Yes	0	50%
Survival		
Yes	100%	40%
No	0	60%

Kassim, S K. *et al* (1999)

Why do we care about chemotherapeutic resistance?

- Resistance is one of the major barriers to successful cancer treatment.
- In Triple Negative Breast Cancer there are less alternatives leading to less options when resistance occurs.
- Approximately 90% of treatment failure in advanced cancers is due to acquired resistance (Cleere 2010).

Exploiting Bcl-2 upregulation with ABT compounds



- BH3 domain mimetic
- Shown to be effective (Ugarenko, 2009, Roberts, 2016).
- Resistance to ABT compounds can occur if Bcl-2 is upregulated further

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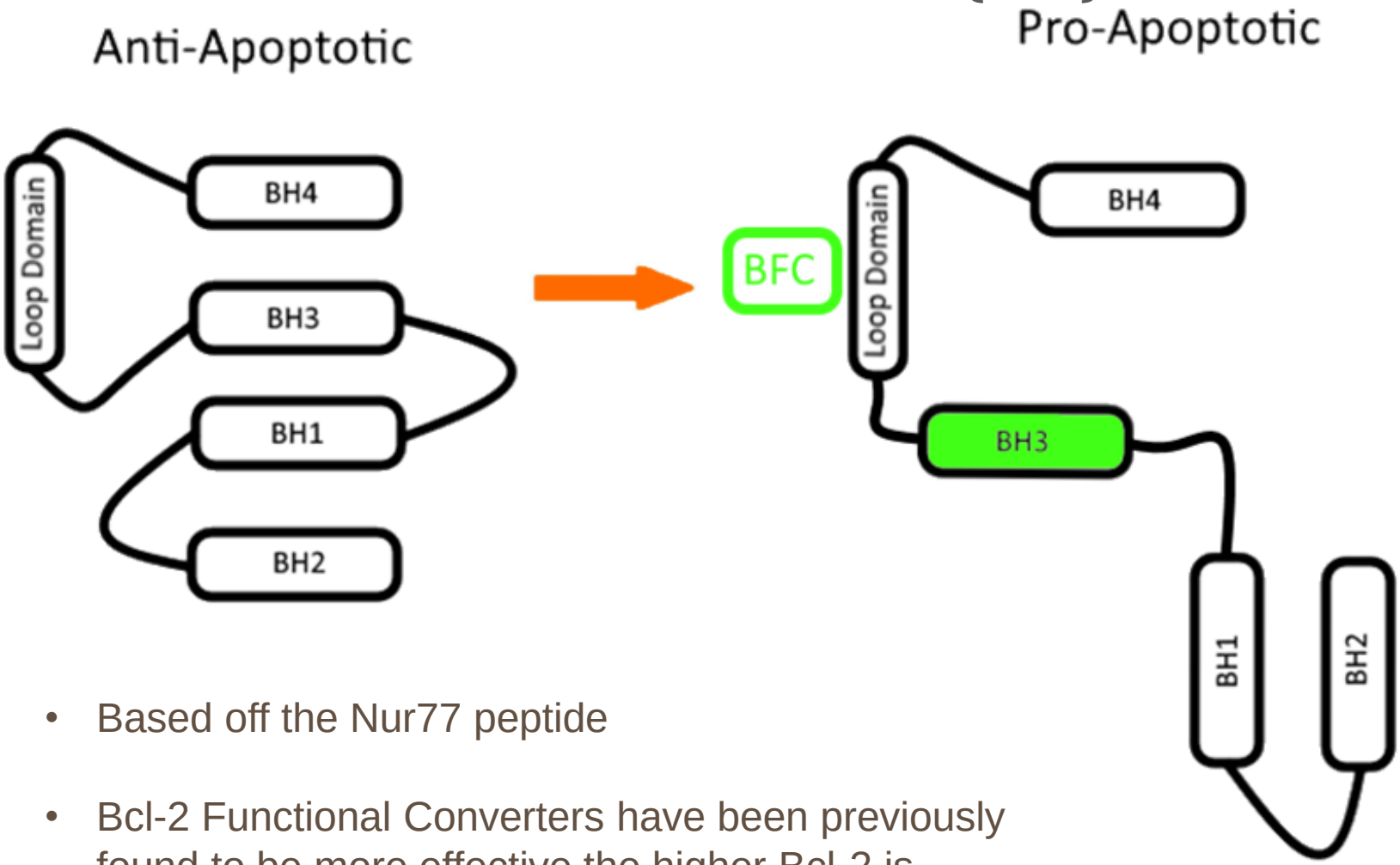
Bcl-2 Functional Converters (BFC)



- Based off the Nur77 peptide
- Bcl-2 Functional Converters have been previously found to be more effective the higher Bcl-2 is upregulated.

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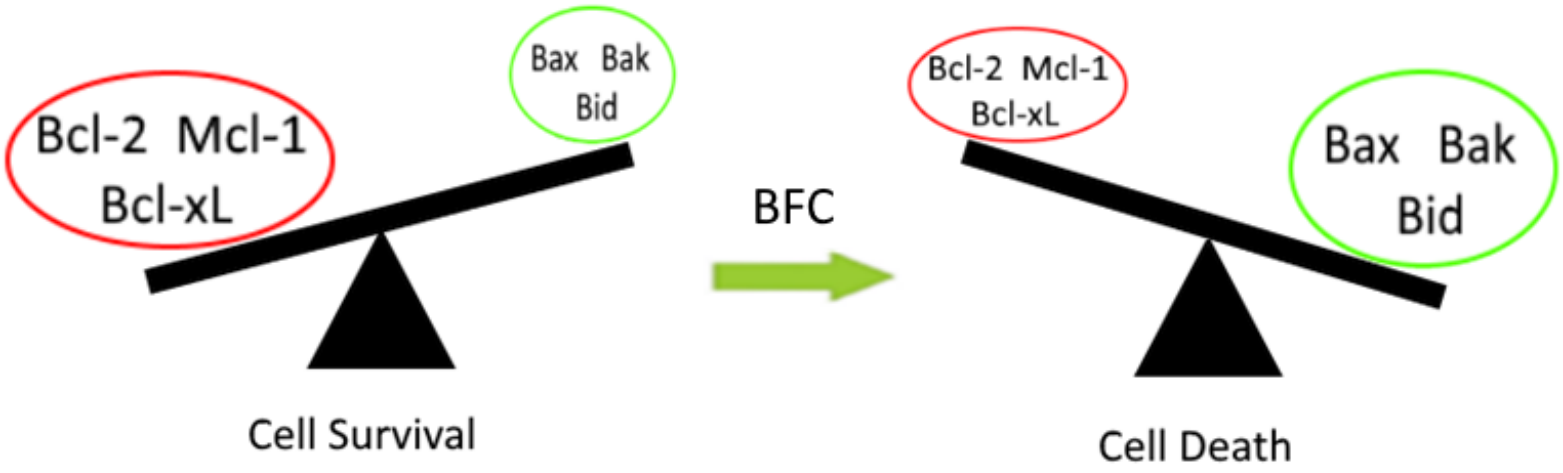
Hypothesis

Bcl-2 is a critical element in chemotherapeutic resistance and the upregulation of the anti-apoptotic members can be targeted using Bcl-2 functional converters (BFC).



