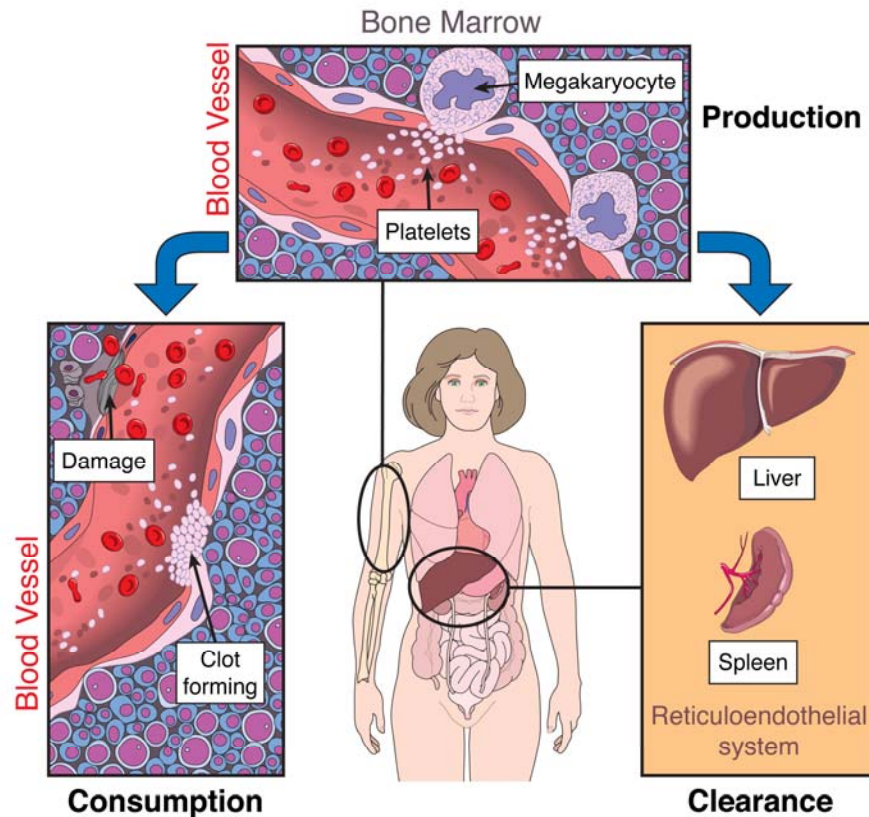

The Bcl-2 protein family is essential for platelet survival *in vivo*

Dr Kylie Mason

Walter and Eliza Hall Institute of Medical Research
The Royal Melbourne Hospital

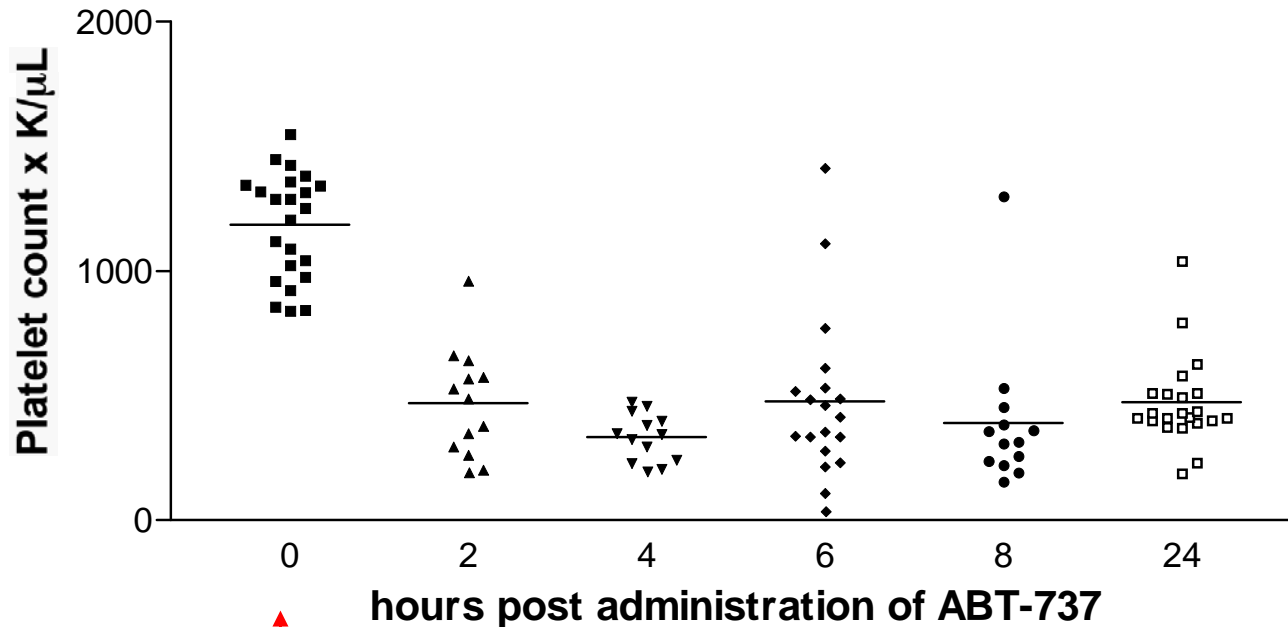


What determines platelet life-span?



The number of platelets in the peripheral blood is balanced between production and consumption

A novel anti-cancer drug, ABT-737, causes acute thrombocytopenia

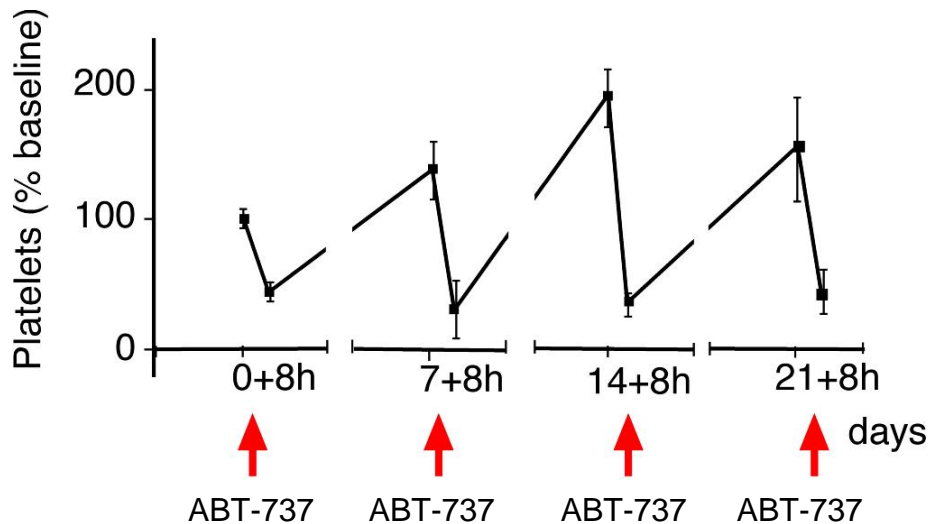


ABT-737

First noted by Abbott
Oltersdorf et al

Could this reflect decreased platelet production?

