Phortress: the smart antitumour agent which induces its own metabolism

In the second of four articles on cancer treatments, Tracey Bradshaw introduces a new antitumour agent, Phortress, currently under clinical evaluation, which offers a novel mechanism of action, with a smart approach to selectivity.

Phortress is the result of multidisciplinary research involving pharmacists, chemists, molecular biologists, pharmacologists and clinicians. It is developed under the direction of Malcolm Stevens, inventor of temozolomide (PJ BPC supplement, October 2009, PB26).

Phortress is currently in phase I clinical trial at the Northern Institute for Cancer Research at Newcastle University. A second phase I site, at Hammersmith Hospital in London, will begin patient recruitment shortly.

Development

Development began following an observation that the growth of MCF-7 human-derived breast cancer cells (a specific breast cancer cell line derived from a patient in 1970) was inhibited by the molecule 2-(4-aminophenyl)benzothiazole — CJM 126 (see Figure 1).

CJM 126 was synthesised as an intermediate compound in a research programme (inspired by nature) to develop polyhydroxylated-2-phenyl-benzothiazoles.

At concentrations below 1µM, growth inhibition of MCF-7 cells was observed. The GI50 value of CJM 126 (the concentration of a test compound required to inhibit cell growth by 50 per cent, which is often used when comparing growth inhibitory activity in cancer research) was less than 10 nM (a comparable value of GI50 for paclitaxel, a mitotic inhibitor used in chemotherapy, is 1nM in MCF-7 cells).2 However, growth inhibition was biphasic and, at CJM 126 concentrations of 3 and 10µM, viable cell colonies could be seen. Synthesis of CJM 126 analogues and structure activity relationship (SAR) elucidation led to identification of molecule DF 203 (2-(4-amino-3-methylphenyl)benzothiazole) (see Figure 1), which demonstrated potent and selective antitumour activity in vitro and in vivo against an enhanced spectrum of tumour types.

DF 203 inhibited the growth of human-derived cancer cells of breast, ovarian, renal and colon origin. Moreover, when human tumours (breast and colon) were grown in immunocompromised mice, DF 203 inhibited their growth. However, in vitro, the biphasic dose response was still observed. At higher concentrations, DF 203 metabolism by cells resulted in production of an inactive hydroxylated derivative, which was able to block antitumour activity of DF 203. To combat this deactivating mechanism, fluorinated analogues were synthesised, yielding the optimised compound 5F 203 (see Figure 1).3 Lipophilicity and poor aqueous solubility associated with 5F 203 necessitated generation of more water-soluble 5F 203 prodrug forms, thus conjugation of amino acids to 5F 203 led to lysyl and alanyl amide prodrugs of 5F 203.

5F 203 is liberated by prodrugs in the presence of cancer cells in vitro and in plasma in vivo. Antitumour activity and selectivity are both retained,4 but the superior pharmacokinetic properties of 5F 203 lysyl amide (Phortress) (see Figure 1) made this the most suitable candidate for phase I clinical trials.

Preclinically, the antitumour efficacy of Phortress (experimental dose and schedule) compared favourably with that of doxorubicin (clinical dose and schedule) in nine human-derived mammary cancer xenograft models cultivated subcutaneously in the flanks of immunocompromised mice. (A xenograft is where tissue or organs from one species is transplanted into or grafted onto another organism, for example, human tumours grown in immunodeficient mice.)5 Significant activity was established in seven

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Figure 1: the chemical structure of synthetic intermediate 1, optimised compound 2 and Phortress

![Chemical structure](https://example.com/structure.png)
out of nine xenografts, with no model demonstrating complete resistance to Phortress. In one model, Phortress significantly outperformed doxorubicin.

**Preliminary clinical data**

Phortress is administered by intravenous injection over a 15-minute period on day one of consecutive 21-day cycles. Nine patients with a variety of cancers, including colorectal, renal cancer and mesothelioma have received Phortress at doses of 3 and 4.25 mg/m². It was generally well tolerated, with mild arthralgia, fatigue and headache being the most common adverse effects.

Indications of stable disease have been demonstrated in four patients. Others withdrew from the trial because of progressive disease and fatigue.

**Mechanism of action**

The amino acid prodrug (Phortress), with its high bioavailability, is readily absorbed and hydrolysed in vivo to liberate 5F 203. This compound is actively sequestered by sensitive cells. Within sensitive cells, 5F 203 binds to the aryl hydrocarbon receptor (AhR), which then activates the production of the p450 CYP1A1 enzyme. This enzyme metabolises 5F 203 to a highly reactive species, which attacks the cell’s DNA through adduct formation, resulting in single- and double-DNA strand breaks and ultimately cell death (see Figure 2 below).

**Summary**

As outlined in the previous science article on an overview of cancer treatments (Pj, 7 November 2009, p511), side effects and toxicity are common in cancer treatments. This is primarily due to the poor selectivity of cytotoxic agents for human cancer cells. Improved selectivity and efficacy of anticancer agents offer real progress in the treatment of cancer and is an important aim of cancer research.

The impressive selectivity of Phortress for susceptible cancer cells arises from its mechanism of action: following the release of 5F 203 from Phortress, it activates AhR signalling and causes induction of cytochrome p450 activity, which metabolically bioactivates 5F 203 to a cytotoxic species at the tumour site. Because the induction and expression of the CYP isoforms is limited (although not exclusively) to cancer cells, the cytotoxic effect is targeted, which minimises systemic toxicity.

**References**


**Figure 2:** The mechanism of action of Phortress

- **Phortress** at doses of 3 and 4.25 mg/m².
- **5F 203** binds to the aryl hydrocarbon receptor (AhR).
- **Increased CYP1A1 mRNA**
- **DNA single/double strand breaks**
- **Cell death**

**Drug metabolising enzyme**

- **Cytosolic AhR translocates to nucleus**