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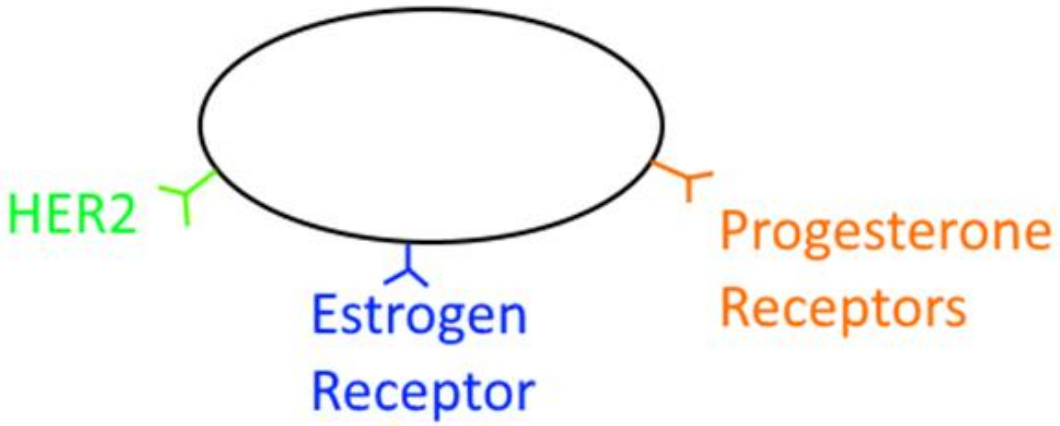
Receptor Positive Breast Cancer



TNBC

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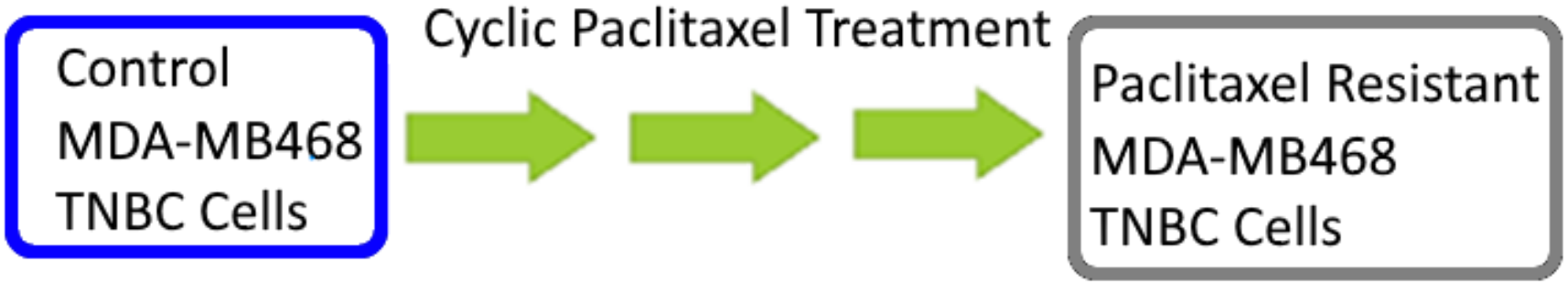
Methods

Generating Resistance

Triple Negative Breast Cancer (TNBC) is the focus of my research. Paclitaxel is one of the main chemotherapeutics used in TNBC treatment.



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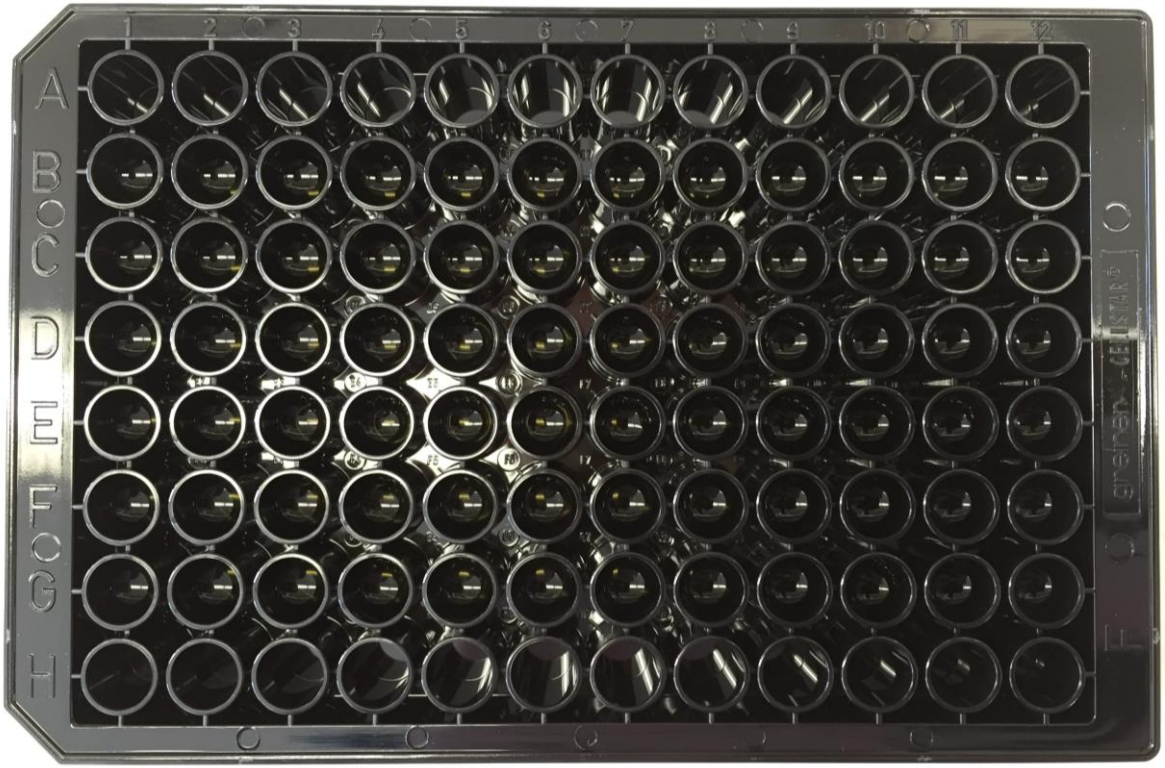
ATP-based cell viability assay

- Measures ATP abundance, a proxy for the amount of viable cells in the culture.
- Treatments in triplicates



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Colony Formation Assay

- Assesses the tumorigenicity of the cancer
- 500 cells per 2 mL well
- 24 hour treatments followed by a 9 day incubation time
- Treatments in triplicates

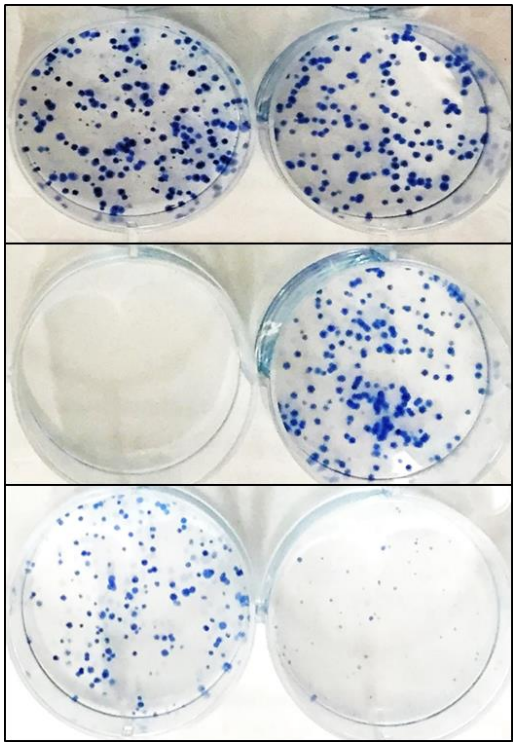


Photo credit: Martin Pearce, 2016

Western Blots

- Used to detect protein levels
- The darker the band the more protein there is
- GAPDH is a protein used as a normalizing Control

