Exploiting Drug Metabolism to Activate Immunity Against Cancer

Dr Rock J. Mancini

Scientia

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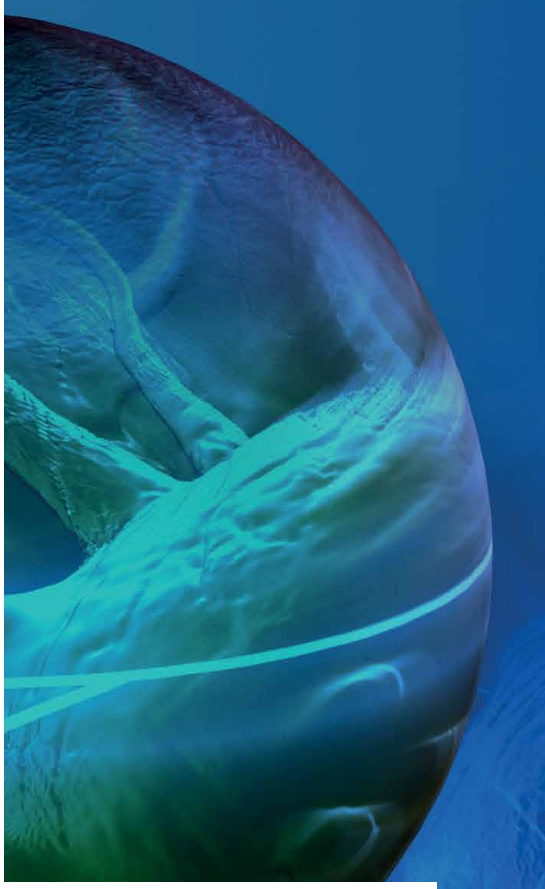


Multidrug resistance is one of the main culprits underlying the failure of chemotherapy as a cancer treatment. Whilst many therapies are initially effective, a considerable proportion of patients eventually incur a poor prognosis and recurrence of malignant spread due to developing drug resistance at a later stage. **Dr Rock J. Mancini**, from Washington State University, has devised an approach that exploits proteins over-expressed in drug-resistant cancers to convert inactive prodrug substrates into active drugs that initiate an immune response targeted at cancer cells.

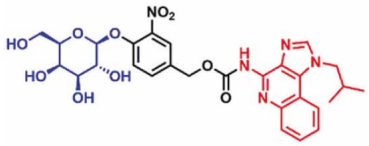
Fighting Multidrug Resistance in Cancer

In recent years, there have been significant advances in the treatment of several types of cancer with chemotherapy and immunotherapy alike. Many cancers are either resistant to specific chemotherapeutic drugs or acquire drug resistance at a later stage, despite initially responding favourably to therapy. However, immunotherapy has also emerged as a new class of cancer treatment with efficacy that is fundamentally orthogonal to acquired chemotherapeutic drug resistance.

There are several mechanisms through which cancer cells develop drug resistance. One commonality across many drug-resistant cancers is that they use molecular transporters that derive energy from intracellular adenosine triphosphate to actively pump cancer drugs out of the cells; a series of biochemical reactions resulting in the mechanisms of drug resistance known collectively as 'drug efflux'.

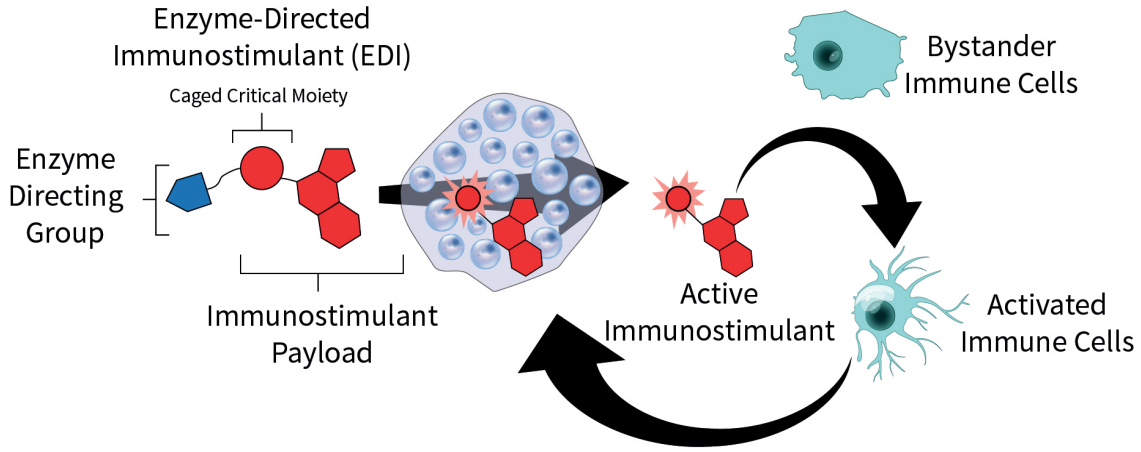
To address this, Dr Rock J. Mancini, Assistant Professor of Organic Chemistry at Washington State University, is pioneering methods that exploit drug efflux to link the immune system, at a chemical level, to drug-resistant cancers. Dr Mancini is working to create an array of smart immunostimulant drugs that are finely modulated by stimuli including enzyme activity, temperature, and disease-specific biomarkers that are present in or on cancer cells.

