Uncovering the molecular pathways dictating the anti-leukemic potency of EAPB02303 of the imiqualines family.

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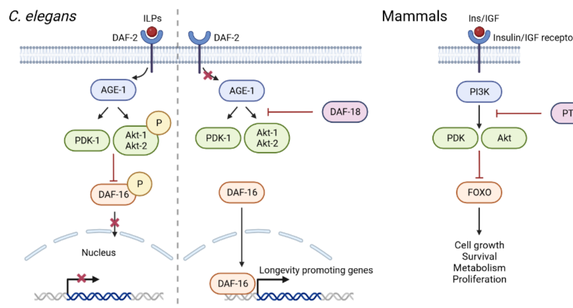
Introduction

Imiqualines, analogues of the immunomodulatory drug Imiquimod, are a family of low molecular weight compounds synthesized by our team and protected by international patents. We focused on the anti-leukemic potency of EAPB02303, lead compound of the second-generation family of Imiqualines, against Acute Myeloid Leukemia (AML), a haematological malignancy characterized by an aberrant differentiation and uncontrolled proliferation of myeloid blasts. AML arises from either mutually exclusive and in some cases co-occurring genetic, epigenetic and/or molecular abnormalities. Among these dysregulated signalling cascades are the PI3K/AKT pathway and the RAS/MAPK pathway, which regulate hematopoietic cell growth and proliferation. Indeed, these pathways are constitutively active in most AML cases and are associated with poor response and decreased overall survival .

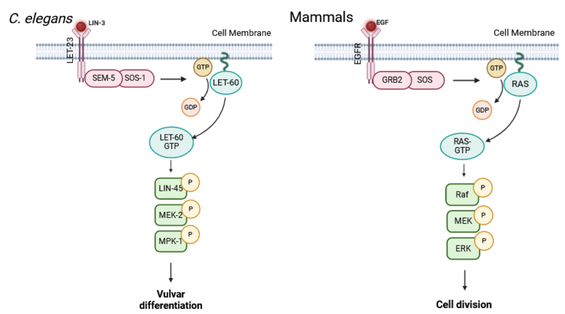
The nematode *Caenorhabditis elegans* is extensively used as a model system for the study of complex molecular processes involved in tumorigenesis. This model presents numerous advantages that render it an enticing small organism for cancer research. In fact, biological processes involved in mammalian cell proliferation and cell signalling are conserved in the worm. Additionally, at least 83% of the *C. elegans* proteome has human homologs, and 72% of tumour driver genes in humans have one or more orthologs in *C. elegans*. Moreover, mutations in key signalling cascades that can promote cancer in humans (namely, PI3K/Akt and Ras/MAPK, among others) are also found in *C. elegans*, and are associated with well-established phenotypes such as longevity and vulva-less or multivulva formation, respectively. Therefore, *C. elegans* is a highly amenable multicellular organism for the in vivo identification of therapeutic targets of anti-tumor agents.

Objectives

We aim to unravel candidate molecular targets of EAPB02303, and putative signalling cascades implicated in the underlying mechanism of action responsible for its potent anti-leukemic activity. The potential targets of EAPB02303 that were revealed *in vitro* using AML cell lines were validated using *C. elegans*. Particularly, we used *C. elegans* as a screening model system to explore the effect of EAPB02303 on PI3K/AKT and Ras/MAPK signalling cascade, a pathway aberrantly activated in human cancers, notably in AML.



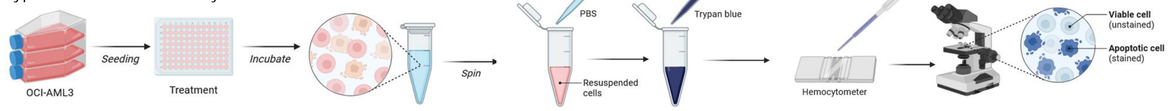
PI3K/AKT pathway in *C. elegans* and its mammalian orthologs.



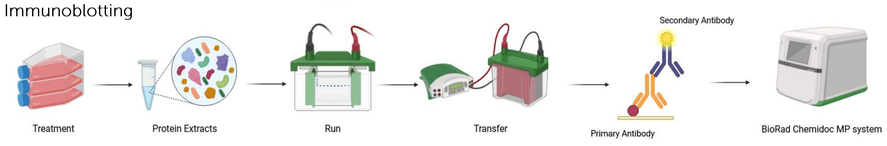
RAS/MAPK pathway in *C. elegans* and its mammalian orthologs