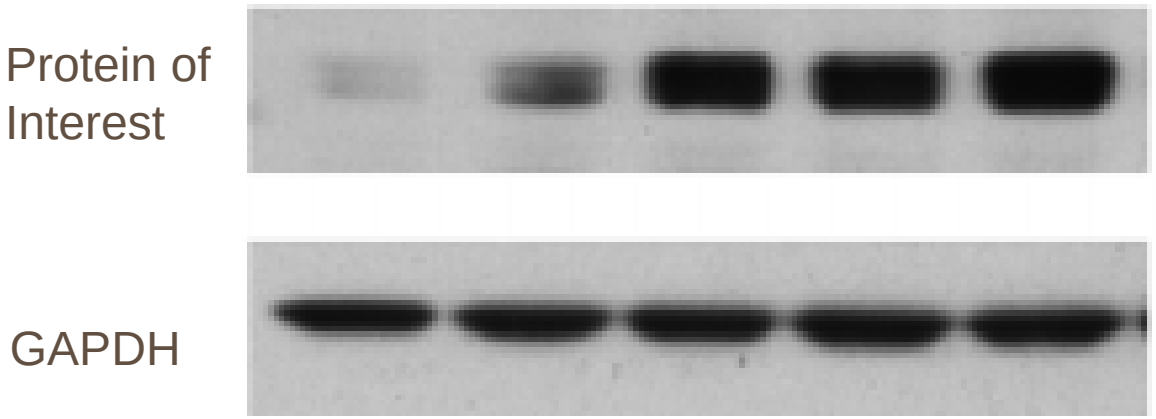
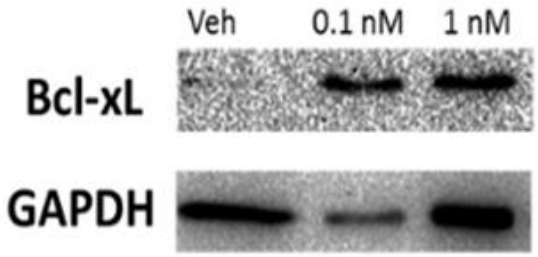
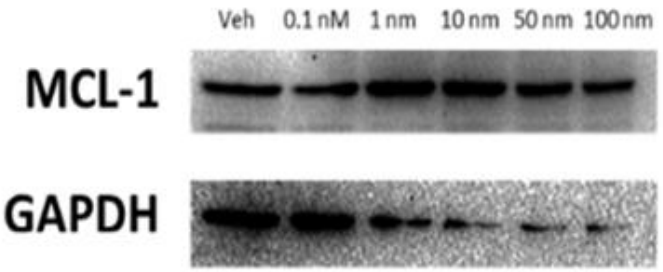


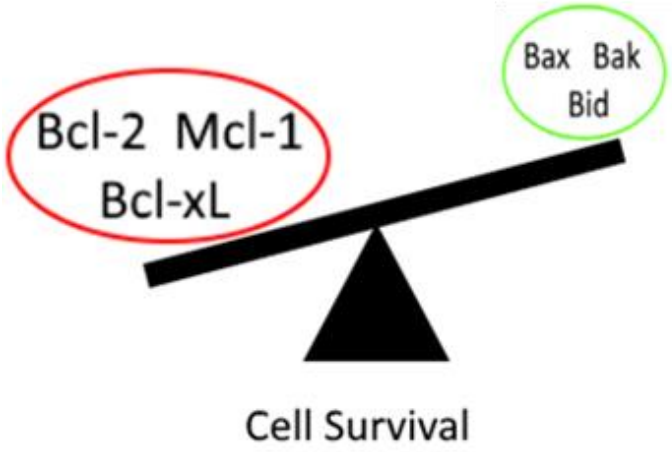
Photo Credit: Sandrine Spriggs, 2015

Results

Paclitaxel treatment upregulates key anti-apoptotic Bcl-2 family members







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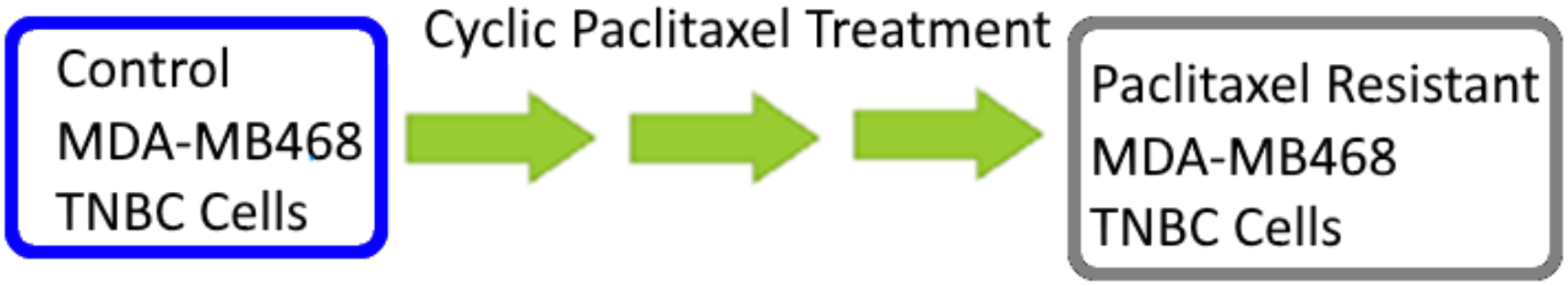
Confirmation of Resistance



