Why Small is Beautiful! Working with Smaller CRO's Can Often Bring Surprising Research Rewards

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Introduction

As an organisation requiring CRO services how would you choose who to employ and what to look for? Cost, speed, disease area expertise, assay development skills, integrated versus specialised boutique companies working in harmony? All factors to be considered when evaluating your progression strategy. We at Aurelia Bioscience, a CRO specialising in building bespoke bio-assays for compound screening, suggest the best return on investment is obtained by bringing together the expertise in each of the smaller boutique organisations, offering greater rewards from broader experience. A company who agreed with this philosophy was e-Therapeutics, who employed Aurelia Bioscience, Fidelta and other specialists to bring added value to their oncology project.

e-Therapeutics aimed to generate novel modulators of the Hedgehog pathway by using their proprietary 'Network-driven Drug Discovery' approach to identify key nodes essential for biological processes. To prosecute this approach, Aurelia developed cell-based assays designed to determine cellular differentiation and cytotoxicity, screening as dose-responses, a subset of 1,100 compounds identified by the e-Therapeutics model. Working closely with Fidelta, a specialist chemistry CRO who designed and synthesised compounds during hits-to-lead iterations, both CRO partners adhered to a one-week design-make-test cycle for compound progression. Hit compounds were used for target validation and mode of action (MOA) studies. These were generated by Aurelia, including collaborating with Horizon Discovery who constructed CRISPR mutant cell lines. The outcome was the successful generation of lead series with novel IP.

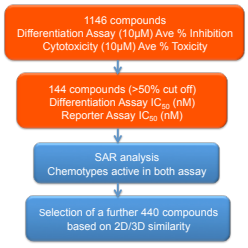
e-Therapeutics have gone on to successfully test these series in *in-vivo* tumour models and are now using this study as an exemplar of their approach.

Project Background

The Hedgehog signal pathway is normally involved in embryogenesis, transmitting signals for cell differentiation at specific times in specific locations through a number of transcription factors including Gli-1. Hedgehog is also important in adults and malfunctions in the pathway lead to a number diseases including cancer, fibrosis and angiogenic disorders. Known as a druggable pathway, Pharma companies have focused on the GPCR-like molecule Smoothened (SMO) as a target for intervention. SMO is on the Hedgehog pathway and while SMO inhibitors (e.g. Vismodegib) are clinically efficacious, tumour evolved resistance restricts there use. Therefore there is an un-met need for compounds that inhibit the Hedgehog pathway while circumventing SMO-induced drug resistance.

E Therapeutics therefore required assays to identify new pathway inhibitors in resistant cancer cell backgrounds.

Initial Project Cascade



Do any of the 1,146 compounds interact with the Hedgehog pathway?

Initially, a commercially available Gli-1-luciferase cell line readout assay was evaluated for use to examine the activity of the 1,146 compounds. However, issues with non-specific luciferase inhibitors resulted in us developing a replacement differentiation assay using C3T1/2 fibroblast cells (Fig.1.). When C3T1/2 cells differentiate they secrete Alkaline Phosphatase (Alk Phos) which can be measured in the media as a marker of differentiation (see Fig. 2.) Compounds were also screened in a cyto-toxicity assay to remove compounds that killed cells and would therefore appear as hits.

Fig.1. Correlation of Gli-1-luciferase reporter assay versus C3T1/2 fibroblast differentiation assay pIC_{50}– for non-interfering compounds there was a excellent correlation

