

# MITOCHONDRIA AND CANCER CELL DEATH



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The best way to treat cancer is to kill it. Indeed, most cancer therapies work by killing tumour cells, be it directly or indirectly. Nevertheless, combined issues of toxicity and resistance limit the effectiveness of anti-cancer therapies. To address these, our research centres on understanding how mitochondria regulate cancer cell death and inflammation, with the ultimate goal of improving cancer treatment.

## Mitochondria, cell death and cancer

Apoptosis requires caspase protease activity, leading to widespread substrate cleavage and rapid cell death. During apoptosis, mitochondrial outer membrane permeabilisation (MOMP) occurs, a crucial event that is required for caspase activation. Following MOMP, mitochondrial intermembrane space proteins, such as cytochrome c, are released into the cytoplasm where they cause caspase activation and apoptosis. Given its key role in controlling cell survival, mitochondrial outer membrane integrity is highly regulated, largely through interactions between pro- and anti-apoptotic Bcl-2 proteins. Cancer cells often inhibit apoptosis by preventing MOMP, often through upregulation of anti-apoptotic Bcl-2 proteins. Importantly, this can be exploited therapeutically – newly developed anti-cancer therapeutics called BH3-mimetics target these apoptotic blocks.

## Therapeutic targeting of BCL-2-regulated cell death in glioblastoma

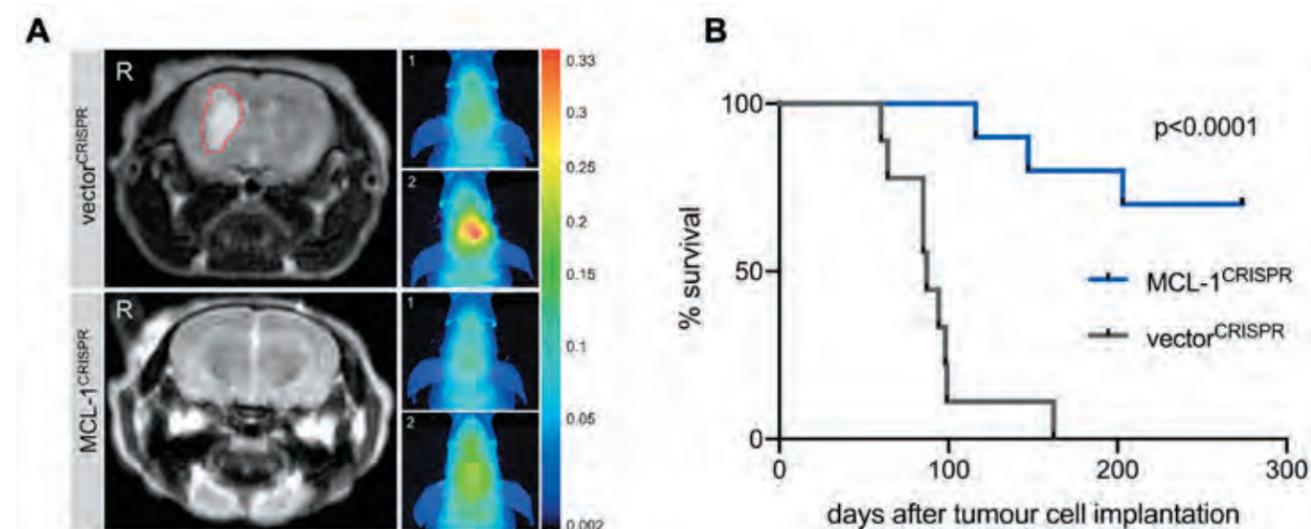
Glioblastoma is an aggressive type of brain cancer with very poor prognosis. Mainstay current treatments entail surgery, radiotherapy and chemotherapy (temozolomide) and unfortunately provide limited long-term benefit. In collaboration with Prof. Anthony Chalmers (Institute of Cancer Sciences), we are investigating whether targeting pro-survival BCL-2 proteins (using BH3-mimetics) either alone, or combined with radiotherapy, may improve therapeutic outcome. Towards this goal, we apply various methods including patient-derived tumour cell lines, *in vivo* mouse models as well as *ex vivo* culture of primary tumour samples. We find that BH3-mimetics can cross the blood brain-barrier to reach effective concentrations. Most importantly, we are finding that glioblastoma often displays dependency on specific BCL-2 family members, including MCL-1 (Figure 1). Future work will

determine the molecular basis for this survival dependency and address the efficacy of combining BH3-mimetics in combination with radiotherapy.