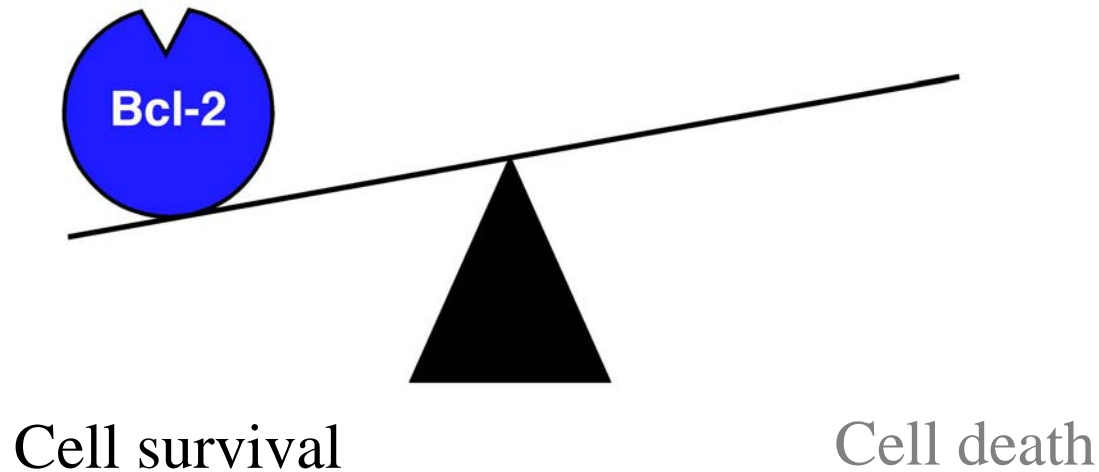
Post ABT-737, platelet production is maintained



Sternal marrow: 4 days post cessation of ABT-737

What is the mechanism of action of ABT-737?

Bcl-2 -like proteins promote cell survival



Pro-survival Bcl-2 proteins are overexpressed in many cancer cells

ABT-737 inhibits pro-survival Bcl-2 - like proteins

ABT-737

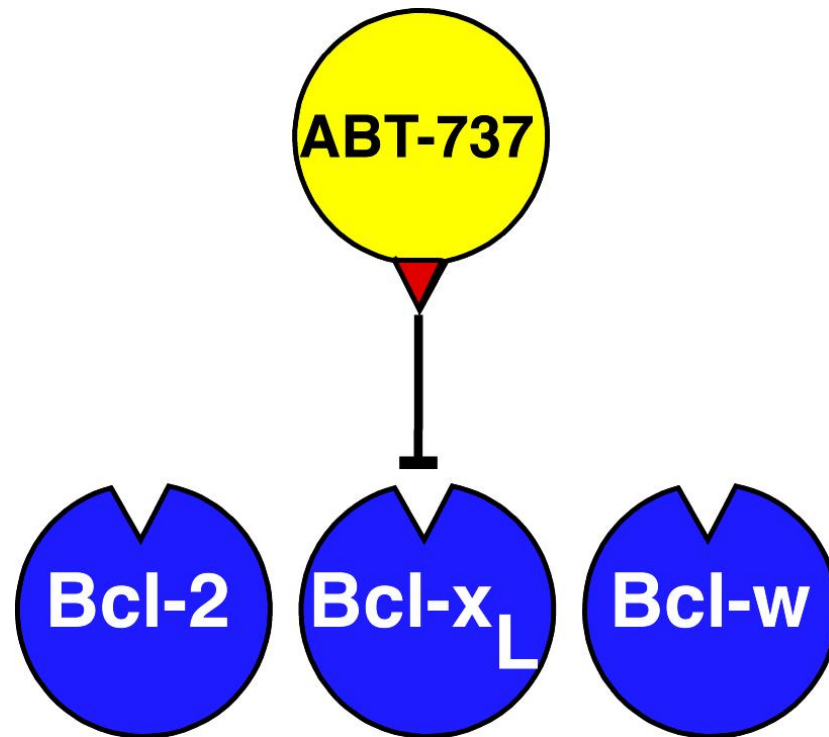


Cell survival

Cell death

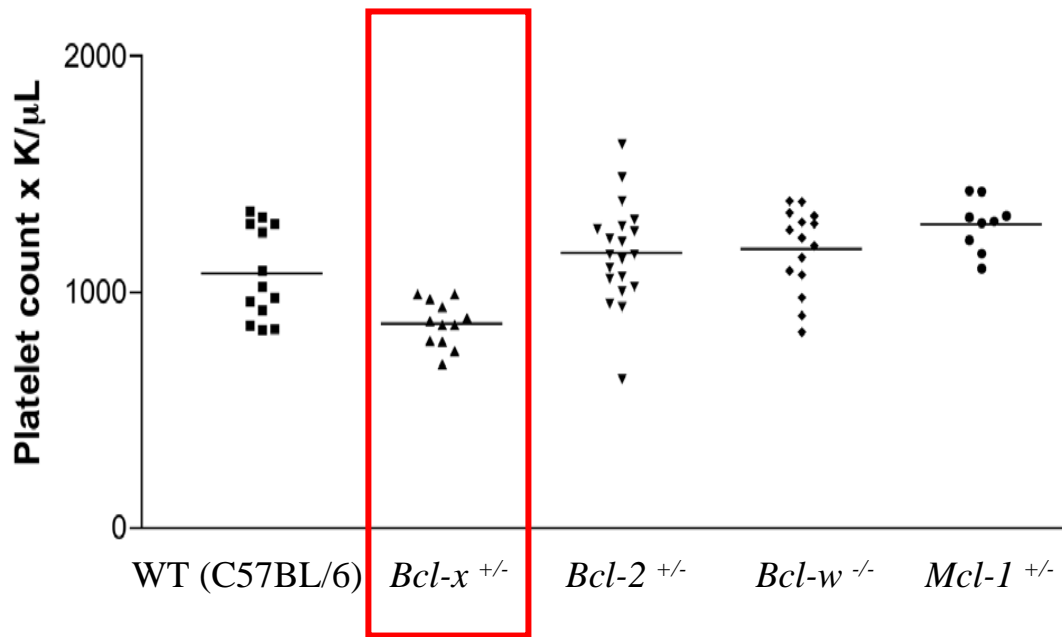
Could this thrombocytopenia be a direct effect
of ABT-737 on platelets?

