Guidelines for applicants regarding target validation criteria

The aim of this document is to give the applicant an overview of some of the requirements expected in order to perform and evaluate the early validation of a target. Applicants should please note that not all of the criteria described are necessary and critical to a successful application - these are guidelines and are provided to serve as such.

1. Cell functional data

The desired outcome is to demonstrate a clear and substantial (>50%) effect on a key cell process that is relevant for the progression of cancer disease states (proliferation, migration, invasion, apoptosis, cell death, cell viability, DNA damage etc).

Technical considerations when investigating target knockdown using siRNA

- Use 3 different siRNA motifs from at least 2 different suppliers if available (data obtained using siRNA from the same supplier is acceptable as long as the associated effect correlates to the level of knockdown)
- siRNA is to be used at low concentrations <20nM with concomitant knockdown levels of >80% (ideally at both the mRNA level and the protein level)
- siRNA used to establish phenotype and biomarker parameters (the phenotypic effects should be observed with 3 siRNA's and correlate with observed knockdown levels)
- For all siRNA studies stringent controls should be included: Mock (reagent only), Targeted control siRNA, non-targeted control siRNA (i.e. Luciferase or GFP), Untreated (media only)
- Results should be normalised/standardised to control values/data.
- Where possible rescue of siRNA phenotype with siRNA resistant mutants should be done
- Results should be obtained in 2-3 cell lines (ideally ones suitable for later *in-vivo* work)

The above can be used to provide early/preliminary target validation, and gain the necessary confidence to progress a project to the *Hit Identification* stage. However it is important to note that target validation is a dynamic process and data packages need to be constantly evaluated and expanded upon. As a result, if a project enters Lead Optimisation or Pre-clinical stage gates

then the biological rationale would need to be significantly enhanced and the package would need to include *in vivo* compound data and ideally human/primary cell data.

2. Target Modulation biomarker evaluation

A biomarker is a clear and unambiguous cellular measure of target modulation; a change in a molecule that results from and can be measured/quantified in response to target inhibition

- The measureable change can be a direct phosphorylation event, a change in the levels of expression or some other processing (acetylation, ubiquitination or metabolite change)
- The modulation needs to be clearly related to the target and ideally to any corresponding phenotypic effect
- Any effects need to be readily and reproducibly monitored
    - Ideally the development of a biomarker should include being able to be monitored in *in vivo* studies
    - Early identification of a biomarker is critical to progress a drug discovery project/programme

A robust translational screening cascade is required to sequentially build evidence that manipulation of the target will confer efficacy in the disease being studied. A key part of this process is the identification of a direct (proximal) biomarker. In cases where pathways downstream of the target are poorly characterised, the use of a distal biomarker may suffice.

- Biomarkers should be validated (i.e. modulation of biomarker upon target inhibition should be clearly observed) by siRNA (or shRNA) where possible
    - 3 siRNA probes against the target (from at least 2 suppliers) plus the following controls: untreated, transfection reagent only, non-targeting control siRNA (Luciferase or GFP), functional off-target siRNA
    - siRNA should be used at as low a concentration as possible to achieve effective knockdown (no more than 20 nM)
    - Knockdown levels of >80% of target mRNA levels. A subsequent drop in protein levels (ideally greater than 85% knockdown optimal) should be confirmed following knockdown but this will not be immediate depending on the stability of the target mRNA

- In addition to siRNA studies, the following should also be explored to increase confidence in the data package

    - tool/standard compounds that are specific for the target (*in vitro* on-target potency of <100 nM), possess >30-fold selectivity over sequence-related proteins within the same target class, readily cell permeable
    - cell lines that over-express the target
    - cell lines that express dominant negative forms of the target

- More than one cell line should be used for validation

- PD Biomarker modulation should be correlated with phenotypic observations at an early stage

For sustained/inducible levels of knockdown, shRNA and CRISPR options would be encouraged.