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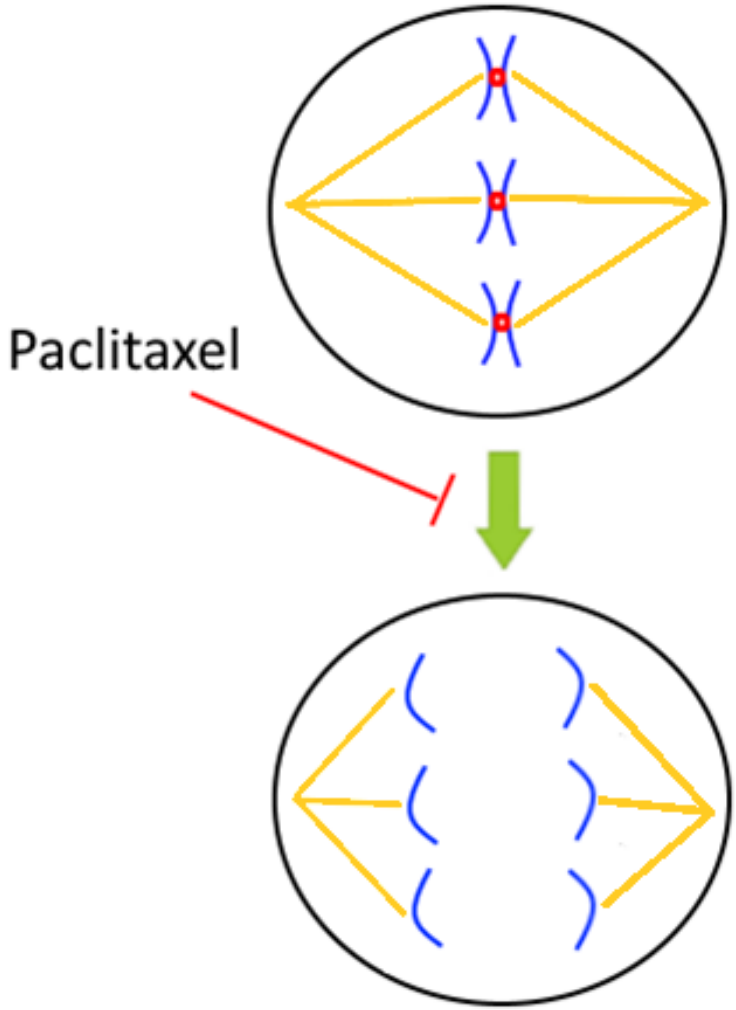
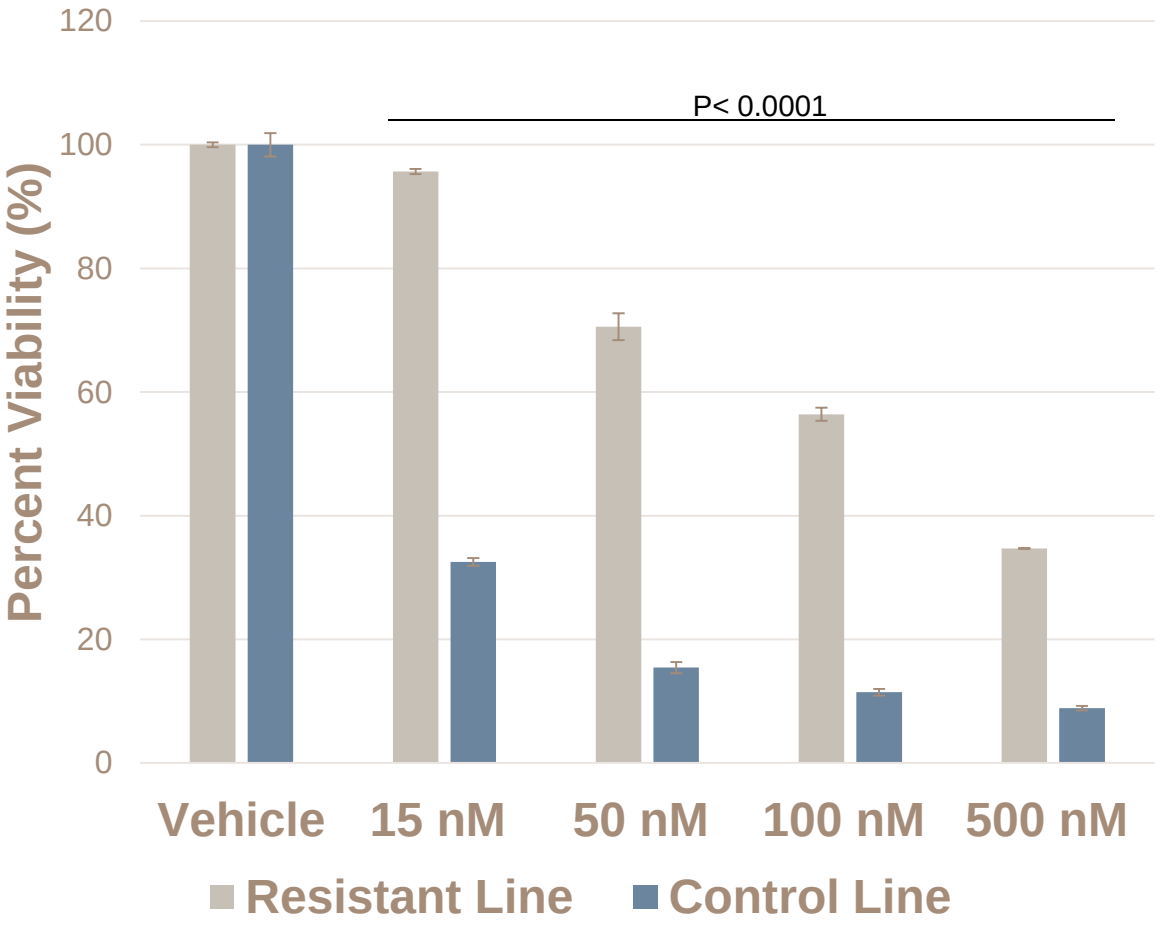
Paclitaxel as a Chemotherapeutic



Resistance to paclitaxel is approximately 2 to 4 fold higher than in the control line in a cell viability assay.



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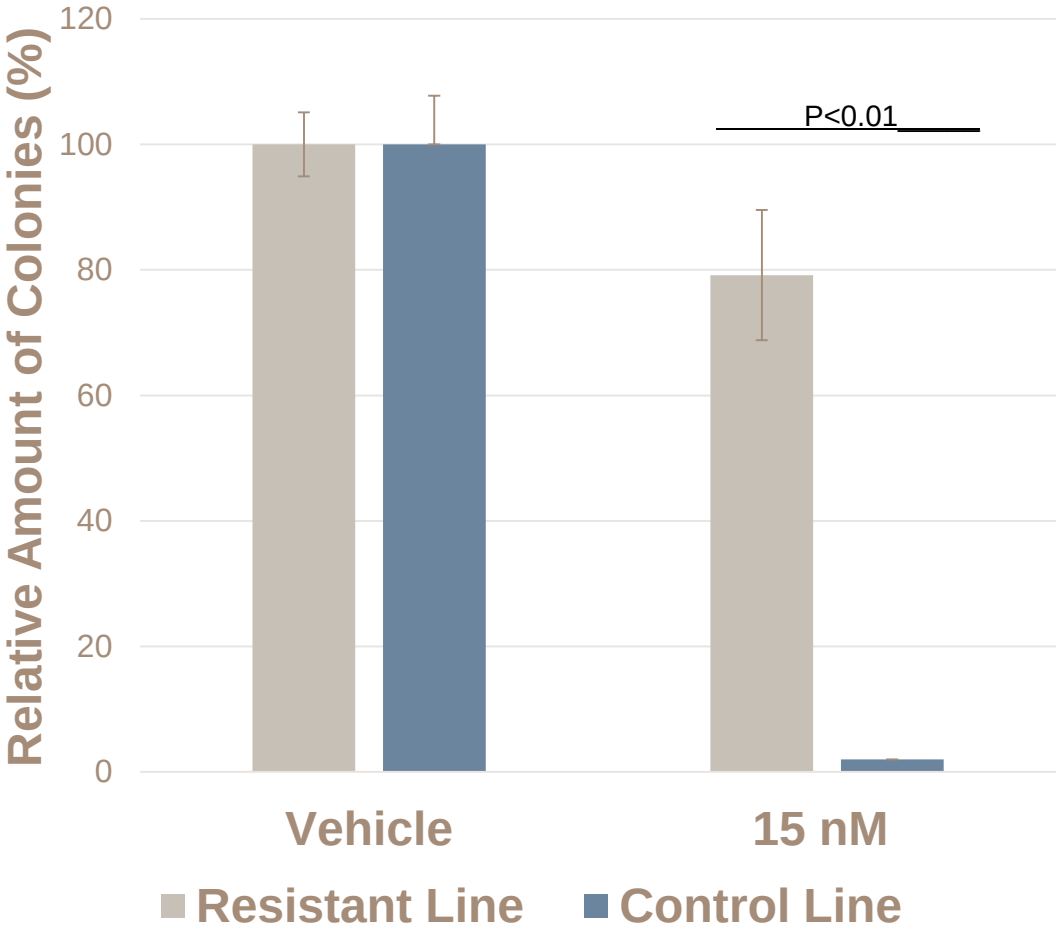
Paclitaxel significantly inhibits the control cells in a colony formation assay, but does not in the developed resistant line.