ABT-737 directly inhibits three pro-survival Bcl-2 proteins



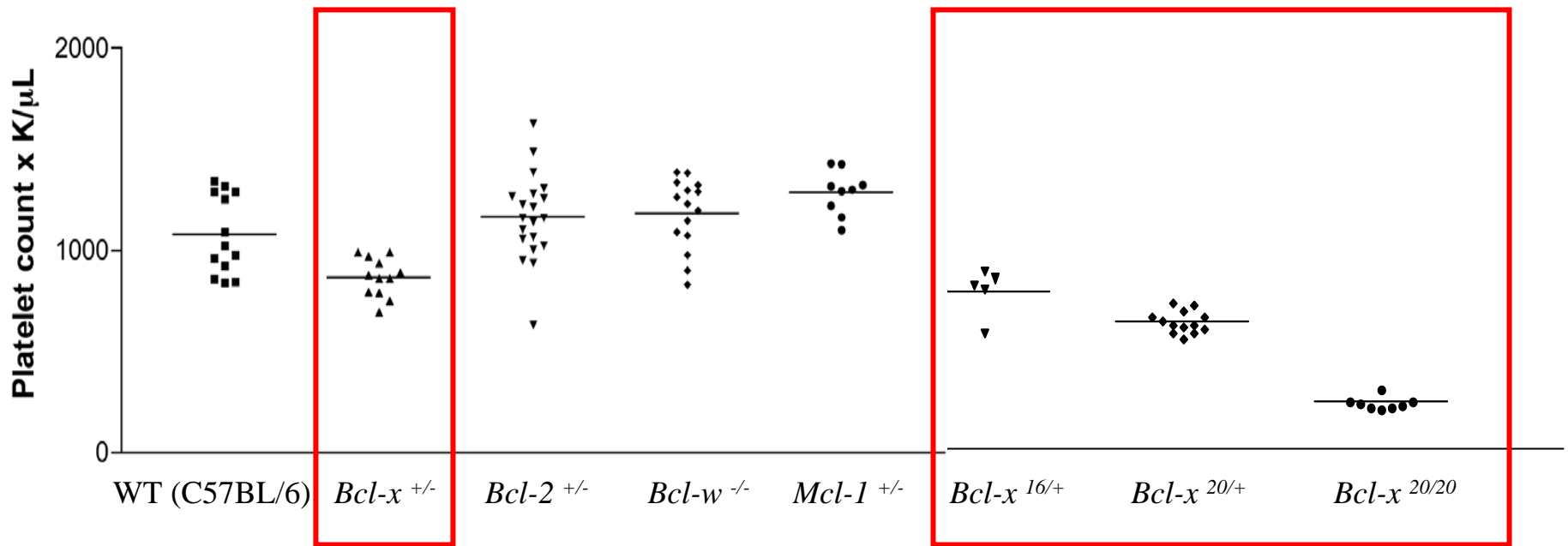
Could targeting one or more of these proteins be responsible for the thrombocytopenia ?