## BCL-2 proteins, metabolism and cancer

BCL-2 proteins have been implicated in the regulation of metabolism, although exactly how they modulate metabolism remains elusive. Moreover, whether metabolic roles of BCL-2 proteins impinges on tumorigenesis is unclear. Applying BH3-mimetics as tool compounds, we have investigated how BCL-2 proteins regulate metabolism, with the aim of investigating whether this contributes to BCL-2 oncogenic effects and/or impinges on the efficacy of BCL-2-targeting BH3-mimetics. Our approach to this question has made use of metabolic flux analyses and mitochondrial activity assays. Interestingly, treatment of cells with the clinically approved BCL-2 inhibitor venetoclax (also called ABT-199) reduces basal oxygen consumption across a panel of cell lines and alters mitochondrial morphology (Figure 2). Importantly, these effects are independent of pro-apoptotic BAX and BAK, thus demonstrating that venetoclax suppresses oxygen consumption independently of the canonical apoptotic machinery. Moreover, we demonstrated that BCL-2-regulated metabolic effects appear solely through inhibition of cell death. Ongoing work aims to understand how venetoclax mediates these effects and define whether these affect its cell killing ability.

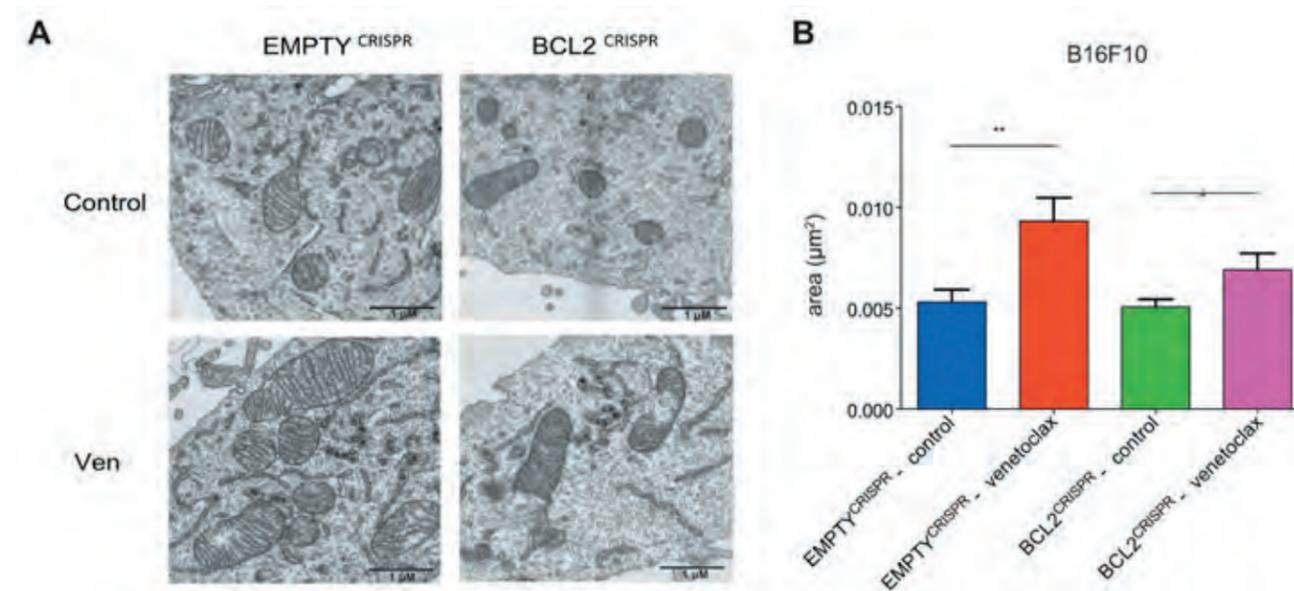
Publications listed on page 111



**Figure 1**

Anti-apoptotic MCL-1 supports glioblastoma growth

A) MCL-1 was deleted by CRISPR/Cas9 genome editing in patient-derived glioblastoma cells expressing iRFP. Following implantation in mice, brain tumour growth was measured by MRI or by iRFP analysis. Tumour cells lacking MCL-1 display strong inhibition in tumour growth  
B) Survival plot showing that mice implanted with glioblastoma cells lacking MCL-1 have a significant survival advantage.



**Figure 2**

Bcl-2-targeting BH3-mimetic venetoclax alters mitochondrial morphology

A) Transmission electron microscopy (TEM) images from B16F10 CRISPR-EMPTY and CRISPR-BCL2 cells after 24h of venetoclax (1µM) treatment. Scale bar=1µm.

B) A random selection of at least 25 mitochondria across different cells and fields was analysed for each condition from images in A).