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<b>PP</b>	Restricted to other programme participants (including the Commission Services)	
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## Description of DL68 with completion date “Month 36”

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### In vivo analysis of AIF death pathways in a defined murine disease model

AIF is a phylogenetically conserved redox-active flavoprotein that contributes to cell death in yeast (*S. cerevisiae*), nematodes (*C. elegans*), mouse and humans, as demonstrated by its experimental knockout or knockdown. AIF has been characterized as a caspase-independent death effector that is activated by its translocation from mitochondria to the extramitochondrial cytoplasm and to the nucleus. In addition, AIF appears to have essential functions in mitochondria controlling oxidative phosphorylation.

We have characterized AIF in *Drosophila melanogaster*. Transgenic expression of *D. melanogaster* AIF (DmAIF) misdirected to the extramitochondrial compartment (but not that of wild-type DmAIF) triggered cell death. DmAIF expressed under the control of an eye-specific promoter triggered the destruction of the fly eye, in a fashion that could not be inhibited by removal of essential fly caspases (Dredd), their obligate co-activators (Dark), or transgenic expression of the caspase-inhibitory baculovirus protein p35. Thus, *Drosophila* AIF can mediate caspase-independent cell death in fly (Joza et al. 2008).

One key issue for AIF research is whether the reported effects are cell-death intrinsic effects and/or dependent and secondary to its function in Oxphos (Pospisilik et al. Cell 2007). We therefore developed knock-in mice into the ROSA26 locus in order to express wild type or mitochondrially anchored AIF in a Cre-regulated fashion. These mice have now been generated and crossed to our AIF<sup>flox</sup> mice (Joza et al. 2006) and Cre deleter lines to generate mice that delete endogenous AIF in a defined tissue and at the same time rescue the endogenous deletion with wild type AIF or AIF that cannot be released from mitochondria.

To distinguish these two possibilities we generated Cre-regulated wild type and mitochondria-anchored AIF knock-in mice on an endogenous AIF mutant background in defined tissues, namely in hepatocytes (Alb-Cre to initially examine hepatocyte death in response to LPS) and in pneumocytes (adeno-Cre inhalations to study the role of AIF regulated oxphos versus death functions in a model of KRas-induced lung cancer).

The role of AIF in liver injury was examined by intraperitoneal injection of a lethal dose LPS/Galactosamine into liver-specific (Alb-Cre) AIF deficient and AIF expressing control mice to induce fatal hepatocyte apoptosis, mediated by TNF $\alpha$ . In first experiments, we observed less apoptosis and liver damage as well as less inflammation and infiltration into the liver tissue of liver-specific AIF deficient mice in comparison to control animals. Next, to distinguish between the oxphos and cell death function of AIF, liver-specific AIF deficient mice with an additional wild type or mitochondria-anchored AIF knock-in were treated with LPS/Galactosamine. Interestingly, there was no difference in survival between the mitochondria-anchored and wild type AIF knock-in mice. These results indicate that a defect in oxidative phosphorylation in liver-specific AIF deficient mice protects from LPS/Galactosamine induced death independently of AIF translocating to the nucleus thereby triggering chromatin condensation, DNA fragmentation and cell death.

We next used a well-defined murine disease model of KRas-induced lung adenocarcinoma to gain insight into AIF function. Hallmarks of cancer growth are increased glycolysis and lactate production and earlier findings suggest that oxidative metabolism may have a key role in controlling cancer growth (Ristow et al, 2006). Since our previous work has

shown that human or mouse cells lacking AIF exhibit an enhanced dependency on glycolytic ATP production, due to reduction of respiratory chain complex I and its components (Vahsen et al. 2004; Joza et al. 2006; Pospisilik et al. 2007), this is an ideal model to genetically assess the Warburg effect on lung cancer.

The lung adenocarcinoma model is based on intranasal infection of AIF<sup>floxed</sup> mice harbouring a conditionally inducible allele of oncogenic *K-ras* with a recombinant adenovirus expressing Cre in order to delete AIF in type II pneumocytes and in the same cell activate oncogenic *K-ras* leading to lung tumor initiation. Strikingly, the first results demonstrate a significant increase in tumor free survival of AIF deficient mice in comparison to control animals after infection with Ad-Cre. We are currently investigating the influence of impaired oxidative metabolism and proapoptotic function on malignant growth using wild type or mitochondria-anchored AIF knock-in mice with a tissue-specific AIF deletion.

## References

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