John Innes Centre

Year in Industry Proposal for 2027/28

Title of Project	Tackling cancer stem cells and restraining the tumor with traditional Chinese medicine
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Project on Offer

Impressive progress has been made in recent years in combating cancer, particularly significant advances in earlier detection and immunotherapy-based treatments. There remains, however, a poor prognosis for cancer patients whose cancer has developed beyond a primary tumor and spread to secondary sites by metastasis. Along with re-occurrence of tumors after treatment, acquired resistance to conventional chemotherapeutics is often seen in a residual population of cancer stem cells, post treatment, highlighting the need to explore novel therapeutics to constrain primary tumors and to target cancer stem cells more effectively.

Medicinal plants offer the potential to address these areas, complementing existing therapies. In fact, sixty-two percent of chemotherapy drugs come from natural products, and plants have been the basis of almost every new class of medication. Plants deal with their environment via chemistry; they synthesise vast numbers of specialised metabolites (such as the diterpenoids, see Fig. 1). Frequently these lie outside areas explored by synthetic chemists, due to difficulty of synthesis or not fitting the profile of a conventional drug target. Medicinal plants offer a huge array of bioactive compounds, many of which are structurally very complex. As a result of how specialised metabolic pathways evolve in plants, they frequently produce an array of analogue metabolites offering huge potential for plant metabolites to generate new drug leads.

The Chinese medicinal plant *Scutellaria barbata* (see **Figure 1**) is widely prescribed as a traditional medicine in many Asian countries (as Ban Zhi Lian, BZL) and has been shown to impact many aspects of cancer biology. Immortalisation of cancer cells gives rise to tumors, as cancer cells have uncontrolled proliferation and switch off programmed cell death (apoptosis and ferroptosis). BZL and selected diterpenoids from *S. barbata* inhibit the uncontrolled proliferation of tumor cells and reinstate programmed cell death, shrinking a tumor. In a phase 1 clinical trial, BZL was found to be safe, well tolerated, and showed promising clinical evidence of anticancer activity in a heavily pretreated population of women with metastatic breast cancer.

More recent studies indicate that *S. barbata* not only affects the biology of primary tumors but also the ability of a tumor to spread by inhibiting epithelial to mesenchymal transition (EMT), a crucial first stage of cancer metastasis, enabling cancer cells to acquire invasive and migratory characteristics. Ethanol extracts of *S. barbata* leaves caused an increase in E-cadherin expression, in an ovarian cancer cell line. E-cadherin is a key component of the adheren junctions, which occur at cell-to-cell junctions, linking the actin cytoskeleton on each bound neighbouring cell. Maintaining high E-cadherin levels would prevent a tumor undergoing EMT and consequent metastasis.

Cancer stem cells (CSCs), represent a small subset of cells in tumors that are characterized by self-renewal and continuous proliferation, lead to tumorigenesis, metastasis, and maintain tumor heterogeneity. CSCs are also characterized by enhanced ability to resist therapeutic treatments. Extracts of *S. barbata* leaves show a significant dose dependent decrease in SOX2 expression, in lung cancer cells. The “degree of stemness” of cancer cells can be ascertained using stemness-associated markers like SOX2. Tumor cells often show optimized SOX2 levels; too little reduces cell viability and tumorigenicity, while overexpression fuels aggressive growth and drug resistance. High levels of SOX2 promote resistance to conventional radio- or chemotherapy.

The student will investigate the effects of the *S. barbata* alcohol-based extracts on two pairs of cell lines, one cancer and one normal. One pair colon of epithelial (CEC) cell lines will be the cancer cell line Caco2 and a normal CEC cell line, CCD-841. Caco2 cells are known to express SOX2 and a knockdown of this was shown to reduce proliferation. We will also use a pair of breast epithelial cell lines; the cancer cell line MCF7 and its normal equivalent MCF10A. MCF7 cells expressing high levels of SOX2 have been shown to proliferate faster

and be more resistant to chemotherapies. The alcohol extract of S. Barbata is rich in diterpenoids (see fig. 1) and Scutebarbatine A has been shown to have clinically-relevant activity in inhibiting the inhibitors of apoptosis. The student will investigate whether effects on induced EMT seen on different cancer cell lines are conserved and importantly cancer specific.