Mice with reduced or mutant *Bcl-x* are thrombocytopenic



Bcl-x^{+/-} mice are more sensitive to ABT-737 induced thrombocytopenia

Mice with reduced or mutant *Bcl-x* are thrombocytopenic

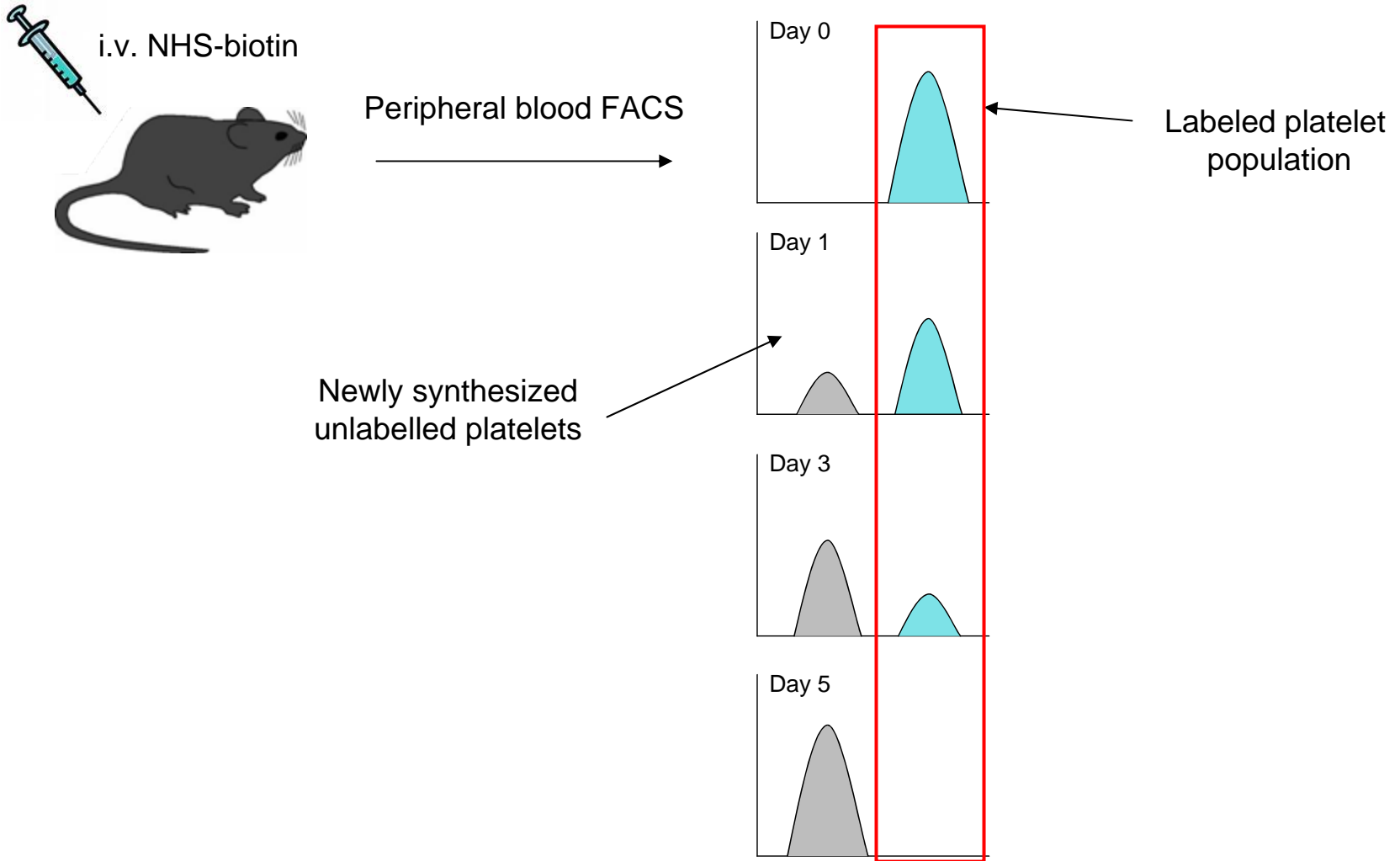


Bcl-x^{+/-} mice are more sensitive to ABT-737 induced thrombocytopenia

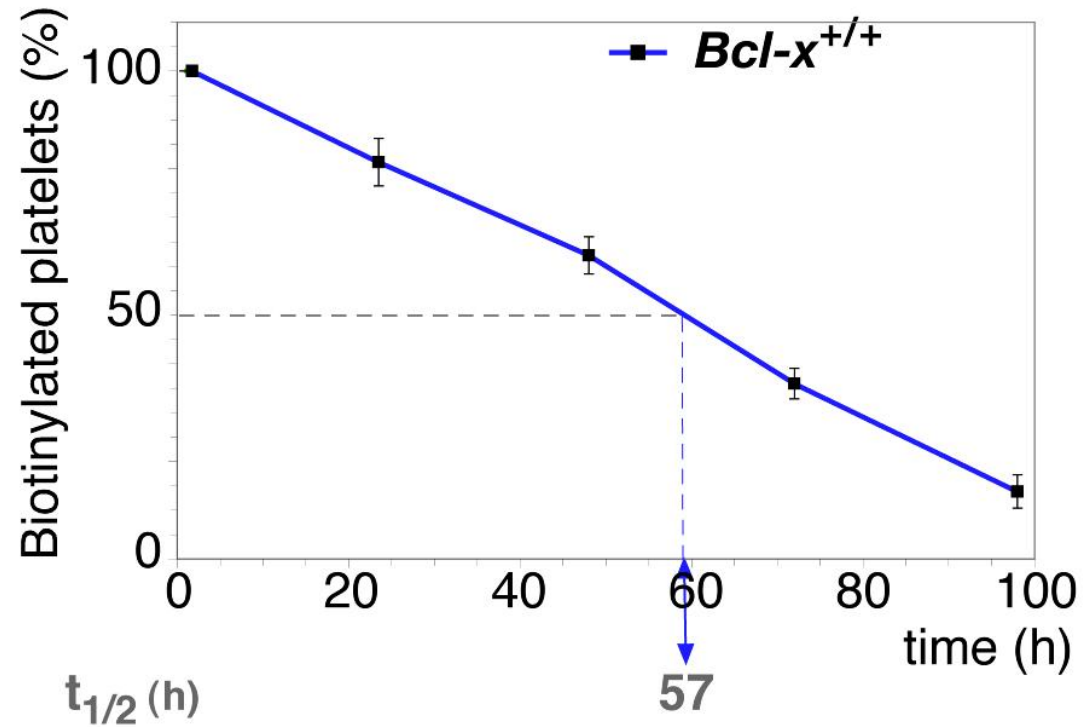
Two independently derived mouse strains (Plt16 & Plt 20) harboring missense mutations in *Bcl-x* are also thrombocytopenic

How does loss of *Bcl-x* reduce platelet numbers?

