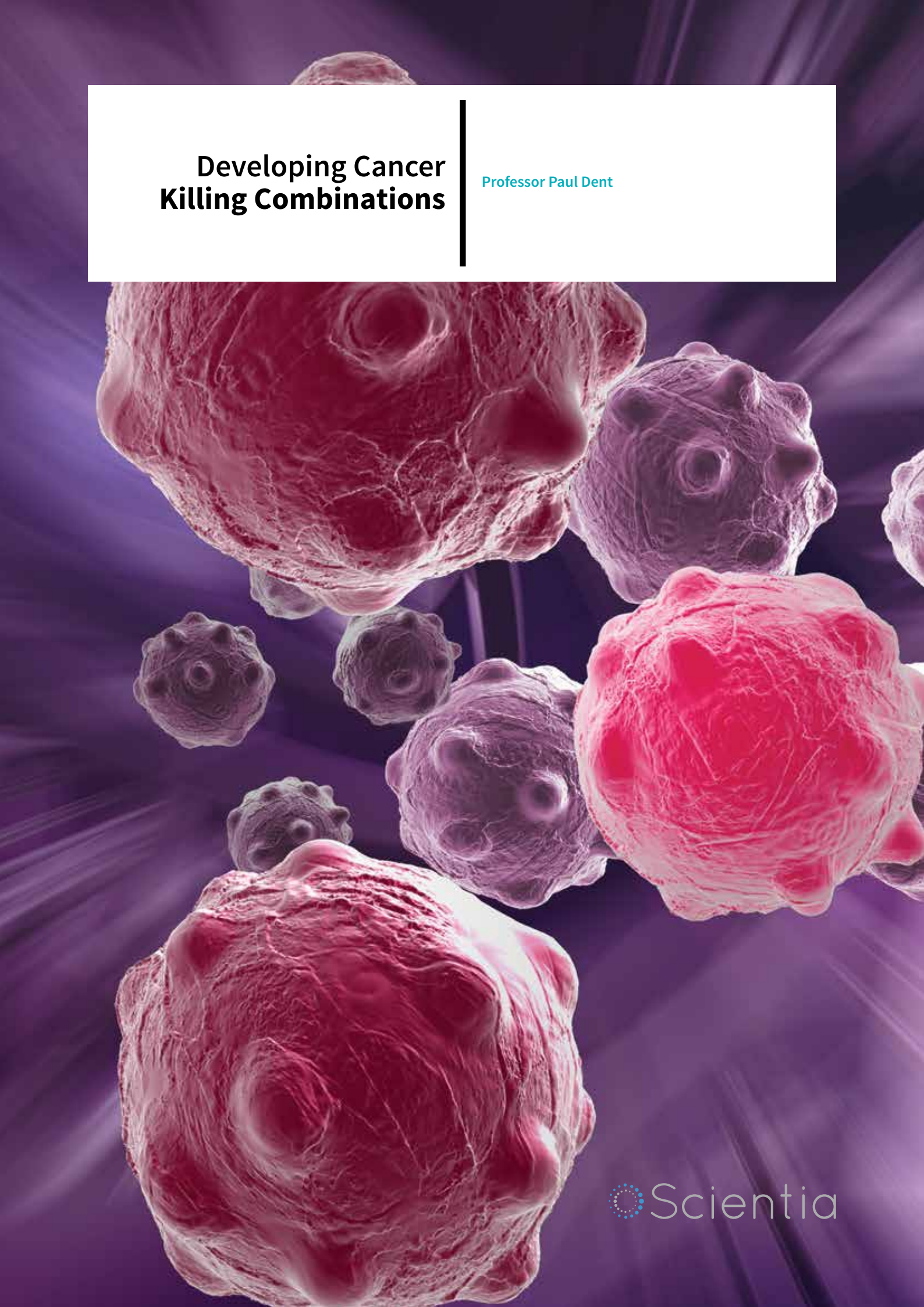
Developing Cancer **Killing Combinations**

Professor Paul Dent



Scientia

DEVELOPING CANCER KILLING COMBINATIONS

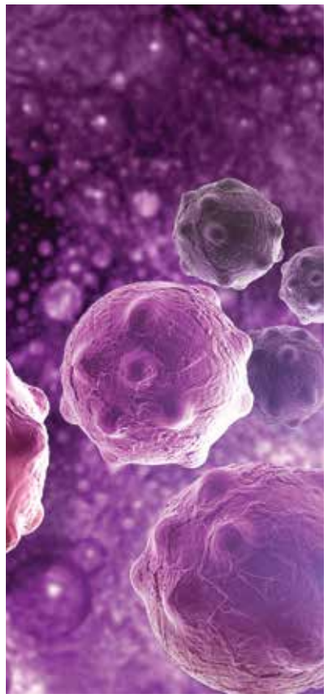
Professor Paul Dent is committed to taking an idea from the bench to the bedside. He is doing just that by designing clinical trials that investigate how drugs can combine and synergise to kill tumour cells.