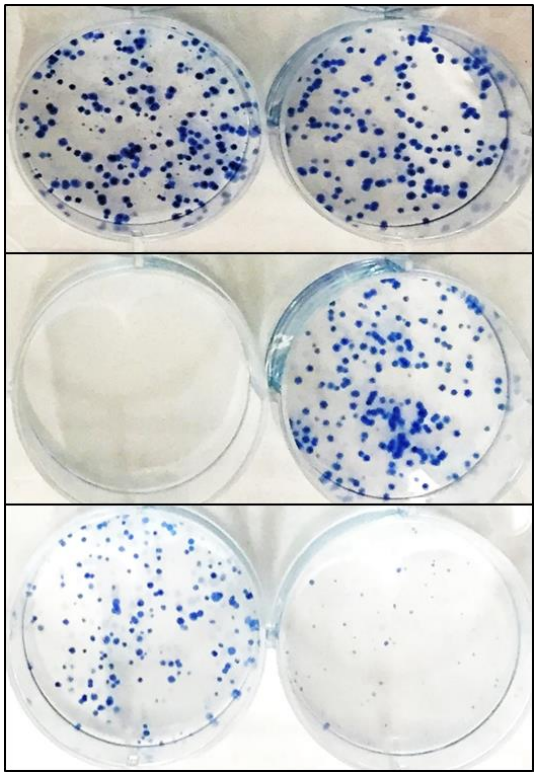


Photo credit: Martin Pearce, 2016

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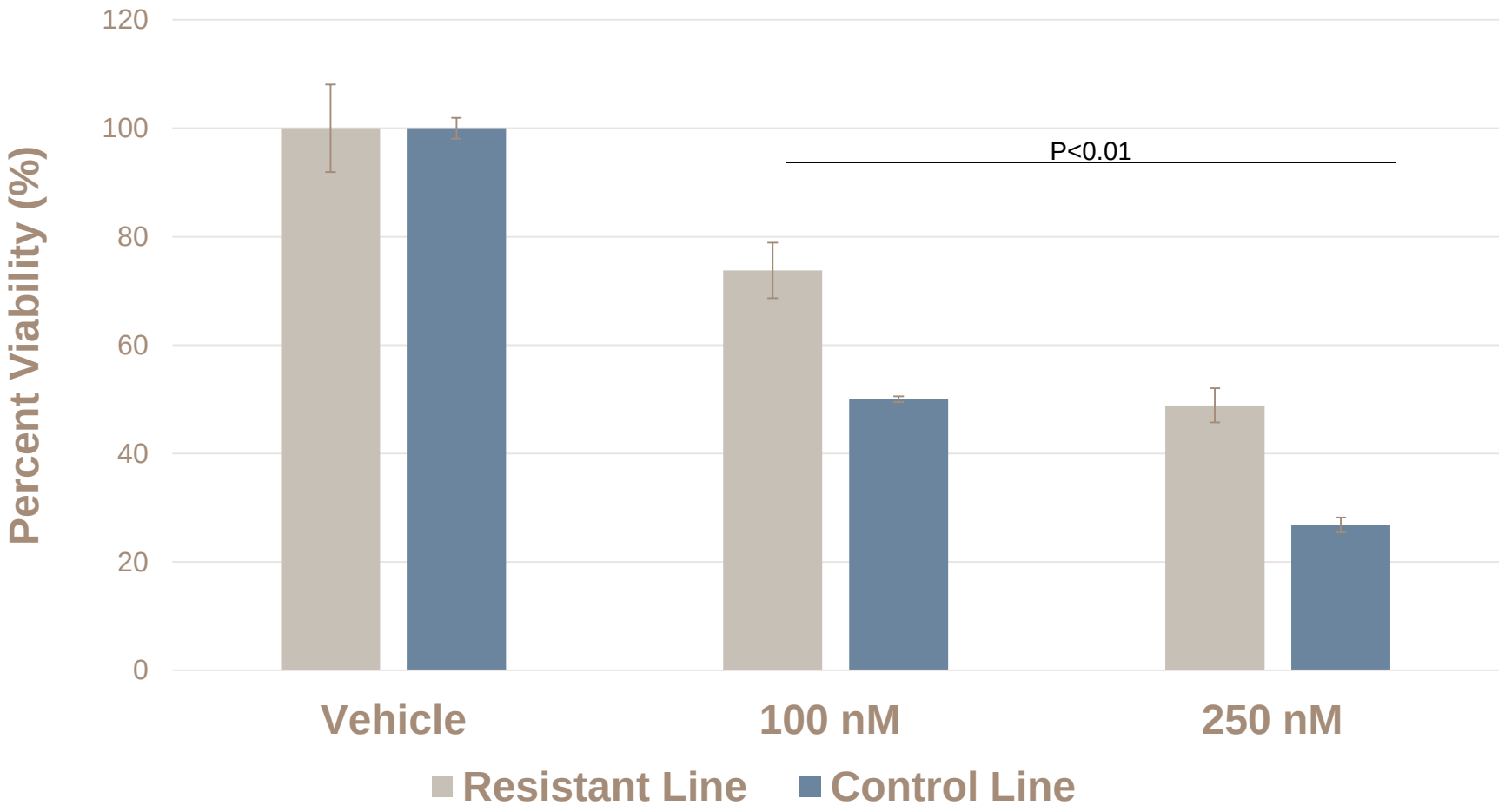
Paclitaxel Resistance Leads to Cross Resistance to Doxorubicin



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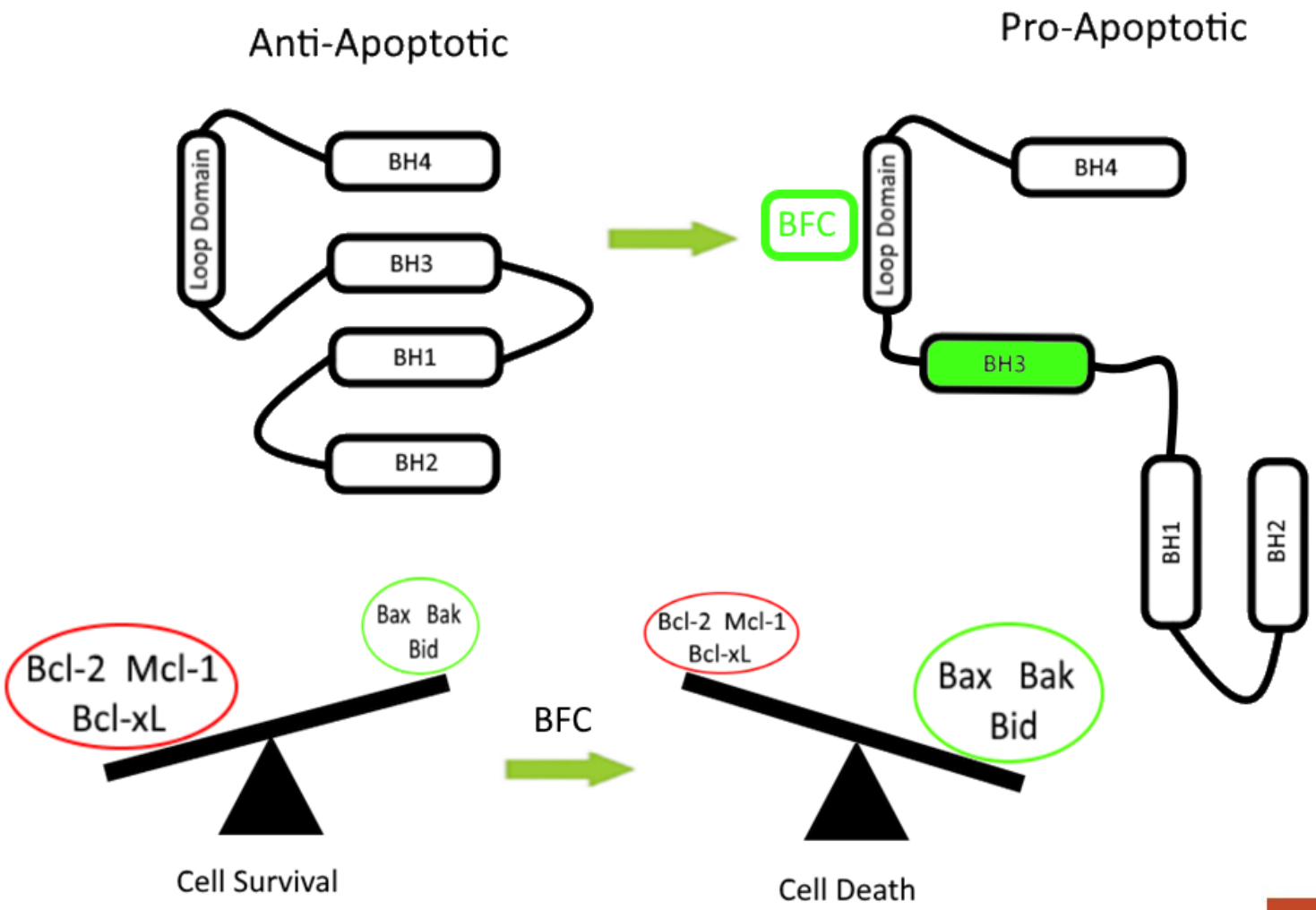
Bcl-2 Functional Converter Treatment