Dr Mancini's team of researchers have devised an ingenious strategy that exploits drug efflux to raise an immune response against cancer cells, an approach that could provide clinical advantage to patients with multidrug-resistant cancers. The approach uses smart immunostimulants that are metabolised by cancer cells, which then secrete immunostimulant drugs via drug efflux, effectively alerting the immune system to the cancer. By coupling cancer cell metabolism and drug efflux to activate the immune system, the team hopes to affect the ability of cancer cells to escape their fate. The hope is that, by selectively activating immune cells in close proximity to cancer cells, this technology will enable a new way in which drug-resistant cancers can be eradicated, and tumour growth and spread can be halted.



Molecular structure of a smart immunostimulant developed by the Mancini Laboratory.

Overview of Bystander-Assisted ImmunoTherapy (BAIT)



A Trojan Horse to Trigger Immunity Against Cancer Cells

Over the past decades, medicinal chemists have contributed to the rational design and production of synthetic immunostimulants, compounds that interact with specific receptors on immune cells, triggering a proinflammatory response. However, immunostimulants are susceptible to diffusion, off-target activity, and other drug resistance mechanisms, including the drug efflux pathway described above.

Dr Mancini's group intentionally uses immunostimulants as drug efflux substrates to hijack the drug resistance mechanism to produce molecules that activate immune cells. Specifically, his research group is recognised as the first to develop enzyme-directed immunostimulants (immunostimulants activated by enzymes present in cancer cell metabolism) and demonstrate that they undergo drug efflux from multidrug-resistant cancers.

Cocking the Molecular Guns

One approach to addressing drug resistance is directed enzyme prodrug therapy (DEPT), which attempts to outcompete drug efflux by increasing the local concentration of therapeutic drugs in the area surrounding tumour cells. In DEPT, enzymes within a solid tumour convert an enzyme-directed prodrug into an active anti-tumour chemotherapeutic. With this approach, there is the potential for diffusion of the active drug away from the target cell, where it can interact with other nearby cells via a phenomenon known as the 'bystander effect'. The bystander effect can be beneficial, resulting in the permanent damage of nearby cancer cells, or unwanted, resulting in overall toxicity from the damage of nearby non-cancerous cells.

Importantly, drug efflux enhances the bystander effect. Dr Mancini and his team created a way to harness the bystander effect for potential therapeutic advantage by replacing the chemotherapeutics typically used in DEPT with immunostimulants. This modification makes the bystander effect a desirable outcome, causing multidrug-resistant cancer cells to activate bystander immune cells.

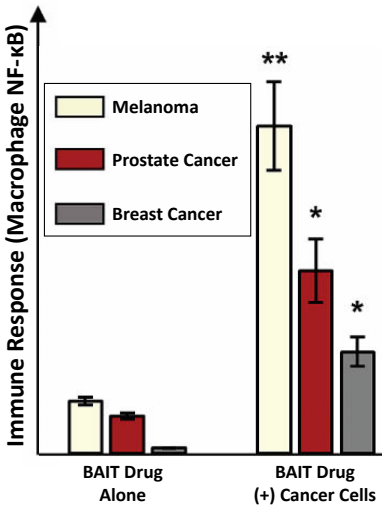
Dr Mancini and his team named their approach 'bystander-assisted immunotherapy' (BAIT). In BAIT, an inert prodrug is first metabolised by enzymes within multidrug-resistant cancer cells to produce an active immunostimulant metabolite. Following this conversion, the immunostimulant is transported to the extracellular space, via drug efflux. Once in the extracellular space, the immunostimulant activates bystander immune cells, resulting in an immune response initiated at the site of the cancer.

The advantage of engaging immune cells to target tumours is that, once they are trained to recognise cancer cells, they have the ability to destroy them with high specificity, leaving healthy cells nearby intact. Given that enzyme-directed immunostimulants are generated within the tumour microenvironment, they are perfectly placed to target tumour cells with high efficacy and low toxicity.

Cancer Takes the Bait

In two different papers, published in 2016 and 2018, Dr Mancini and his team reported the creation of the first enzyme-directed proimmunostimulants in the form of imidazoquinoline-pyranosides, substrates for hydrolase enzymes, such as the alpha-mannosidases expressed by cancer cell metabolism. Effectively acting like a piece of cheese in a mousetrap, the BAIT prodrug is harmless and inert until it is eaten (metabolised) by cancer cells, ultimately initiating an anti-cancer immune response.