Project decision – screen using differentiation assay

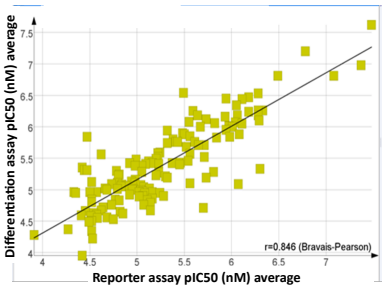
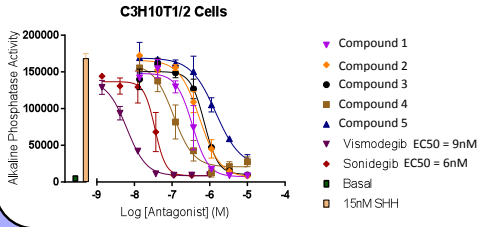
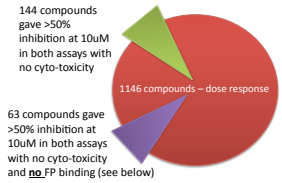


Fig.2. Inhibition of differentiation by compounds. Sonic Hedgehog (SHH) is a ligand that induces differentiation while Vismodegib and Sonidegib inhibit differentiation. Test compounds also inhibit differentiation in a dose dependent manner.





Do hits bind to SMO?

Fig. 4. Fluorescence Polarisation assay developed using membranes from cells expressing the SMO receptor and a fluorescence tagged SMO ligand - Cyclopamine

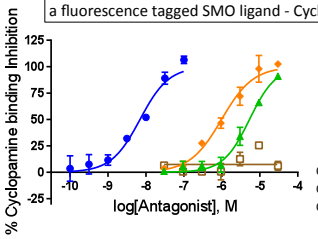
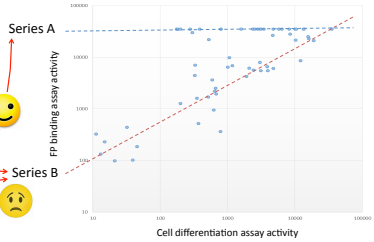


Fig. 5. Correlation of hits from FP assay and differentiation assay – ideal compounds = Series A – no SMO binding



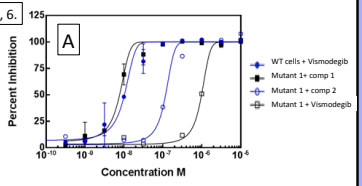
Cell-based hits were screened in a binding assay to remove compounds that bind to SMO. Series B bind to SMO and were therefore excluded from further study while Series A which were active in the differentiation assay, non cyto-toxic AND did not bind SMO were taken forward for further evaluation and chemistry

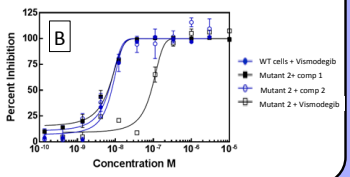
Do hits interact with known SMO mutations identified in drug resistant patient populations?

Fig. 6.

A number of mutations in SMO, a key Hedgehog pathway protein, are known to exist in the patient population and promote drug resistance. Horizon Discovery generated 3 mutant cell lines (using CRISPr) containing some of the mutations in the same cell background used in the differentiation assay. Active compounds were tested in these cells to determine if mutations affected antagonist activity (Fig. 6.A. & B.).

Mutations of single amino acids in the SMO sequence derived from patient data significantly influenced the bio-activity of Vismodegib. In addition, the activity of some (compound 1 – Fig. 6A) but not all compounds (compound 1 & 2 – Fig.6B) was altered, suggesting that some compounds may bind to and activate mutated forms of SMO and therefore may have anti-cancer properties in this refractory patient population.





Summary and Conclusion

- Aurelia successfully validated an existing Gli-1 – luciferase cell-based assay that was subsequently discounted due to compound interference and replaced by a cell differentiation assay
- Aurelia developed cyto-toxicity assays to remove compounds that caused cell death
- Aurelia developed a fluorescence polarization assay to discount compounds that bound to SMO
- Aurelia evaluates the activity of compounds in a number of knockout cell lines developed to investigate if hits were active against known drug resistant SMO mutants in the patient population
- Aurelia collaborated very successfully with Fidelta to deliver a weekly design-make-test cycle, testing compounds simultaneously in a number of assays allowing rapid progression of the med chem program