The Drug Discovery Unit operates at the interface between discovery biology and clinical development. We provide a mechanism by which fundamental biology being undertaken within the Beatson Institute can be translated into new medicines for patients where there is a clear unmet medical need. We work collaboratively with the Institute's research scientists to identify areas of biology fundamental to the progression of cancer and target these to develop novel medicines that will positively impact the lives of patients.

Drug discovery is a multi-faceted scientific endeavour, involving expert scientists in cancer biology, chemistry, biophysics, biochemistry, structural sciences and clinical research. High quality, reproducible science is central to drug discovery and we have developed a collaborative team with a robust approach to rapidly progressing novel therapeutic opportunities. Working closely with Beatson scientists and clinicians, we have identified a number of exciting new targets that are being progressed in our laboratory.

Identifying new cancer target is the key initial step in the drug discovery process. Once this happens, a number of parallel drug discovery activities ensure a rationalised progression through discovery research to generate a new drug, ready to test in patients. We have built a focused capability that enables us to execute even the most challenging projects and successfully progress them through the drug discovery process. In order to understand how to modulate the function of a target (typically a protein) that is important in the progression of cancer, we need to understand how it works and how best to stop it working. By identifying molecules that interact with our target of interest we can evaluate their potential to modulate target function and gain valuable insight into their importance in cancer.

Drawing on the world-class research at the Beatson Institute is a key remit of the Drug Discovery Unit. As such, we interact closely with research groups within the Institute to identify novel and exciting targets for translation into the drug discovery paradigm. In collaboration with Professor Stephen Tait, we are aiming to identify small molecule inhibitors of the caspase proteases that can be combined with standard-of-care chemotherapeutics to promote a caspase-independent cell death in tumours.

Targeting cancer cells to die by Caspase-Independent Cell Death (CICD)

Caspase-Independent Cell Death (CICD) is an emerging mechanism by which cancer cells die when exposed to anti-cancer therapies. The first committed stage in cell death is permeabilisation of the mitochondrial outer membrane (a process called MOMP). BH3 mimetics such as ABT-737 induce MOMP, which leads to the subsequent activation of the caspase cascade. By its nature, apoptosis is limited to the specific cells affected by treatment and furthermore, can promote oncogenesis in remaining cells, leading to recurrence. Once cells undergo MOMP, this is a point of no return for cell viability and will result in cell death. However, seminal work in Stephen Tait's group has demonstrated that a block of the caspase cascade (notably caspase-9) causes cells to be re-routed from a rapid apoptotic death to a delayed caspase-independent cell death (CICD) that is pro-inflammatory. Blockade of caspase 9 in vivo and in vitro leads to activation of pro-inflammatory M1 macrophages and immune clearance of tumours, which is not seen in immune-compromised mice.

Working closely with Professor Stephen Tait and his group, we have made excellent progress in developing a robust screening cascade that utilises our in-house biophysics, crystallography, biochemistry and cell biology expertise. By performing drugability and structural assessments of the caspase family, we have identified potentially druggable binding sites. With that in mind, we have developed a strategy to screen for small molecules that could perturb the function of these caspases.

To facilitate our drug discovery efforts, we have developed and validated biochemical assays for measuring activities of a number of caspases. All assays use a tetra-peptide substrate (e.g. DEVD for caspase 3 and 7) labelled with the fluorescent molecule amino-4-trifluoromethylcoumarin (AFC). Cleavage of this fluorophore leads to a concomitant increase in the fluorescence signal, allowing for detection of caspase activity modulation. We have utilised a small panel of commercially available caspase inhibitors to validate these assays and show their suitability for compound screening (Fig. 3). By screening an in-house library of ~750 reversible compounds, we have identified a number of chemical hits for caspase 3/7 inhibition and have shown the assay is suitable for a larger high-throughput screening campaign.

We have characterised appropriate cellular assay systems to measure caspase inhibition and changes in cell viability, and therefore CICD. When used alone the BH3 mimetic ABT-737 induces MOMP, caspase activation and a rapid apoptotic form of cell death (Fig. 4). Treatment
with a pan-caspase inhibitor (such as Emricasan, Q-VD-OPh or Z-VD-fmk) in combination with ABT-737 prevents cells from dying via apoptosis but instead promotes a delayed, non-apoptotic cell death that is independent of caspase 3, 7 and 9 activity (Fig. 4). Moreover, these HCT116 cells undergoing CICD upregulate the expression of a number of cytokines and chemokines, such as CSF2, CXCL1 and TNFα, involved in pro-inflammatory signalling and immune cell activation (Fig. 5).

Taking a multi-disciplinary approach, which is the foundation of all drug discovery projects, has enabled rapid target validation and assay development in the caspase project. Working with Stephen Tait provides the depth of expertise in this complex area of cell death in cancer, helping to guide the key biological questions that will enable us to target this cellular process in the most effective manner. This model of close collaboration is the basis of all drug discovery projects at the Institute and ensures even the most challenging targets are approached with a strong and far-reaching capability that allows for efficient and successful progression of promising new treatments in cancer.

Publications listed on page 84

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**Figure 3**
Biochemical Assay Development: Reaction time course and substrate Km for Caspase 3.

**Figure 4**
Caspase inhibition with Q-VD-OPh in combination with ABT-737 leads to a non-apoptotic cell death in HCT116 cells that is independent of caspase 3, 7 and 9 activity.

**Figure 5**
CICD upregulates the expression of pro-inflammatory cytokines and chemokines involved in pro-inflammatory signalling and immune cell activation.

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**ADVANCED TECHNOLOGIES**