Maximising the potential of cancer treatments

Professor Paul Dent and his colleagues at the Massey Cancer Centre are leaders in the field of applied translational cancer research. Over the last decade, Professor Dent and his team have researched and contributed novel treatment strategies for a number of different forms of cancer, including hepatoma, soft tissue sarcoma, glioblastoma, pancreatic carcinoma, renal cell carcinoma and breast cancer. Other trials have been open to include all solid tumor patients. This work continues today and focuses on the development of therapeutic interventions which combine two or more drugs to kill tumour cells. The two primary drugs being investigated in clinical trials are pemetrexed and sorafenib. So what are these drugs and how do they work?

Pemetrexed is an anti-folate drug which is used in the treatment of advanced and metastatic non-small cell lung cancer. Anti-folate drugs block the action of folic acid which then inhibits cell division and the production of proteins, DNA and RNA. Specifically, pemetrexed inhibits an enzyme called aminoimidazole-carboxamide ribonucleotide formyl-transferase (AICART). This leads to a series of biochemical events which ultimately stimulates autophagy (the destruction of damaged cells).

Sorafenib is a multi-kinase inhibitor which is used to treat liver and kidney cancers. Multi-kinase inhibitors block the action of certain enzymes (or kinases) which add phosphate groups to particular proteins in a process known as phosphorylation. This is important because the overexpression of these kinases can cause a number of diseases, including cancer. Sorafenib works by inhibiting a proto-



Ongoing preclinical experiments show that it could be possible to pinpoint exactly how the cancer cells are developing resistance to therapies, which might eventually allow oncologists to develop in real time a personalised therapy designed to overcome drug resistance in an individual patient's tumour

oncogene called RAF-1. Proto-oncogenes are genes which could potentially cause cancer if activated by mutations or changes in protein expression. RAF-1 is associated with the ERK1/2 pathway which plays a crucial role in the regulation of the cell cycle. The inhibition of RAF-1 reduces levels of a protein called MCL-1 which is associated with increased levels of apoptosis (programmed cell death).

Because pemetrexed and sorafenib exert their effects through different pathways, it was hypothesised that together they could lead to a synergistic increase in tumour killing. Through clinical trials, Professor Dent and his team are investigating how best to combine these two drugs in order to achieve clinically significant outcomes in the fight against cancer.

Beginning Phase I Clinical Trials

You may have heard the term 'clinical trial' before, but what exactly do the different phases of a clinical trial involve? Phase I involves testing the new treatment on a small group of participants in order to determine the optimal dose, identify adverse effects and evaluate its overall safety. If this is successful, the treatment is then given to a larger group to further assess its safety and effectiveness in Phase II. Phase III compares the treatment to other common interventions and confirms its efficacy in large groups of participants. Phase IV looks at outcomes and side effects associated with long term use and occurs once the drug is on the market.

Between 2011 and 2014, Professor Dent and his colleagues carried out a Phase I study (ref: NCT01450384) to determine the safety and tolerability of a combination of pemetrexed and sorafenib in patients with advanced solid tumours. The study, run by Dr Andrew Poklepovic, used a '3 + 3' design to explore the effect of various doses of pemetrexed with continuous sorafenib. '3 + 3' designed studies involve incremental dose escalation in order to determine the maximum tolerated dose of a drug. Three patients will be enrolled in the first cohort with the lowest dosage. If no patient experiences dose limiting toxicity (side effects which are serious enough to prevent further treatment), three more patients can be enrolled in the next cohort at a higher dose. If one or more patients develops dose limiting toxic effects, three more participants will be added to that

dose cohort. The development of adverse effects in more than one patient in a group suggests that the maximum tolerated dose has been surpassed and that the treatment should be stopped or de-escalated. The researchers in this study slightly adapted this design in a novel way which allowed for the escalation or de-escalation of one or both drugs depending on the dose limiting toxicities observed. 36 patients with various forms of cancer were treated over the course of Professor Dent's study with breast cancer being the most common diagnosis amongst participants. Participants were divided into two groups: 24 in cohort A and 12 in cohort B.