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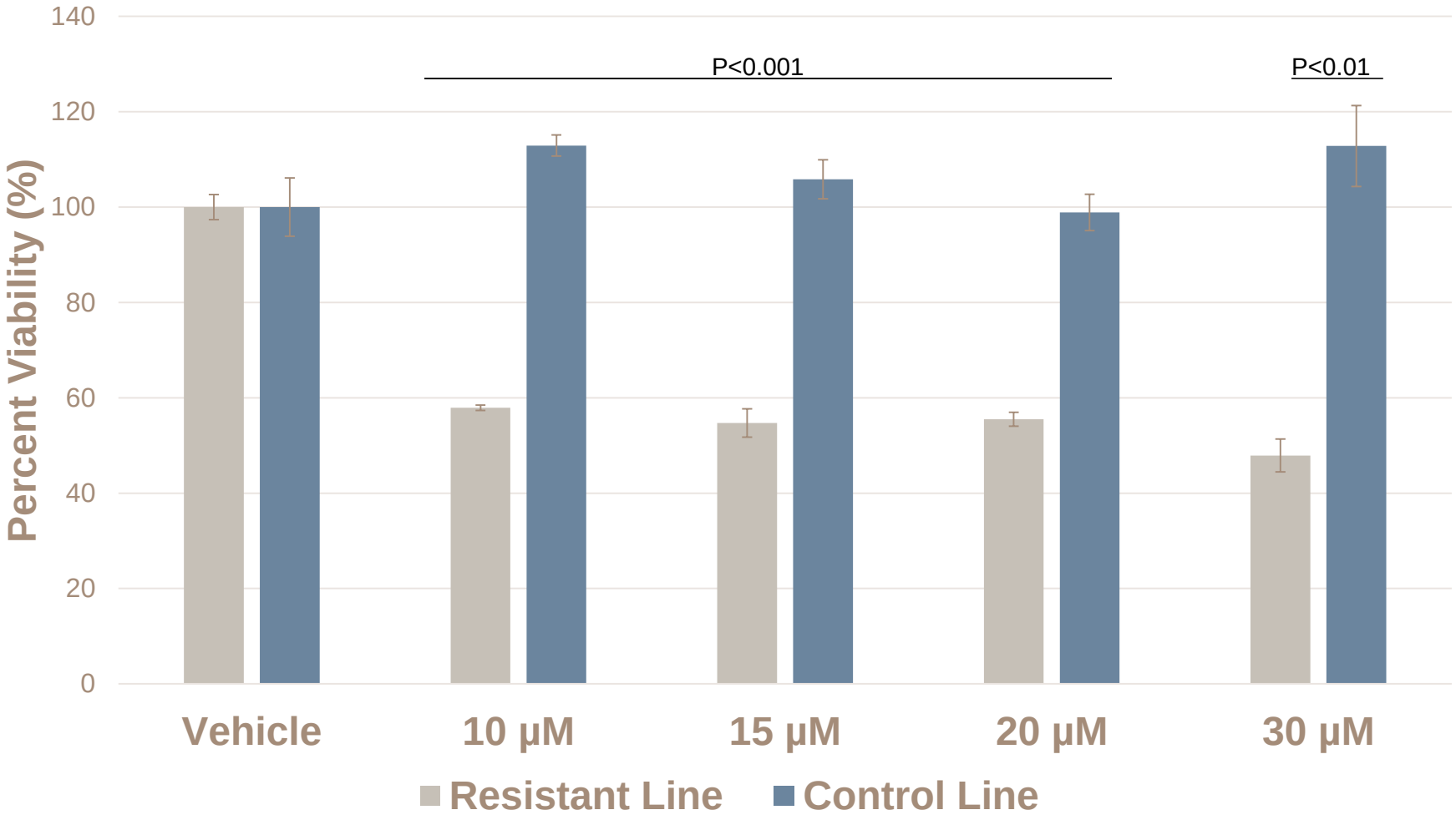
Bcl-2 functional converters in low glucose medium



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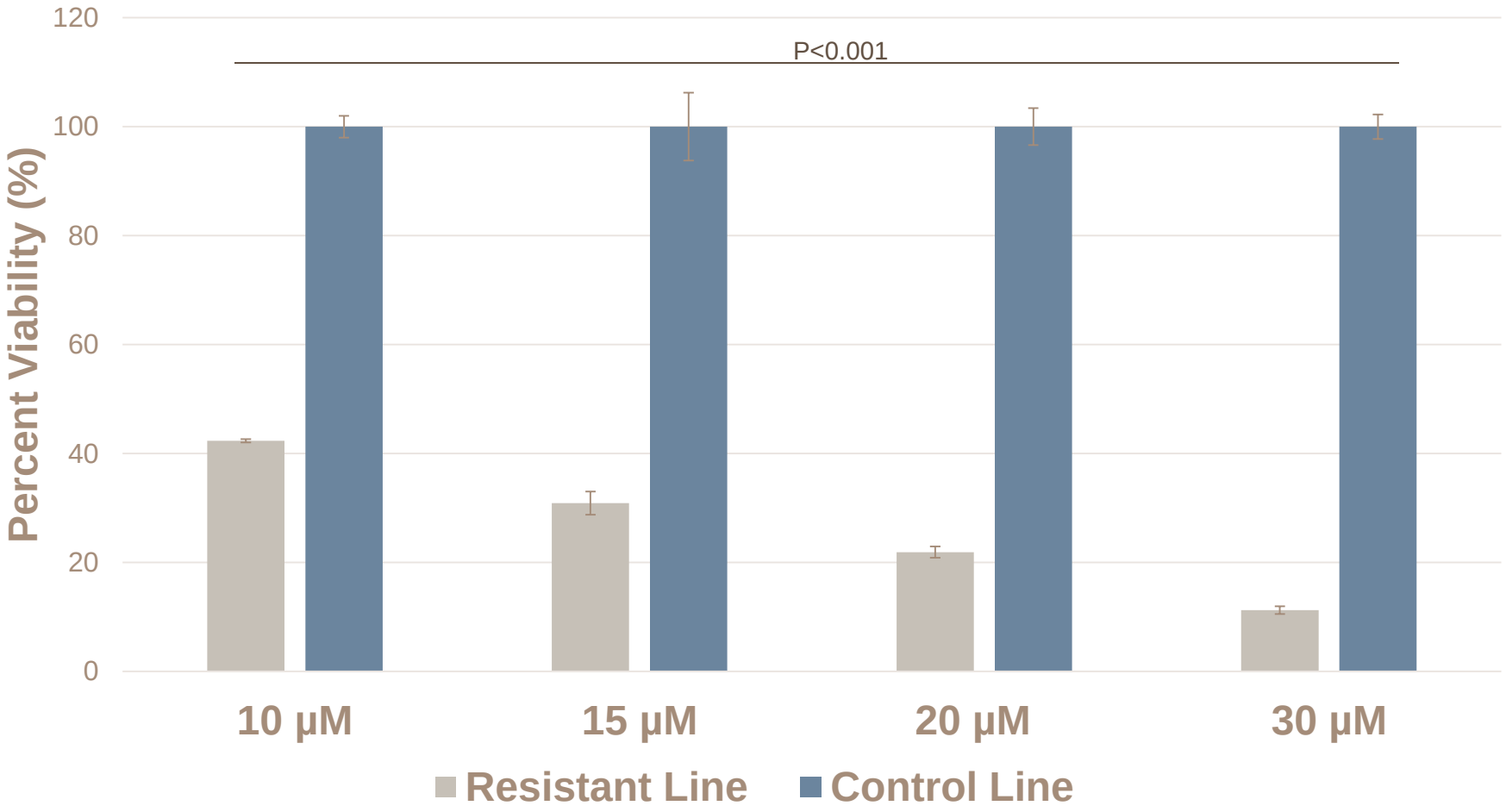
Bcl-2 Functional Converters in 1% FBS medium



