Novel p21-disrupting peptides to prevent quiescence and resistance to chemotherapy

Supervisors:

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Research Summary

Lung cancer is the deadliest cancer worldwide and non-small cell lung cancer (NSCLC) accounts for 85% of cases. 80% of these are inoperable and 5-year survival rates are ~15%. We need new mechanisms to reduce the burden of this disease. Cell proliferation is driven by Cyclin/cyclin-dependent kinase (CDK) activity. High expression of CDK inhibitor p21Cip1/Waf1 correlates with a poor NSCLC prognosis. Our work suggests that high p21 expression allows NSCLC cells to enter quiescence - a reversible, non-proliferative state, which allows cancer cells to resist chemotherapy. We hypothesise that by preventing p21 binding to and inhibiting CDKs, we can prevent NSCLC cell quiescence and reduce chemotherapy resistance. We will develop **first-in-class stabilised peptides that inhibit p21 binding to CDK ("p21 tides").** Our approach has the dual advantage of increasing fractional killing in the primary tumour and reducing the possibility of tumour relapse by eliminating a source of dormant tumour cells.

The student will develop novel p21-tides based on the following timeline:

- **Year 1 – Identification of binding peptides**
  Expression of recombinant p21 and design and synthesis of p21-tides. Testing of peptide affinity, CDK2 kinase activity inhibition and competition with p21 (AB). Crystallisation of p21 in complex with CyclinA/CDK2 (CA). Cell uptake of fluorescent p21 peptides (ARB).
- **Year 2 – Optimisation of peptide structures and *in vitro* validation of stapled peptide derivatives**
  Synthesis, binding, and structural analysis of stapled p21-tides (AB/CA). Analysis of p21-tide interaction with CyclinA/CDK2 and impact on stability and cell uptake (CR/ARB).
- **Years 3-4 – Validation of peptide activity in cells and identification of susceptible tumour types**
  Validation of p21-tides using cellular assays to (i) monitor changes in cell cycle dynamics (phenotypes will be compared with p21KO NSCLC cell lines) and (ii) quantify response to chemotherapy in the presence and absence of p21-tides (ARB). Immunofluorescence staining of NSCLC tissue sections to identify p21-dependent quiescent cells *in vivo*. Expansion into Renal and Liver cancers to (i) determine if p21-dependent quiescence exists in these tumours and (ii) identify p21-dependent quiescence *in vivo* (ARB).

The student will receive world-class, interdisciplinary training in chemical biology, biophysical techniques, cell and molecular biology, structural biology, quantitative single cell imaging and

analysis, and tumour histopathology and immunostaining. The Barnard group has peptide synthesis and biophysical analysis equipment providing rapid access to peptide libraries and binding assays and has expertise in targeting protein- protein-interactions with stapled peptides. The Barr group has validated fluorescent reporter NSCLC cell lines and imaging methods to quantify peptide activity in cells. The Alfieri lab has expertise in the structural biology of cell cycle proteins. The crystal structure of p27 bound to CyclinA/CDK2 has been solved making the acquisition of a homologous structure of both p21 and stapled peptide analogues highly feasible.

By working across multiple labs across the two institutions, the student will be trained in a range of practical techniques based on physical sciences (synthesis, characterisation, biophysical analysis), biochemistry (*in vitro* assay development, structural biology), molecular biology and medicine (cell-based compound analysis towards *in vivo* models). Through association with the Institute of Chemical Biology (Faculty of Natural Sciences), the Institute of Clinical Science (Faculty of Medicine), the MRC-LMS and the ICR, the student will have access to a wide range of transferable skills training programmes in teamwork, science communication and project management.

Literature references

1. Barr AR*[1], Cooper S*, Heldt FS*, Butera F, Stoy H, Mansfeld J, Novak B, Bakal C[1] (2017), DNA damage during S-phase mediates the proliferation-quiescence decision in the subsequent G1 via p21 expression. *Nature Communications*, Mar 20; doi: 10.1038/ncomms14728
2. Russo AA, Jeffrey PD, Patten AK, Massague J, Pavletich NP (1996), Crystal structure of the p27Kip1 cyclin-dependent-kinase inhibitor bound to the cyclin A-Cdk2 complex. *Nature*, Jul 25;382(6589):325-31
3. de Castro GV, Worm DJ, Grabe GJ, Rowan FC, Haggerty L, de la Lastra AL, Popescu O, Helaine S, Barnard A* Characterisation of the key determinants of Phd Antitoxin Mediated Doc Toxin Inactivation in *Salmonella*. *ACS Chem. Biol.* 2022, 17, 1598.
4. Koliopoulos MG, Alfieri C, (2022), Cell cycle regulation by complex nanomachines. *FEBS J*, Sep;289(17):5100-5120.
5. Swadling JB, Warnecke T, Morris KL, Barr AR (2022). Conserved Cdk inhibitors show unique structural responses to tyrosine phosphorylation. *Biophysical J.* May 25; S0006-3495(22)00417-9. doi: 10.1016/j.bpj.2022.05.024

Person specification

This project is suitable for a talented graduate or undergraduate student with life sciences, or chemistry background. The standard minimum entry requirement is a relevant undergraduate honours degree (First or 2:1). Applications are invited from talented graduates or final year undergraduates. We particularly welcome British applicants from Black and minority ethnic backgrounds, as they are underrepresented at PhD level within Imperial and The Institute of Cancer Research.

The studentship will be registered at Imperial College London with affiliate status at the Institute of Cancer Research. The student will have access to both institutions and benefit from the world

class research infrastructure and expertise across the two institutions. The student will become a member of the CRUK Convergence Science Centre PhD cohort which is a unique group of students working across distinct disciplines to tackle the big problems in cancer. A unique convergence science training programme will provide the skills and language to navigate different disciplines.

Funding and Duration

Studentships will be for four years commencing in October 2023. Successful candidates will undertake a four-year research training programme under the guidance of a supervisory team of world-class researchers. Students will receive an annual stipend, currently £23,000 per annum, and project costs paid for the four-year duration. Convergence Science PhDs cover tuition fees for UK students only. Funding for overseas fees is not provided, international students are invited to apply subject to outlining how they will meet the difference in tuition fees.