Individuals in cohort A were given escalating doses of pemetrexed every 14 days with continuous doses of sorafenib twice daily. Although no dose limiting toxicities were observed at lower doses, higher doses induced a number of adverse side effects amongst participants. High blood pressure, inflammation of the mucous membranes, cytopenia (reduction in number of blood cells) and gastrointestinal symptoms were included amongst the dose limiting toxic effects observed. Therefore, it was determined that pemetrexed at any dose combined with continuous sorafenib was not tolerable and the drug schedule was adjusted for cohort B. The new treatment protocol involved administering sorafenib only on the first five days in each two week pemetrexed cycle. Intermittent dosing was found to be far more tolerable with no dose limiting toxicity and fewer dose delays or modifications required amongst participants. As a result, phase II (ref: NCT02624700) will involve a schedule of pemetrexed 750 mg/m2 every 14 days with sorafenib 400 mg given twice daily on days one through five.

So now that the tolerable dose has been determined, how effective is the actual protocol against advanced solid tumours? Of the 33 participants who were assessed for anti-tumour activity, 20 experienced stable disease or tumour regression. Let's break that down: disease progression was stabilised in 15 patients with responses lasting up to one year; four patients had a partial response (meaning their tumour shrank by at least 30 per cent); and one patient had a complete response in which all traces of the tumour had gone. The treatment was found to be particularly effective in cases of breast cancer. All partial and complete responses occurred in breast cancer patients and 58 per cent of breast cancer patients (and all patients with triple negative breast cancer) experienced stable disease or response from the treatment.

The third piece in the puzzle

Professor Dent and his team have undertaken a number of pre-clinical studies in order to determine if a third drug could be added to enhance the killing effect of pemetrexed and sorafenib. Previous studies by Professor Dent indicated that the addition of the drug vorinostat to sorafenib prolonged stable disease in patients with liver cancer. Animal studies showed that the addition of AR-42 (a drug in the same family as vorinostat) to pemetrexed and sorafenib significantly reduced tumour growth and increased survival when compared to pemetrexed and sorafenib alone.

The team explored the possibility of adding an ERBB1/2/4 inhibitor to the combination of pemetrexed and sorafenib after a surprising discovery. The ERBB proteins are known as receptor tyrosine kinases which are enzymes with the ability to phosphorylate a protein and influence cellular function. The overexpression of ERBB signalling, particularly ERBB1/2, is associated with solid tumours and has previously been thought to protect cancerous cells from treatment. However, using multiplex assays (which allowed the team to analyse the activities of enzymes in tumour cells), Professor Dent and his colleagues were able to the determine that, contrary to expectation, ERBB1 was activated in response to pemetrexed and sorafenib. When tested, lapatinib and afatinib (both ERBB1/2/4 signalling inhibitors) enhanced the lethality of pemetrexed and sorafenib in a variety of cell lines.

Another finding of the study was that in order for the combination of pemetrexed, sorafenib and afatinib to be effectively lethal, there needed to be elevated endoplasmic reticulum stress. Endoplasmic reticulum stress signalling leads to the unfolding of proteins and autophagy. Therefore, the team investigated the upstream signalling pathways that control autophagy and found that the knockdown of certain regulatory proteins protected cancer cells against pemetrexed and sorafenib. The triple drug combo facilitated autophagy by increasing the expression of these key regulatory proteins through endoplasmic stress signalling.

Endoplasmic reticulum chaperones (proteins which assist in the folding of molecular structures) also played a significant role in the tumour killing ability of pemetrexed and sorafenib. The overexpression of the chaperone GRP78 protects cancer cells from pemetrexed and sorafenib because it diminishes the ability of the drug combination to reduce protein expression. By combining pemetrexed and sorafenib with an ERBB1/2/4 inhibitor such as afatinib, researchers were able to rapidly reduce the expression of GRP78 and other chaperone proteins.

Finally, reduced GRP78 expression correlated with increased phosphorylation of the endoplasmic reticulum stress mediator eIF2α (an essential factor in protein synthesis). These processes collectively promoted autophagy and the release of toxic lysosomes in in vitro models of disease.

Where do we go from here?