In the system developed by Dr Mancini's laboratory, alpha-mannosidase converts the prodrug substrate into the active immunostimulant imiquimod. The studies were conducted *in vitro* on cultured prostate cancer cells, and the enzyme-mediated production of imiquimod resulted in the activation of macrophages and dendritic cells, providing robust evidence that the



Data taken from Ryan et al., Comparing the immunogenicity of glycosidase-directed resiquimod prodrugs mediated by cancer cell metabolism, Acta Pharmacologica Sinica, 2020, doi: 10.1038/s41401-020-0432-4

mechanisms of drug efflux in cancer cells can be exploited via BAIT. Like most cancer immunotherapies, BAIT offers the advantage of specifically targeting one undesired type of cell, unlike other chemotherapeutic agents that cause high levels of toxicity due to their lack of specificity.

Dr Mancini and his team used cell cultures to demonstrate that enzyme-directed immunostimulants only target those cultures that overexpressed the corresponding proteins associated with multidrug-resistant cancer cells. This indicates that this method triggers a cancer-specific immune response without causing inflammatory toxicity, and could be used alongside blockers of immunosuppression to improve efficacy. Preliminary *in vivo* mouse studies demonstrate that enzyme-directed proimmunostimulants are well-tolerated and do not cause systemic inflammatory toxicity in otherwise healthy mice. The team is currently testing the efficacy on a model of drug-resistant breast cancer in mice, providing evidence that immunostimulants produced by the enzyme-directed BAIT method are more effective when compared to directly administered parent immunostimulant.

Comparing the Immunostimulant Effect Across Different Cancer Cell Lines

In optimizing the BAIT approach, Mancini and his group compared the immunogenicity of several enzyme-directed prodrugs that all have the imidazoquinoline immunostimulant resiquimod embedded in their structure. Like imiquimod, resiquimod is a ligand for immunogenic toll-like receptors. This compound was chosen for its potency at nanomolar concentration, rather than simplicity, as was the case in earlier iterations. The cancer types studied were skin cancer, prostate cancer, and breast cancer, because they are among the most frequently diagnosed cancers in the USA.

The group published their latest results in 2020 which confirm that immunogenicity across different cancer cell lines is due to the enzymatic conversion of substrate prodrug into the immunostimulant resiquimod, liberated following drug efflux. The team compared cancer cell metabolism of the prodrug to adding the enzymes responsible for metabolism separately, both of which resulted in increased production of active immunostimulant and the activation of immune cells. It was established that melanoma cells *in vitro* showed the greatest extent of immunogenicity, followed by prostate cancer cells and breast cancer cells.

Not surprisingly, the group established that different cell lines displayed different levels of enzyme expression and activity. Those differences in glycosidase activity and drug efflux across different cancer cell lines will provide insight into which factors affect the cancer-mediated activation of immune cells, serving as a powerful diagnostic tool to predict the efficacy of BAIT in specific cancers.

Optimizing BAIT Drug Design and Other Future Developments

The team is currently optimising a lead enzyme-directed immunostimulant for further studies *in vivo*. Dr Mancini and his group also hope to confirm the observation that cancer cell drug efflux, a trait linked to drug resistance, is the most important factor that drives efficacy of the prodrugs. To test this hypothesis, his lab has spent the past months growing cells that are resistant to chemotherapy, a process that involves adding chemotherapeutic drugs to the cell culture and selecting the resistant cancer cells that survive. Overall, the findings suggest that BAIT is best suited for cancer cells that overexpress proteins associated with drug efflux, in particular multidrug-resistant cancers that do not respond to chemotherapy. However, to understand the process at a molecular level, more studies will be needed to characterise all of the possible routes of efflux, as the immunostimulant might be a substrate for many different transport proteins involved in drug efflux.

Given that the efficacy of traditional chemotherapeutics is attenuated by mechanisms of drug resistance, Dr Mancini's research efforts will continue to provide tools to interrogate the immune system, shedding light on how immune cells can be activated and initiate a targeted response against cancer. In the long term, the team has the ambition of contributing significantly to the field of medicinal chemistry by designing the next generation of immunotherapeutics and cancer vaccines.

Meet the researcher

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Dr Rock J. Mancini is Assistant Professor of Organic Chemistry at Washington State University. Dr Mancini obtained his PhD in Organic Chemistry from the University of California, Los Angeles, in 2012. He subsequently trained as a synthetic immunologist from 2012 to 2015 while pursuing his postdoctoral studies at the University of California, Irvine. Since starting his tenure track position in 2015, his research interests have evolved to incorporate organic chemistry, chemical biology, and polymer chemistry. The research focus of his laboratory is the development of methods to both control and interrogate the immune system, aimed at creating the next generation of immunotherapeutics and vaccines. Dr Mancini mentors several junior researchers in his team and encourages them to reach their full potential as scientists by acquiring a deep understanding of basic and applied research processes, from hypothesis generation to its proof of concept and ultimate dissemination to the scientific community.

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FURTHER READING

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