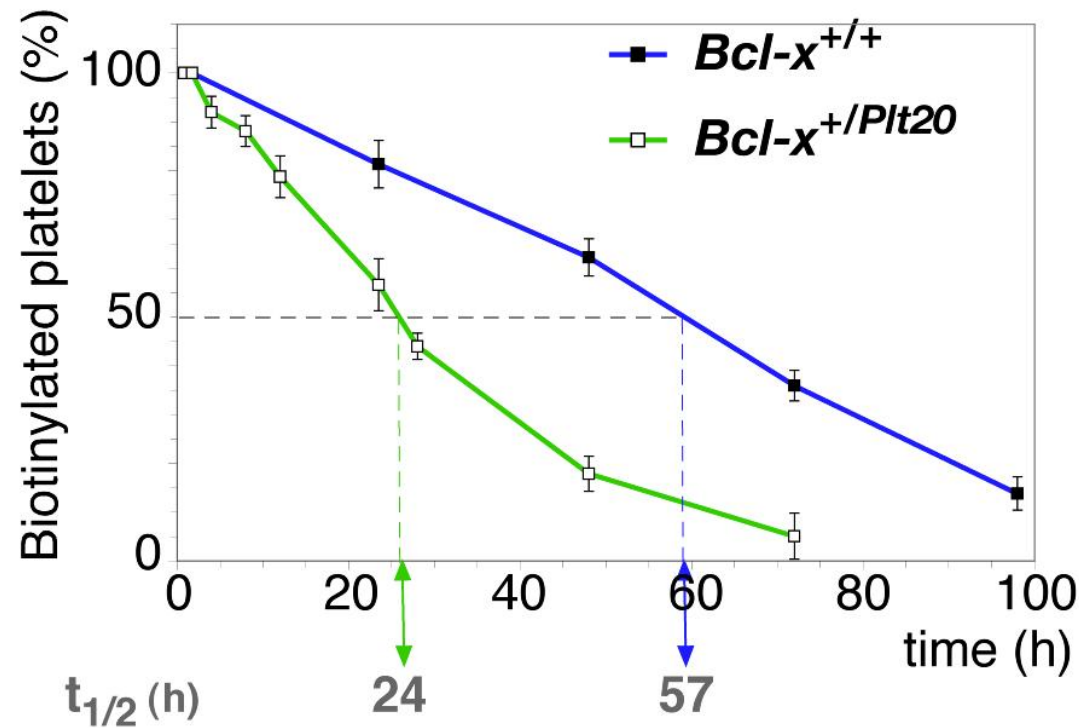
Measuring platelet life-span *in vivo*



Half-life of wild-type platelets

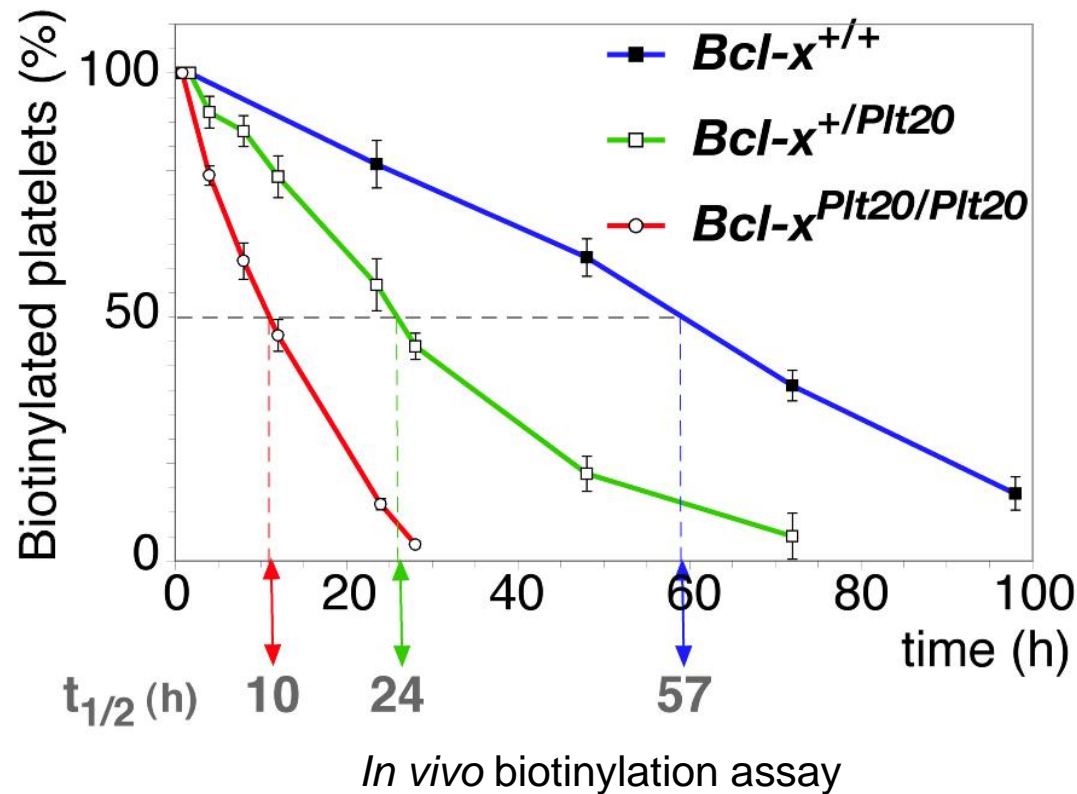


Mutations in *Bcl-x* cause a dose-dependent reduction in platelet life-span



In vivo biotinylation assay

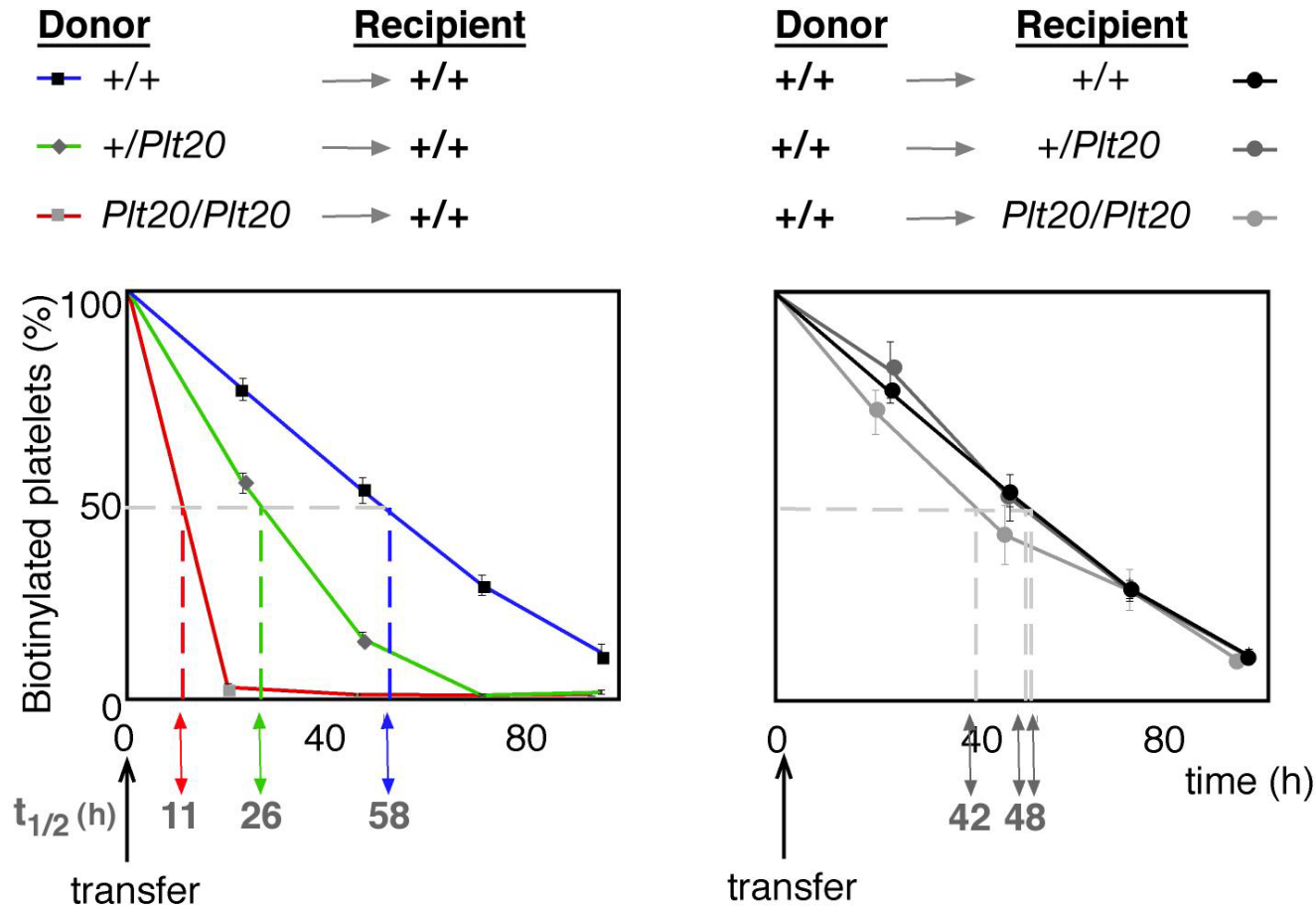
Mutations in *Bcl-x* cause a dose-dependent reduction in platelet life-span



Are these changes intrinsic to the platelet or host ?