The clinically used drug Fasudil has been identified as having the same pro-apoptosis mechanism as Scutebarbatine A and X and had a close similarity in silico (see fig. 1). The student will use Fasudil to explore if it can affect EMT and cancer stemness in these cell lines also. Recent published evidence, for Fasudil treated MCF-7 cells, showed an effect on other cell to cell junction proteins and a strong anti-tumor effect. Using a known drug means results are of particular importance as the route to clinically use can be fast tracked.

The student will investigate SOX2 and E-cadherin expression levels in the treated cells by both flow cytometry and immunohistochemistry (with microscopy). Flow cytometry will allow the student to quantify the number of SOX2 expressing cells with the treatments in cancerous and non-cancerous cell lines. Staining with specific antibodies (immunohistochemistry) will allow visualisation of any expression changes of SOX2 and E-cadherin with treatments. Further studies on the number of actively proliferating treated cells will be done by flow cytometry, using the proliferation antibody marker Ki-67.

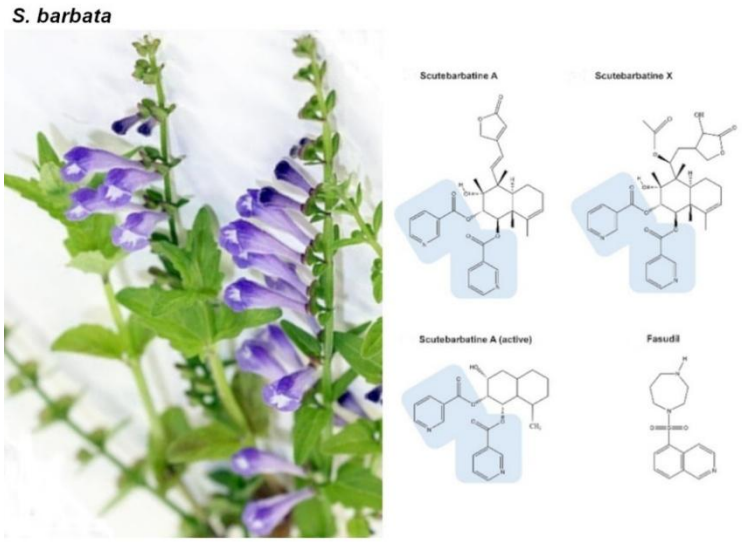

Figure 1. Left: the *S. barbata* plant and Right: examples of the most abundant diterpenoids; Scutebarbatine A and one of its analogues Scutebarbatine X, the proposed active part of these diterpenoids and the drug Fasudil. Bioactive niacin groups are shaded in blue.

The student will have access to the JIC flow cytometry and bio-imaging platforms and will work with a post-doctoral scientist experienced in using human cell lines, flow cytometry, microscopy and apoptosis assays.

The student will gain experience of working in a research lab and in designing experiments. They will work as part of a team that can offer support and advice and will be encouraged to take the initiative wherever possible. We offer an experience of research with applied objectives. The student will be encouraged to attend group meetings, departmental and institute seminars, and also gain skills in presenting their own results.

References: Li H, et al, The genomes of medicinal skullcaps reveal the polyphyletic origins of clerodane diterpene biosynthesis in the family Lamiaceae. Molecular Plant, 2023; 16, 549-570. Tomlinson ML et al. Diterpenoids from *Scutellaria barbata* induce tumour-selective cytotoxicity by taking the brakes off apoptosis. *Medicinal Plant Biology* 1:3 (2022); Sun, J. et al. Chemical Constituents, Anti-Tumor Mechanisms, and Clinical Application: A Comprehensive Review on *Scutellaria barbata*. *Molecules*, 2024, *29*(17), 4134. Beljkas M, et al., Pioneering first-in-class HDAC-ROCK inhibitors as potential multitarget anticancer agents. Future Med Chem. 2025 Feb;17(4):393-407.

PERSON PROFILE *2 bullets max per box*	**Essential**	**Desirable**
Education *e.g. degree subject*	A-levels in science subjects Undertaking a degree in Biological/Chemical Sciences	Plants Metabolism/Biochemistry
Specialist knowledge and skills *e.g. course module*		Cell biology, Cancer biology,
Relevant experience *e.g. lab/research work*		Some experience of research Enjoying working in a lab
Interpersonal and communication skills *e.g. teamwork and record-keeping*	Good record keeping Enjoying teamwork	
Additional requirements *e.g. outdoor working*		Interest in learning high-tech analytical techniques.