Methods

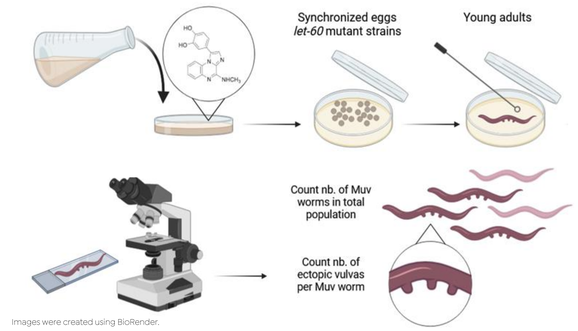
I. Trypan blue exclusion assay



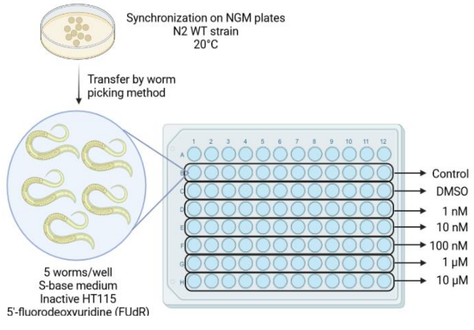
II. Immunoblotting



III. Muv phenotype

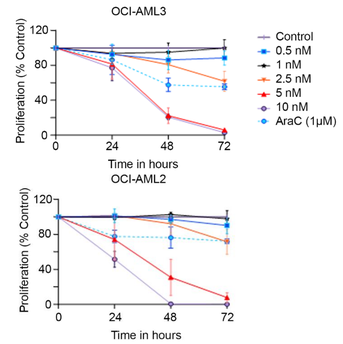


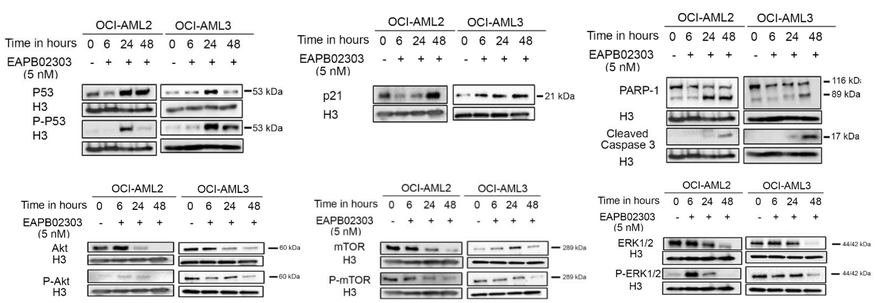
IV. Lifespan Bioassay



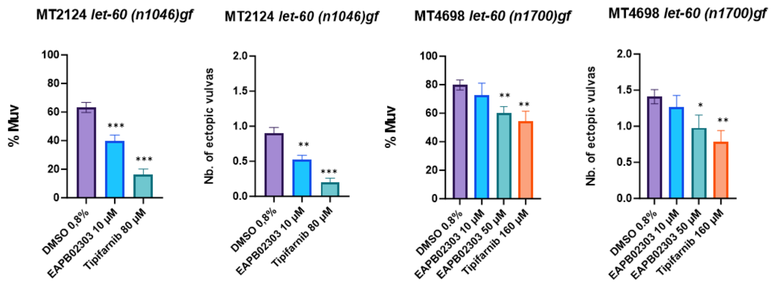
Results

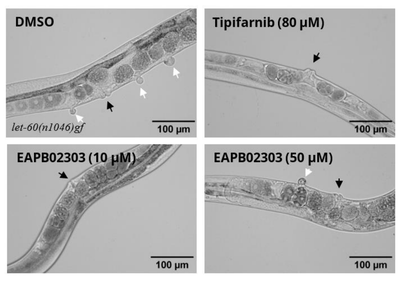
I. EAPB02303 significantly decreases the proliferation of AML cell lines, induces apoptosis and downregulates the expression of protein components of the PI3K/AKT and RAS/MAPK pathways.



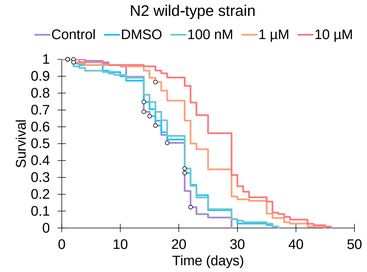


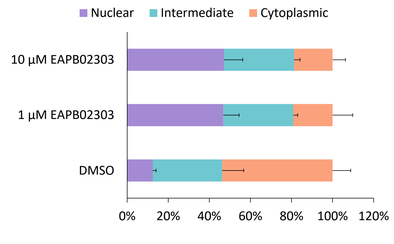
II. EAPB02303 potently reduces the multivulva phenotype caused by *let-60/Ras* overactivation mutation in MT2124 and MT4698 mutant strains.

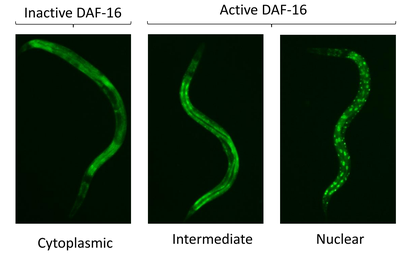




III. EAPB02303 extends lifespan of N2 wild-type adults and leads to the nuclear translocation and subsequent activation of DAF-16







Conclusion

In summary, we uncovered for the first time the mode of action of EAPB02303 against AML *in vitro*, and we validated these targets using a simple *in vivo* model system. Our data suggests that this original compound has a PI3K/AKT and Ras-inhibitory effects conferring a significant anticancer activity against human neoplasms linked with upregulated signalling activity through these pathways and oncogenic or *Ras* mutations.

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