Changes in half life are platelet intrinsic

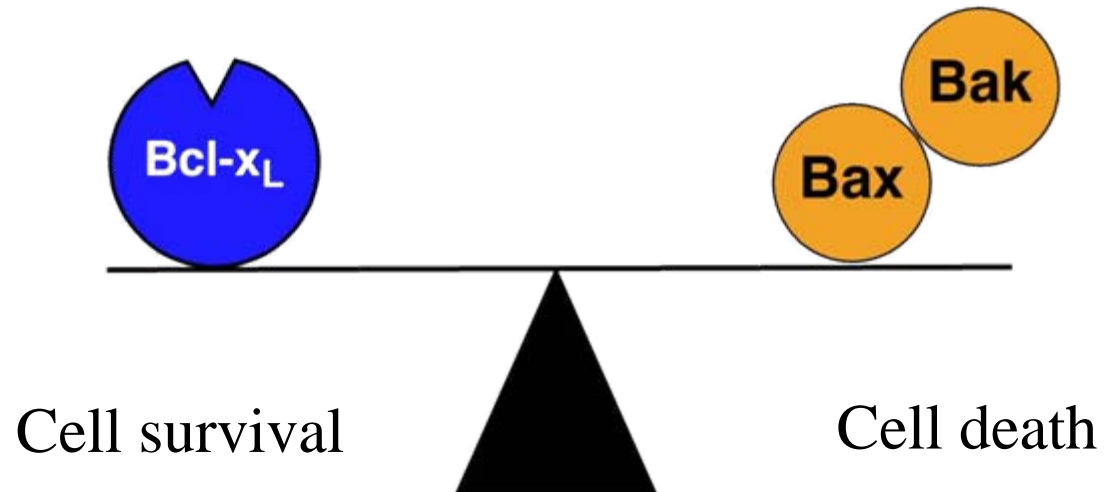
Reciprocal adoptive transfer of biotinylated platelets



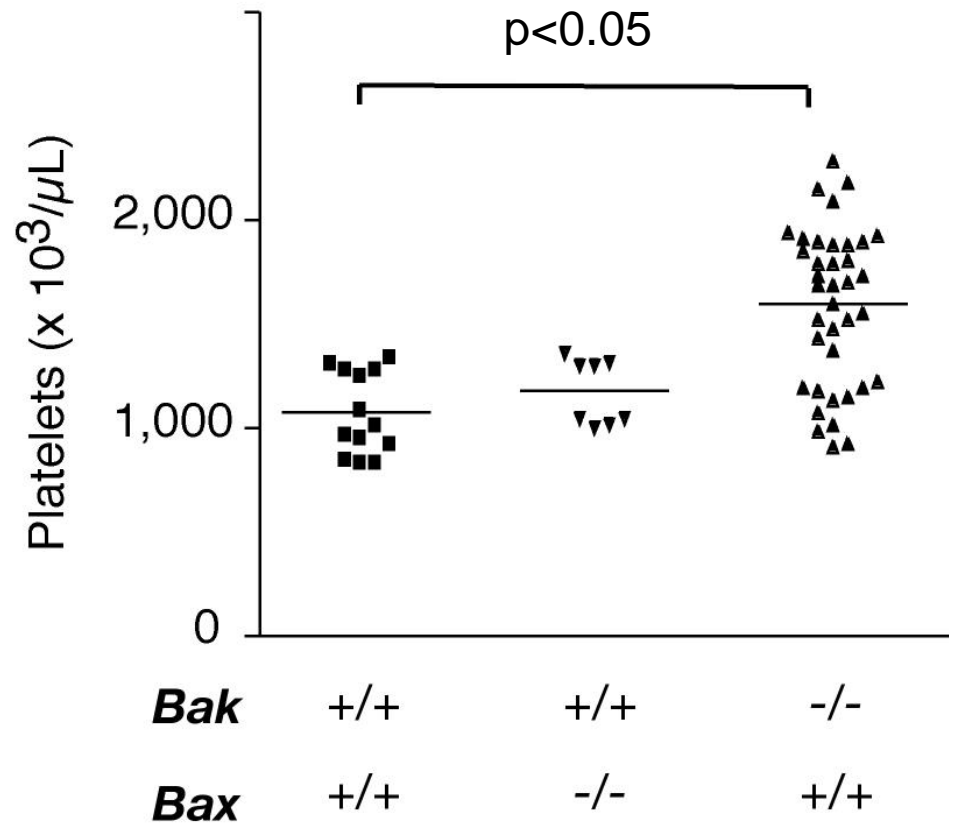
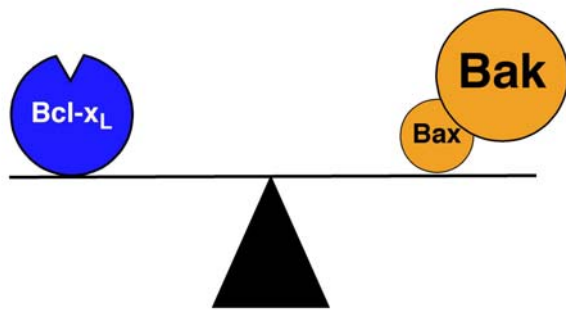
Bcl-x_L: the key to platelet survival

- Reduced Bcl-x = thrombocytopenia
- Production - normal
- Destruction - increased (shorter platelet half life)

What is the downstream target by which Bcl-x_L acts in platelets?