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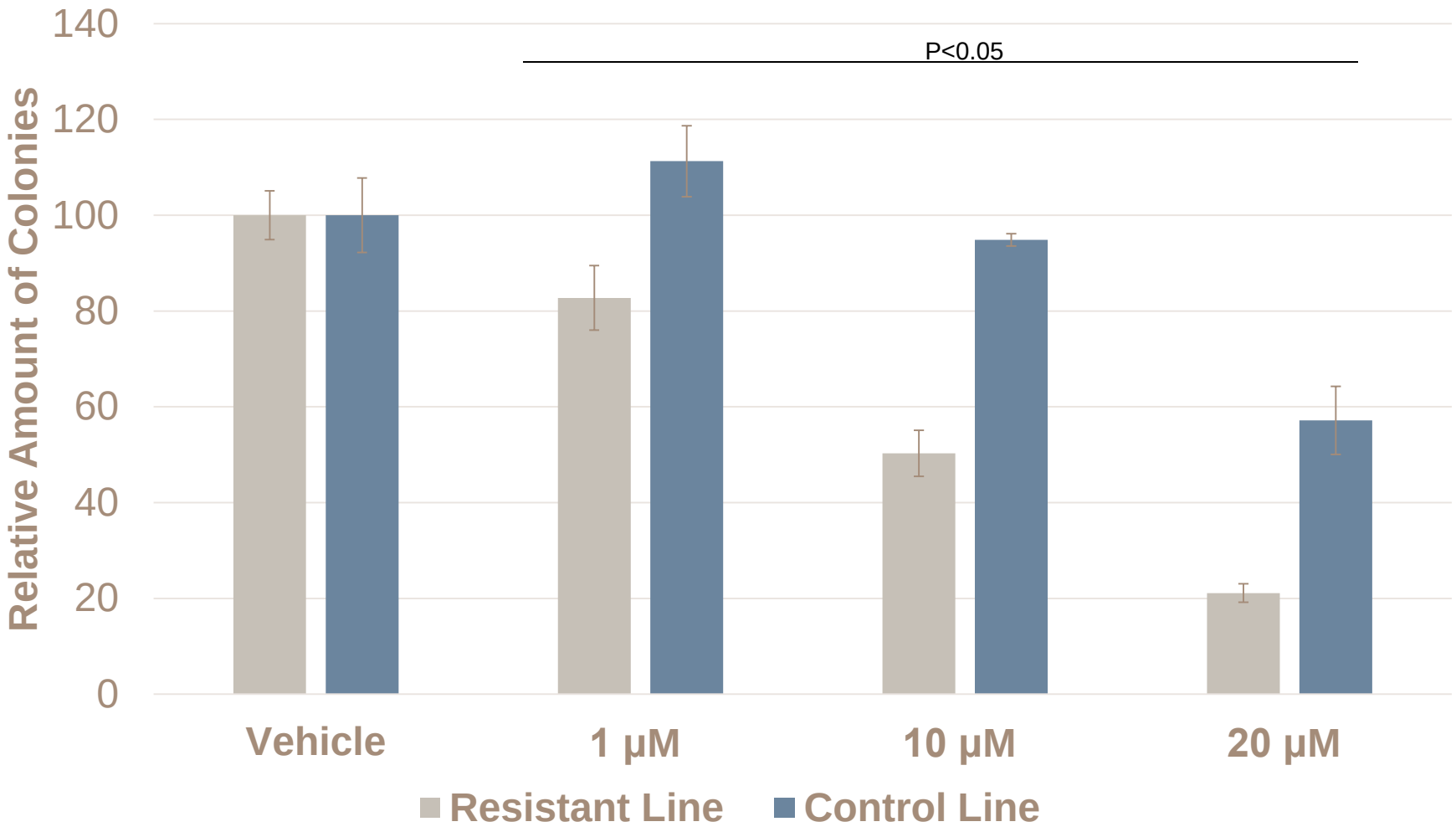
Bcl-2 Functional Converters are effective at reducing the amount of colonies formed (a measure of tumorigenecity)



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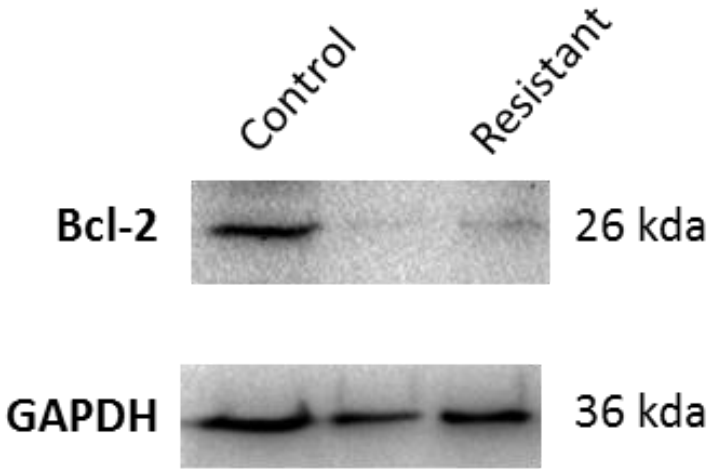
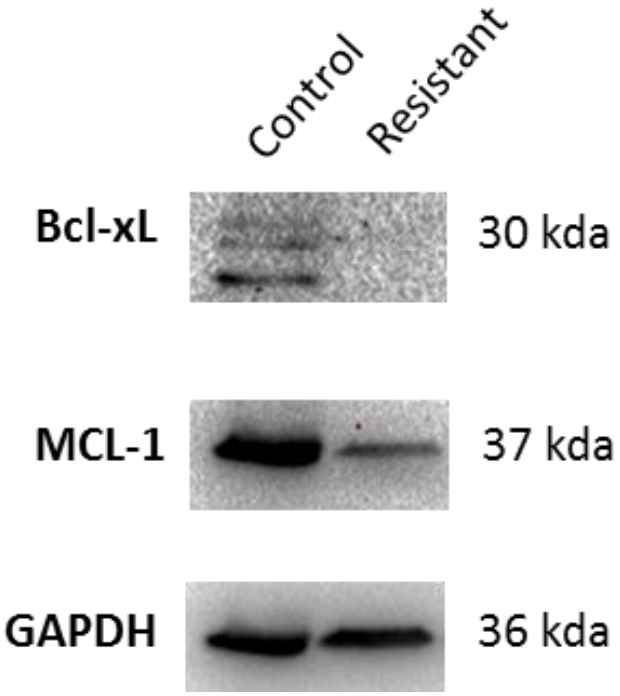
Paclitaxel resistance lead to a decrease in all key anti-apoptotic protein levels

