Establishing a multiparametric imaging assay for the identification of novel CDK inhibitors

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Transition through the mammalian cell cycle is co-ordinated by distinct cyclin/cyclin dependent kinase (CDK) heterodimeric complexes. These complexes regulate the function of effector proteins including transcription factors that promote cell cycle phase transition. Specific cyclin/CDKs regulate distinct phases of the cell cycle at particular times to ensure DNA synthesis and the transfer of DNA to daughter cells is regulated. Because of the central role cyclin/CDKs have in controlling the cell cycle, their regulators and substrates are often deregulated in cancer. Although CDKs have been targeted by drug discovery for many years, identification of specific cyclin/CDK inhibitors has been a longstanding challenge.

Here we describe the development of a multiparametric high-throughput cell-based imaging assay that can be used to assess compound effects on cell cycle phase, cell viability and phosphorylation of specific CDK substrates. Nuclear staining intensity is used to determine cell cycle phase, allowing phosphorylation of CDK substrates to be quantified at different phases of cell cycle progression. A number of previously described CDK inhibitors have been validated using this imaging algorithm, with important differences in potency against key CDK substrates. This work has facilitated the identification and characterisation of specific cell cycle inhibitors.