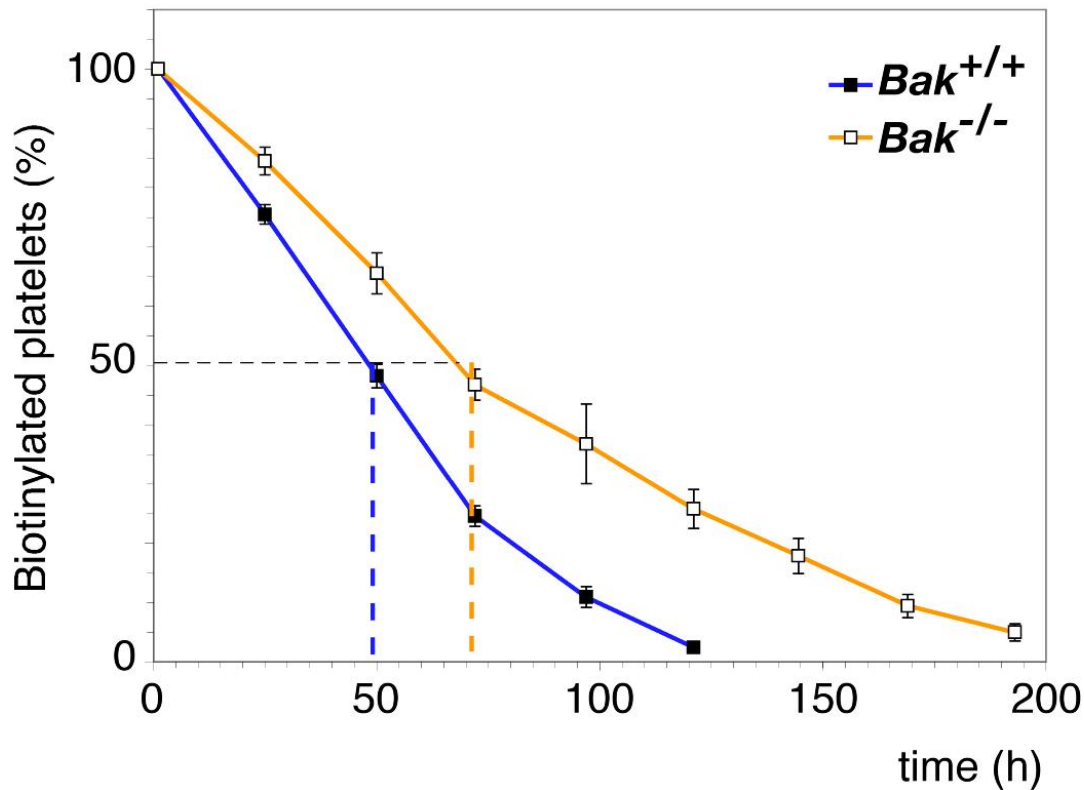


Mice lacking Bak exhibit elevated platelet counts



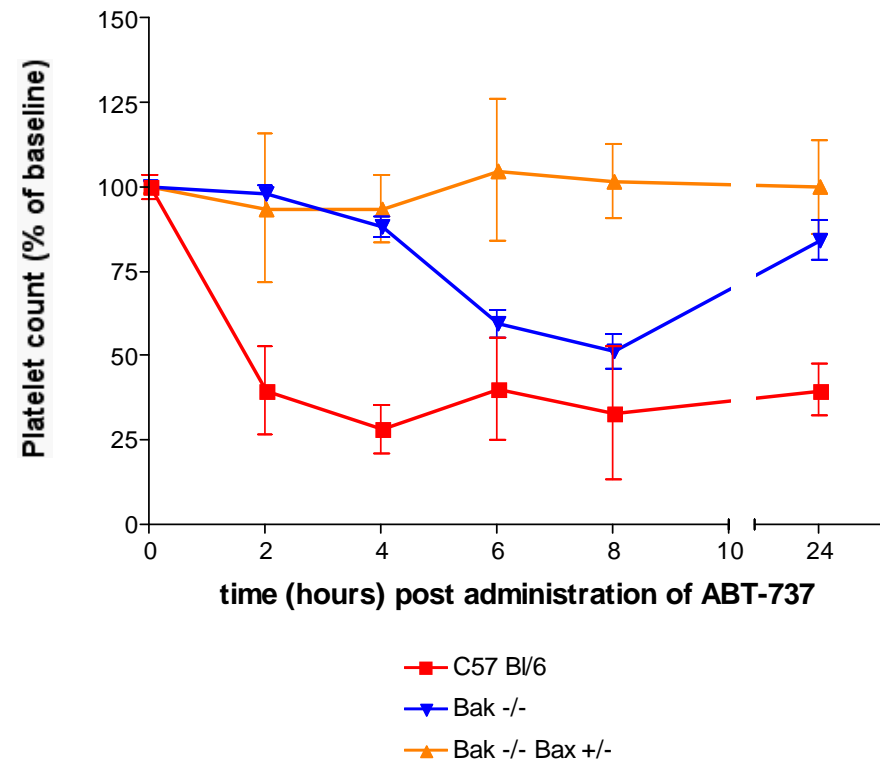
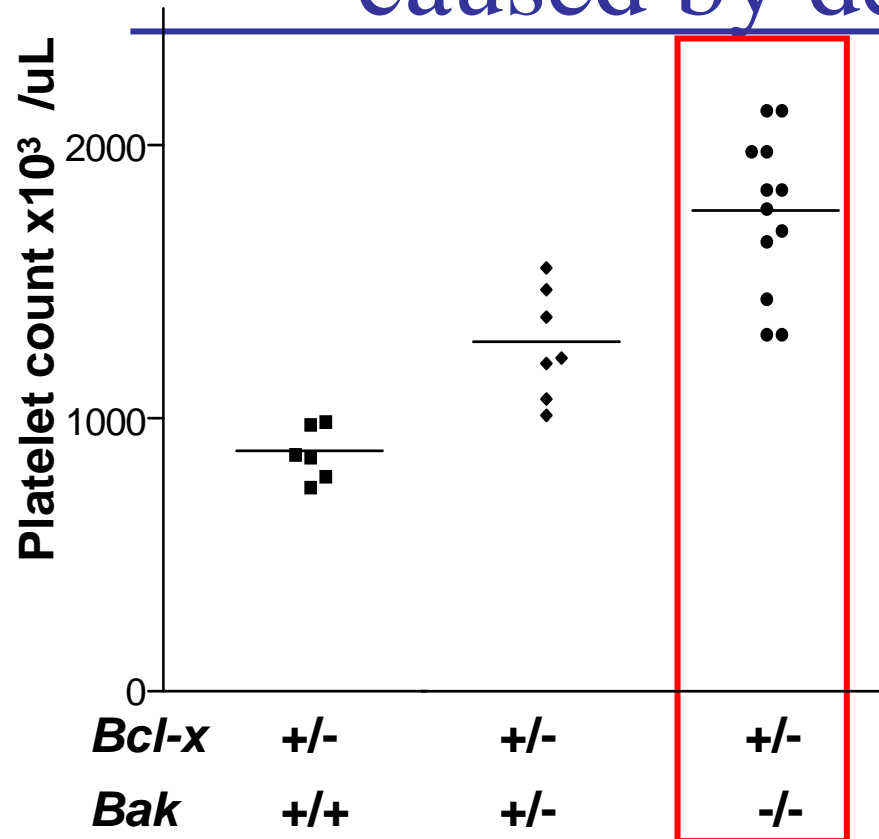
By extension, does loss of Bak extend platelet half life?

Platelet life-span is extended in Bak deficient mice



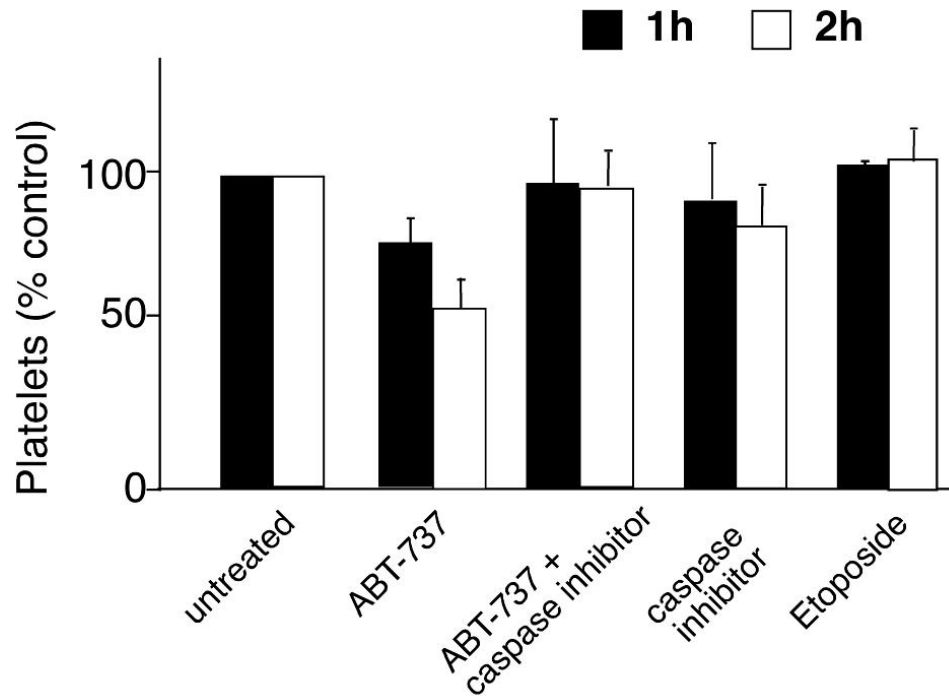
Can loss of Bak correct the thrombocytopenia seen in $Bcl-x^{+/-}$ mice?

