DevelopMed

Work Plan Template

A Work Plan maps out what you will be doing over the course of your proposed research project, including the various aspects of your research as well as your training and career development.

The project should be divided into suitable *Work Packages*, wherein each work package should include a summary of its specific aims, tasks, deliverables (a measurable and tangible outcome of the project), milestones (checkpoints throughout the life of the project which identify when a notable point has been reached in the project) and timeline.

Example Work Package:

|  |  |  |  |
| --- | --- | --- | --- |
| Work Package number: | 1 | Start date: | Month 1 |
| Work Package Title: | Proteomic evaluation of BRAF binding partners | End date: | Month 10 |

**Objective:** Cell lines generated in WP2 will be used as a platform to assess the binding partners of the various BRAF variants.

**Description:**

*Task 1.1: Test whether BRAF proteins have different interaction partners (Months 1-10):*
Protein lysates will be extracted from cell clones expressing each BRAF variant. A single purification step will be carried out allowing isolation of BRAF-bound protein complexes in their native form with a high purity. Expected interactions will first be validated using IP and Western blotting (WB).

*Task 1.2: Test whether these proteins have different interaction partners after treatment with MAPK-pathway targeted therapies (Months 1-10):*
The cell lines expressing the TAP-tagged BRAF variants will be treated with a clinically relevant dose of BRAFV600E-targeted inhibitor for 20 min, 1 hour, 3 hours and 9 hours, after which the protein extraction, purification and bioinformatic analysis protocol described above will be performed. Protein complexes will be evaluated to assess and map different proteomic interactions in cells expressing different BRAF variants before and after treatment with BRAFV600E-targeted inhibitors.

**Deliverables:**

D1.1 – Treatment of isogenic cell lines with BRAF inhibitors.

D1.2 – Validation of selected PPIs in BRAF-V5 immunoprecipitates using IP and WB

**Milestones:**

M1.1 – Identification of protein subcomplexes that specifically bind to BRAFWT, BRAFV600E and p61BRAFV600E

M1.2 – Validation of selected BRAF-protein interactions