- These proteins are the targets for BFCs

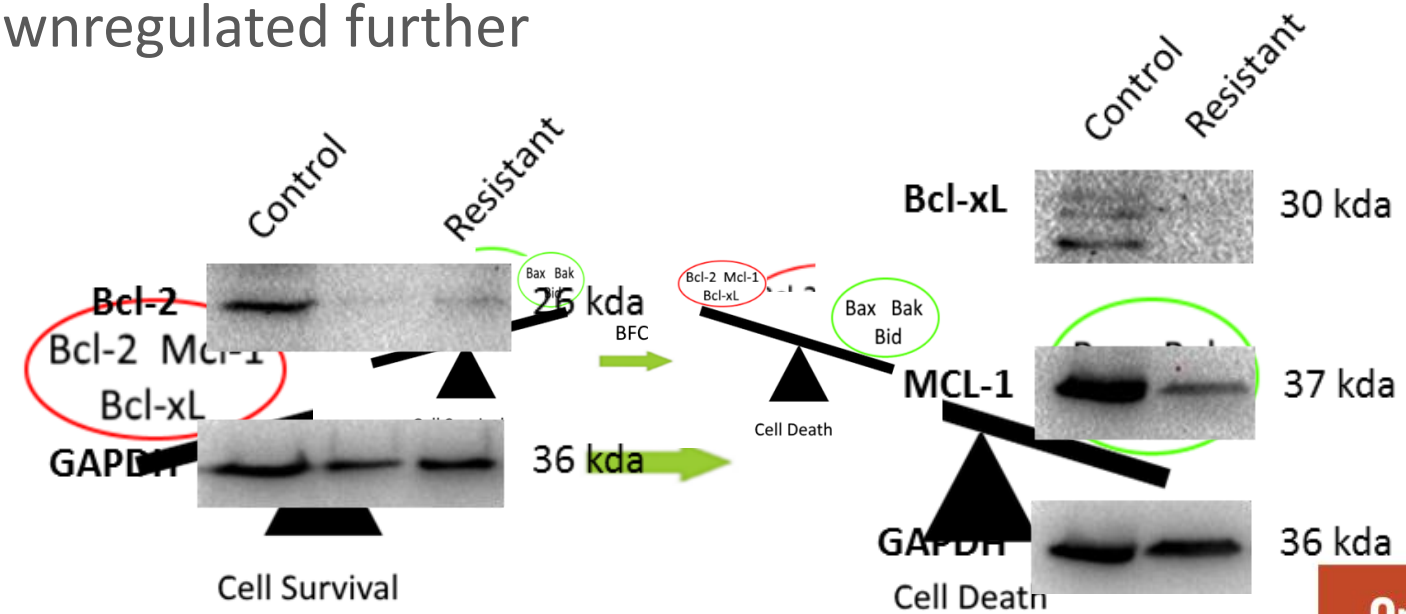




Discussion

Bcl-2 was downregulated in paclitaxel resistant cancer

- Previous targeting had been based on Bcl-2 upregulation
- Bcl-2 functional converters were effective with Bcl-2 downregulation and this presents a new targetable phenotype.
- This phenotype may be because pro-apoptotic proteins are downregulated further



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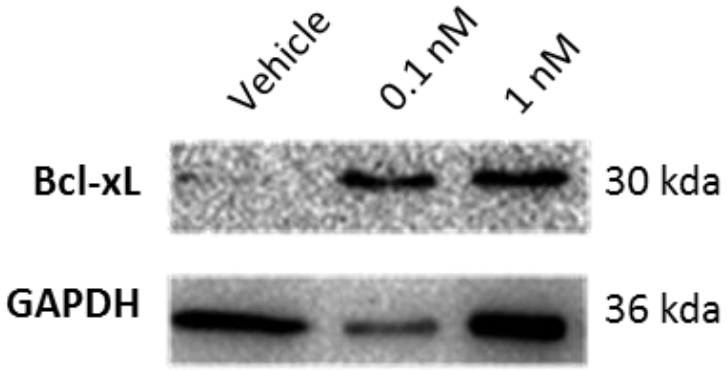
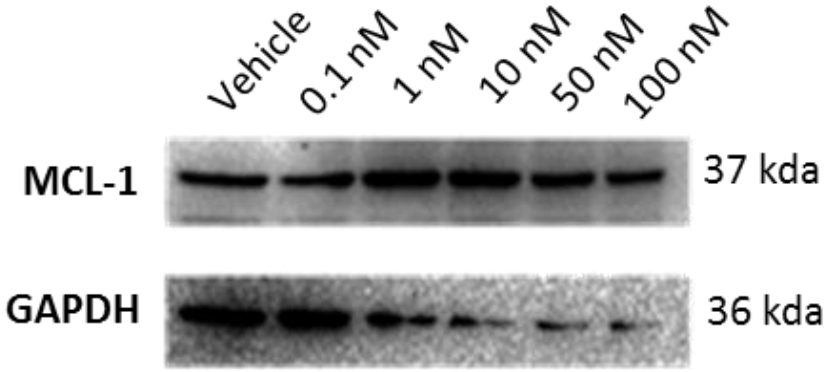
Bcl-2 Functional Converters may act synergistically with Paclitaxel

- Bcl-2 anti-apoptotic protiens are upregulated in response to paclitaxel.
- Bcl-2 functional converters can targeted this upregulation.
- A combination therapy could result in synergistic treatment.





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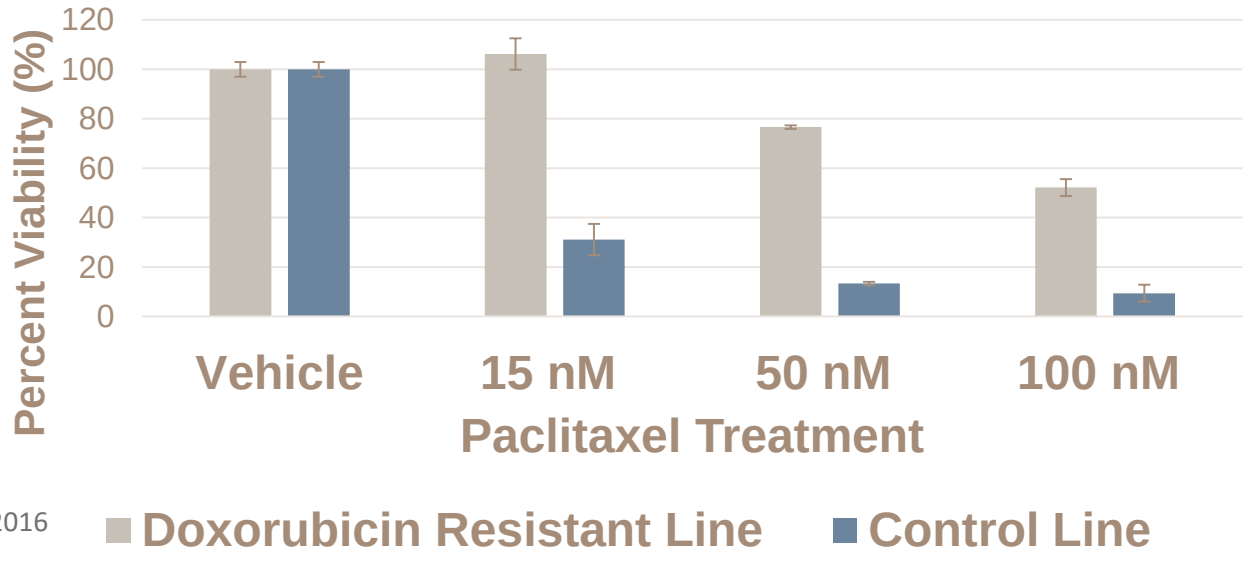
Paclitaxel resistance in Triple Negative Breast Cancer leads to cross-resistance to the other major player in triple negative breast cancer treatment.

- This leads to a decrease in treatment options.
- The lack of non-resistant treatment options furthers the need for resistance targeting.
- Triple Negative Breast Cancer that was resistant to doxorubicin also showed cross resistance to paclitaxel.



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Bcl-2 Functional Converters are effective in nutrient limiting environments

- Cancer tumors tend to have harsh conditions surrounding them (Weber and Kuo 2012).
- We need to test these harsh conditions in order to simulate real tumor conditions.
- Bcl-2 functional converters were very effective in both limited mediums tested, suggesting a viable treatment option for triple negative breast cancer in the clinic.

Key Findings

- Bcl-2 downregulation is a potential target for Bcl-2 functional converters
- Bcl-2 functional converters are effective in the selective killing of resistant cancer cells
- Paclitaxel resistance can lead to cross resistance to other major chemotherapeutics.

Acknowledgements

The Kolluri Lab

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Questions?