Loss of Bak rescues the thrombocytopenia caused by decrease in *Bcl-x*



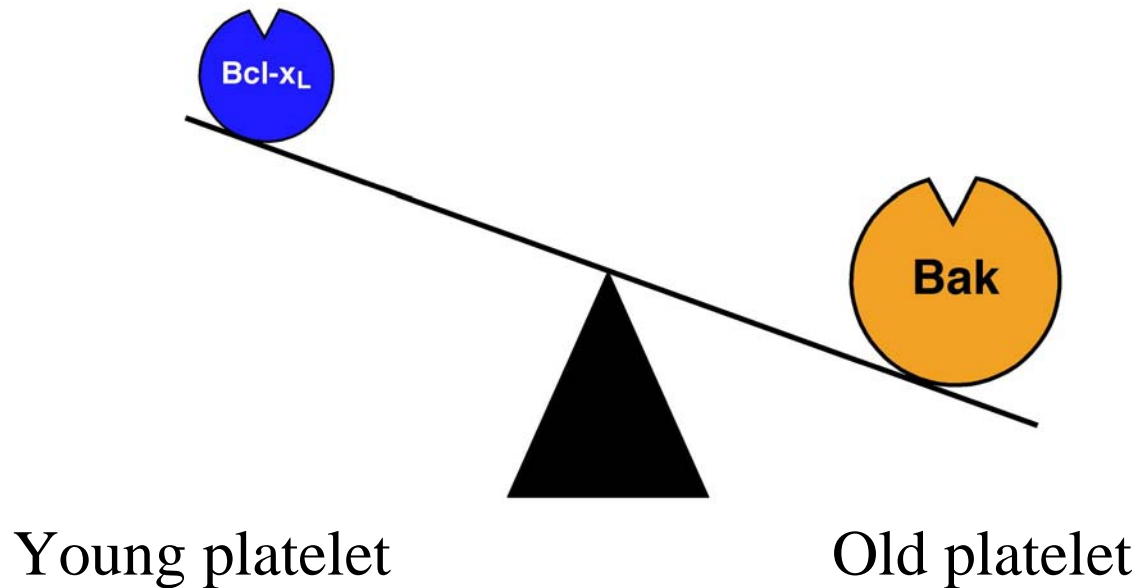
...and ameliorates the effect of ABT-737

Human platelets also demonstrate susceptibility to ABT-737



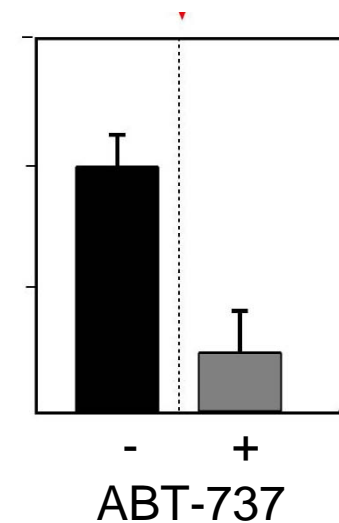
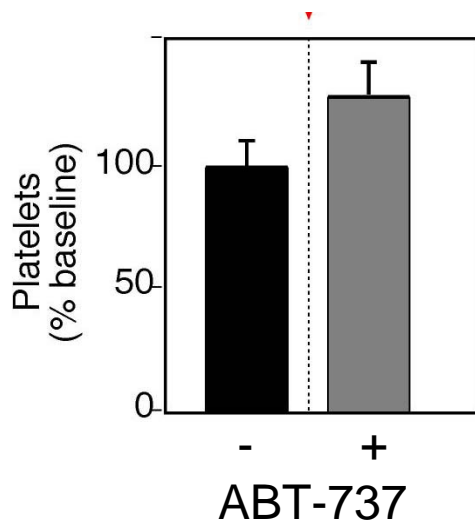
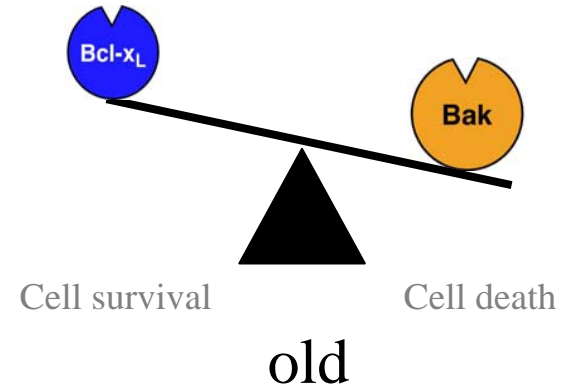
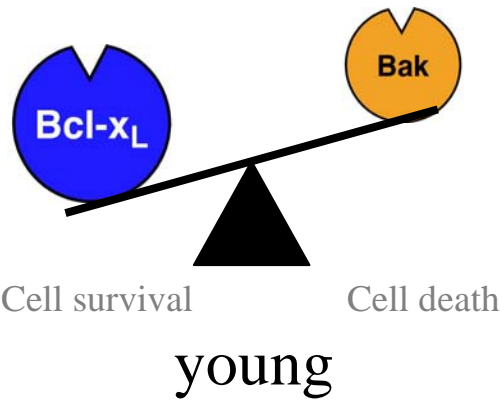
That is dependent on the classical apoptosis pathway

The Bcl-x_L : Bak axis controls platelet life span



If so, young platelets should be more resistant to
ABT-737

Young platelets are more resistant to ABT-737



Implications for disease and therapy

- Marker of bioactivity for drugs targeting Bcl-x_L
- Potential role of mutations in disorders of platelet number: thrombocytopenia / thrombocytosis
- Induction of Bak-mediated platelet apoptosis may be useful in thrombocytotic (e.g. essential thrombocythaemia) or pro-thrombotic states (e.g. atherosclerosis)
- Does the Bcl-x_L/Bak pathway contribute to platelet function?

Applications for platelet transfusion

- Potential to modulate pathway to improve platelet (blood bank) storage *ex vivo*
 - *Potential for inhibition of platelet death ex vivo by inhibition of Bak = extend life span of platelets*



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RMH

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Cancer Council Victoria

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