FINAL REPORT

Project: 302265 – Call identifier FP7-PEOPLE-2011-IEF

Project goal and results:

Despite the dramatic advances of conventional and targeted anti cancer therapies, many cancers still remain incurable. The overarching goal of this project is to deliver to the pharmaceutical industry a pre-clinical model of the role of the p53 tumour suppressor in response to existing and novel anti-cancer drugs for both therapy and collateral effects on normal tissues. The p53 protein is one of the most commonly mutated genes associated with cancer. To determine the therapeutic relevance of p53 for both treatment of cancer and protection of individuals from chemotherapeutic and radiological injury, we have created a mouse model that will allow acute targeting of p53 function.

p53 function is largely regulated by the stability of the protein and we have employed a novel technology that allows us to reversibly interfere with p53 protein stabilization. This technology relies on the fusion to p53 of a heterologous degradation sequence (degron) that is regulated by the plant hormone, auxin, or its chemical derivatives.

As refereed to the Annex I of the Grant Agreement and the research methodology and approach, the main tasks for the project are:

1. Extensive *in vitro* validation of the auxin-induced degron (AID) protein regulation technology.
2. *In vivo* validation of the AID technology by generating a suitable reporter.
3. Generation of the final *in vivo* p53 switchable model, and consequent analysis.

No major deviation from the programme of work occurred. The researchers involved in the project successfully completed task 1 and 2 and are in the process of fully completing the project.

Task 1. We have completed a series of *in vitro* experiments to determine the minimal degron sequence that mediates efficient degradation of the target protein and the efficacy of synthetic auxin analogues. We confirmed that the degron tag itself did not affect p53 function in the absence of auxin in mouse embryo fibroblasts (MEFs) lacking the endogenous p53 gene. We also demonstrated that p53 stabilization in tumour cells could be prevented by auxin-dependent degradation of the p53-AID protein. These *in vitro* data confirm that p53 protein stability can be regulated ectopically by a plant hormone that is very unlikely to have any other effects on mammalian cells.

Task 2. We generated a transgenic mouse model that expresses and AID-tagged green fluorescent protein (GFP) to validate auxin-dependent degradation of the reporter protein, GFP-AID. GFP-AID has a half-life of 22 hours similar to the wild type GFP protein and this is reduced to approximately 40 minutes in the presence of auxin. Unfortunately, initial analyses of GFP-AID half life in mouse tissues in the absence and presence of auxin administration have been inconclusive due to difficulties in detecting GFP-AID (low transgene expression).

Task 3. As indicated on our proposal (sections B1.2 and 4.3) we were aware of potential problems that might arise at this stage and to fully pursue the project aim, while minimising delay, we adopted an additional strategy to regulate p53 function. Other experiments indicate that a tetracycline response element (TRE) can be inserted into a gene's control region to elicit tetracycline–dependent regulation of gene expression. We have conducted a series of experiment to define the optimum position for TRE insertion into the p53 gene locus, and successfully generated 2 different p53 TRE model organisms. We are currently generating experimental cohorts of TRE-*p53* mice and hope to address task 3 in the next months. We will address the therapeutic utility of inactivating and activating the p53 gene initially in a preclinical model of skin cancer. If successful, we will extend these studies to other cancers, including breast and pancreas.

Conclusion:

We have clearly demonstrated the utility of the AID system for regulating protein level *in vitro* and begun to elucidate the role of p53 stabilization in normal and tumour cells. We have also investigated other methods of gene regulation for p53 and we propose that both these methods provide rapid and reversible activity of endogenous functions without the confounding issues of overexpression in traditional transgenic and compensation in gene deletion animals that can be applied to numerous other genes that are implicated in human cancer.

2: Section A (public): DISSEMINATION MEASURES

The data generated in this project were disseminated to the scientific community at national and international (2014: 64th Lindau Nobel Laureate Meeting -DE, Genes & Cancer 30th -UK) scientific conferences.

After completion of Task 3 the data will be submitted to a peer-reviewed international scientific journal increasing the overall impact of the project and maximizing the dissemination to the whole scientific community.