PUBLISHABLE SUMMARY

Acute promyelocytic leukemia (APL) represents 10% to 15% of acute myeloid leukemias in adults. It is caused by a variety of chromosomal translocations into the retinoic acid receptor-α (RARα) gene. The t(15;17)(q22;q11.2) is the most common translocation and gives rise to promyelocytic leukemia PML-RARα fusion protein. PML-RARα is a transcriptional repressor that associates with a corepressor complex leading to transcriptional repression and thus, promyelocytic differentiation blockage. All-trans retinoic acid (RA), a derivative of vitamin A and the physiologic ligand of RARα, is able to elicit complete remission of APL and has been successfully used in clinical treatment of APL. Pharmacologic concentrations of RA trigger the dissociation of the correpressor complex and the recruitment of the coactivator complex, and lead to changes in gene expression, APL cell differentiation, but also degrade the fusion via proteasome by the retinoic acid-bound RARα moiety of PML-RARα. It has been suggested that while transcriptional activation control differentiation, this may not be the primary basis for the therapeutic efficacy of RA in clearing APL-associated t(15;17). Indeed, only the leukemia initiating cells (LICs) clearance is responsible for the remission of mouse APL and high concentrations of RA are required for complete PML-RARα catabolism and full LIC clearance (Figure 1). This proposal aimed at dissecting the molecular basis of RA response in APL through two main objectives:

1. **Purification of the oncogenic PML-RARα complex from murine leukemia cells:** the origin of transcriptional repression is not yet clarified. The binding of co-repressor proteins, enhanced by the homo-dimerisation of the oncoprotein is thought to be the basis for repression, but also the binding of chromatin-modifying proteins such as histone deacetylases (HDACs) and other post transductional modifications. Through this proposal we have developed proteomic approaches and mouse models in order to identify new partners in the PML-RARα complex *in vivo* (Figure 2).

2. **Analysis of PML-RARα-dependent target gene repression:** It has been shown that PML-RARα can behave as a transcription activator in response to RA. In order to study the gene repression due to the oncogenic complex, and the transcriptional activation in response to RA we proposed methods of expression and epigenetic analyses. During this project we have revisited the modifications in gene expression, combining Real-Time-PCR and Chromatin Immunoprecipitation (ChIP), imposed by PML-RARα onto its primary target genes (*tgII*, *rarb* and *cyp26*).

Through our first objective, we have developed an APL mouse model expressing a tagged version of PML-RARα, ER-Ty-Ty-PML-RARα, which has allowed us, for the first time, to isolate, *in vivo*, the PML-RARα complex, and after identifying partners of the oncoprotein, such as SUMO-2 and Daxx through the PML domain and sub-units from the Polycomb repressive complex 2 (PRC2), such as Suz12, as well as RXRα and an aberrant recruitment of HDAC3 through the RARα domain, both *ex vivo* essential partners of PML-RARα. The epigenetic approaches applied in the objective 2 showed significant low levels of H3 and H4 acetylation at the promoters of *tgII*, *rarb* and *cyp26* genes, as well as a clearly elevated methylation at these gene bodies, comparing with normal myeloid progenitors. RA treatment was able to significantly increase acetylation in both histones, but not decrease DNA methylation at the PML-RARα binding sites or at these target genes, respectively. This acetylation was correlated with the RA-induced genes, suggesting a main role of the HDACs in APL pathogenesis. We then decided to silence HDAC3, one of the identified partner (from the objective 1), and we could observe that the hipoacetylation observed in untreated cells was reverted. Valproic acid (VPA) causes hyperacetylation of the N-terminal tails of histone H3 and H4 *in vitro* and *in vivo*, thus, to assess the biological relevance of these findings we treated mouse models of APL with VPA and we could

observe that the inhibition of HDAC3 is able to complete differentiate APL blast through the activation of gene expression, without any effect over the PML-RARα protein levels. However, the resulted terminal differentiation did not have any affect over the LIC clearance, and consequently in APL cure. We then carried out expression analyses with RA and other retinoids unable to degrade PML-RARα in *ex vivo* and *in vivo* APL mouse models, and we have observed that both types of retinoids were identical re-activating the primary target genes. However, we could not observe any beneficial effect in terms of LICs clearance when we treated APL mice with these non-degrading retinoids, thus, differentiation is insufficient for APL eradication, while PML/RARA loss is essential.

In summary, the implementation of this proposal has significant improved the **molecular understanding of the APL** disease:

- The identification *in vivo* of PML-RARα partners may address new **therapeutic targets for APL and other RARA fusion-mediated cancers.**
- The HDAC3 recruitment by PML-RARα has a main role in APL pathogenesis; however, the reversion of the consequent epigenetic alterations, after "epi-drugs" treatment, could not be relevant in APL cure at least as monotherapy.
- The obtained results establish a model that **uncouple retinoic induced degradation and PML-RARα or RARα transactivation**, definitively establishing the role of PML-RARα degradation in RA-induced response, **opening the new perspectives for designing efficient APL therapy** (Figure3).

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