IMAGING THE LOCALISATION AND SUBCELLULAR INTERACTIONS OF THE PAN-mTOR INHIBITOR AZD2014 IN THE LIVE CELL

Abdullah R Ahmed[1*], Ray J Owens[2], Christopher D Stubbs[1], Anthony W Parker[1], Richard Hitchman[3], Rahul B Yadav[3], Chris Hawes[4] and Stanley W Botchway[1]

1 Central Laser Facility, Research Complex at Harwell, STFC, Rutherford Appleton Laboratory, Harwell Campus, OX11 0FA, UK
2 The Oxford Protein Production Facility, Research Complex at Harwell, Rutherford, Appleton Laboratory, Harwell Campus, OX11 0FA, UK
3 Evotec (UK) Ltd, 114 Innovation Drive, Milton Park, Abingdon, OX14 4RZ
4 Oxford Brookes University, Headington Campus, Oxford, OX3 0BP, UK
***Email: abdullah.ahmed@stfc.ac.uk**

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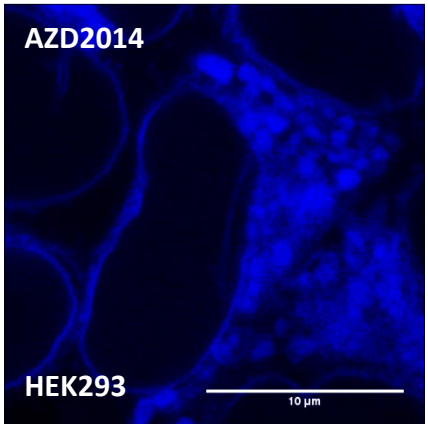

Figure 1: 405 nm confocal image of AZD2014 in living HEK293 cells.

The mammalian Target of Rapamycin (mTOR) pathway co-ordinates the availability of nutrients, growth factors and the energy status of the cell with the activation (phosphorylation) of its downstream target proteins [1]. These substrates are responsible for regulating functions such as cell proliferation, protein synthesis, autophagy and longevity. The mTOR protein exists in two complexes: Complex 1 (mTORC1) and Complex 2 (mTORC2). The broad functionality of the mTOR protein makes it an attractive drug target to fight diseases such as cancer where mTOR signalling is dysfunctional or hyper-activated. First generation mTOR inhibitors such as rapamycin and clinically approved analogues (rapalogs) have proven to be inefficient, usually targeting only mTORC1. Novel second generation ATP-competitive inhibitors such as AZD2014 provide greater selectivity by targeting both mTORC1 and mTORC2 [2]. Although AZD2014 is currently undergoing active clinical trials, its mode of action within a live cancer cell environment is unknown. To investigate the mechanism of AZD2014 we have studied its cell uptake and localisation in living cells (Fig 1). Furthermore, we report AZD2014 interaction with mTORC1 (GFP tagged fluorophore as acceptor) using FRET-FLIM microscopy. Together, our findings indicate that AZD2014 may cause inhibition through allosterically hindering the complex by directly and physically interacting with mTORC1 in lipophilic structures. Our results demonstrate how FRET-FLIM can be utilised to screen for potentially new mTOR drug targets for cancer treatment.

References

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