So what effect does this drug combination have in a living organism? In vivo models of breast cancer were exposed to lapatinib or vandetanib (another type of signalling inhibitor) for five days which boosted the lethality of pemetrexed and sorafenib without any evidence of toxicity to normal cells. Similar findings were observed in non-small cell lung tumours when afatinib was added to pemetrexed and sorafenib. These findings are particularly pertinent in the face of treatment resilient disease states. Other drugs, such as flavopiridol and copanlisib, also show promise in subverting secondary drug resistance mechanisms. Cells treated with the combination of pemetrexed, sorafenib and afatinib showed greater sensitivity to both the above drugs, suggesting beneficial synergistic effects. These researchers firmly believe that resistance mechanisms to the drug combinations can be overcome. In light of this, they are currently submitting a new grant to fund a phase I cancer trial in all solid tumour patients using the combination of pemetrexed, sorafenib and afatinib.

It is clear that Professor Dent and his team are not done yet. A phase II study testing the combination of pemetrexed and sorafenib in patients with recurrent or metastatic triple negative breast cancer is now open at Massey Cancer Centre and Professor Dent encourages people to participate, particularly if they live in the United Kingdom.

Meet the researcher

Professor Paul Dent
Professor and Universal Chair in Signal Transduction
Department of Biochemistry & Molecular Biology
Virginia Commonwealth University

Professor Paul Dent is Professor and Universal Chair in Signal Transduction in the Department of Biochemistry and Molecular Biology in Virginia Commonwealth University. After receiving a 1st for his BSc degree in Biochemistry from Newcastle University, he went on to do a PhD at the University of Dundee in Scotland. After graduation, Professor Dent became a Postdoctoral Fellow in the University of Virginia, US, and later set up a laboratory dedicated to developmental cancer therapeutics. Through securing funding from the National Institutes of Health and the Department of Defence, Professor Dent and his colleagues have explored the ways in which drugs can be combined to kill tumour cells. Clinical trials have focused on breast, brain, liver and pancreatic cancers respectively and the team are preparing further studies into colon cancer and other solid tumours. Professor Dent has also served as Assistant Editor-in-Chief for the academic journal Cancer Biology & Therapy.

CONTACT

E: Paul.Dent@vcuhealth.org
T: (+1) 804 628 0861
W: http://www.biochemistry.vcu.edu/Faculty/Dent.html

KEY COLLABORATORS

Laurence Booth, Virginia Commonwealth University
Jane L. Roberts, Virginia Commonwealth University
Mehrad Tavallai, Virginia Commonwealth University
Andrew Poklepovic, Virginia Commonwealth University
John Chuckalovcak, Bio-Rad Laboratories Ltd.
Daniel K. Stringer, Bio-Rad Laboratories Ltd.
William P. McGuire, Bio-Rad Laboratories Ltd.
Antonis E. Koromilas, Lady Davis Institute for Medical Research
David L. Boone, Indiana University School of Medicine-South Bend

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REFERENCES

L Booth, JL Roberts, M Tavallai, J Chuckalovcak, DK Stringer, AE Koromilas, DL Boone, WP McGuire, A Poklepovic and P Dent, [Pemetrexed + Sorafenib] lethality is increased by inhibition of ERBB1/2/3-PI3K-NFκB compensatory survival signalling, Oncotarget, 2016, 7, 23608–32. DOI: 10.18632/oncotarget.8281

L Booth, T Albers, JL Roberts, M Tavallai, A Poklepovic, IO Lebedyeva and P Dent P, Multi-kinase inhibitors interact with sildenafil and ERBB1/2/4 inhibitors to kill tumor cells in vitro and in vivo, Oncotarget, 2016. DOI: 10.18632/oncotarget.9752

A Poklepovic, S Gordon, DA Shafer, JD Roberts, P Bose, CE Geyer Jr, WP McGuire, MB Tombes, E Shrader, K Strickler, M Quigley, W Wan, M Kmieciak, HD Massey, L Booth, RG Moran and P Dent, Phase I study of pemetrexed with sorafenib in advanced solid tumors, Oncotarget, 2016, DOI: 10.18632/oncotarget.9434

L Booth, B Shuch, T Albers, JL Roberts, M Tavallai, S Proniuk, A Zukiwski, D Wang, CS Chen, D Bottaro, H Ecroyd, IO Lebedyeva and P Dent, Multi-kinase inhibitors can associate with heat shock proteins through their NH2-termini by which they suppress chaperone function, Oncotarget, 2016, 7, 12975–96.

MD Bareford, MA Park, A Yacoub, HA Hamed, Y Tang, N Cruickshanks, P Eulitt, N Hubbard, G Tye, ME Burow, PB Fisher, RG Moran, KP Nephew, S Grant and P Dent P, Sorafenib enhances pemetrexed cytotoxicity through an autophagy-dependent mechanism in cancer cells, Cancer Res., 2011, 71, 4955–67.