An *in vitro*/*in vivo* platform to test the anti-tumor activity of tyrosine kinase inhibitors in medullary thyroid cancer

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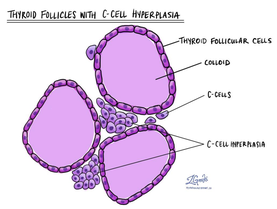
INTRODUCTION

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor, originating from calcitonin-producing parafollicular C cells of the thyroid gland. The clinical course of patients with MTC is variable, ranging from indolent to extremely aggressive forms. RET proto-oncogene alterations represent the most frequent events that lead to the development of both sporadic and inherited forms. Surgery is the only curative treatment for MTC, but advanced MTC are often unresectable and unresponsive to radiotherapy and chemotherapy. Cabozantinib (CAB) and Vandetanib (VAN) are two multi-target tyrosine kinase inhibitors (TKIs) currently used as first-line treatment for unresectable, progressive and symptomatic MTC. Although these drugs increased progression-free survival, drug discontinuation due to either disease progression or toxicity has been reported in about 40-55% of patients. Therefore, new therapeutic strategies are urgently required.



From https://www.mypathologyreport.ca/medullary-thyroid-carcinoma/

In the last decades, zebrafish (*Danio rerio*) has emerged as a powerful alternative model for the preclinical study of several human diseases, including cancer. Its intrinsic peculiarities, such as high fecundity, external fertilization, transparency, rapid development, embryo permeability to small molecules, together with easy genetic manipulation and low maintenance cost, make zebrafish an essential tool in biomedical research.

From
https://www.brainfacts.org/in-the-lab/animals-in-research/2020/researching-regeneration-through-the-zebrafish-100520
https://www.pinterest.it/pin/553590979195727653/

OBJECTIVE

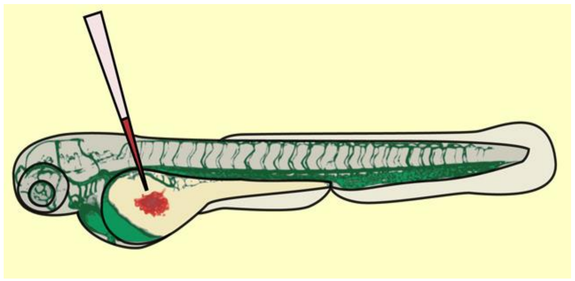
The aim of this study was to adopt an *in vitro* and *in vivo* platform to investigate the antitumor activity of TKIs in MTC, comparing the effects of CAB and VAN, with SPP86, a novel RET specific inhibitor.

MATERIALS AND METHODS

In vitro experiments were performed in two human MTC cell lines, TT and MZ-CRC-1, characterized by C634W and M918T RET mutations respectively. After six days of incubation, the effects of **VAN**, **CAB** and **SPP86** on cell viability, cell cycle, and apoptosis of TT and MZ-CRC-1 cells were evaluated *in vitro* using MTT assay, DNA flow cytometry with propidium iodide, and Annexin V-FITC/propidium iodide staining, respectively.

Our *in vivo* preclinical model was based on the implantation of human MTC cells in transgenic *Tg(fli1a:EGFP)*^{y1} zebrafish embryos at 48 h post-fertilization (hpf), that express the fluorescent protein EGFP in the endothelium, allowing the *in vivo* visualization of the entire vascular tree. MTC cells were labeled with a fluorescent viable dye, resuspended in Phosphate Buffered Saline (PBS) and grafted into the sub-peridermal space of 48 hpf *Tg(fli1a:EGFP)*^{y1} embryos.

As control of the injection, we considered embryos injected with only PBS. The tumorigenic potential of MTC implanted cells was evaluated by the quantification of tumor-induced angiogenesis as early as 24 h post-injection (hpi).



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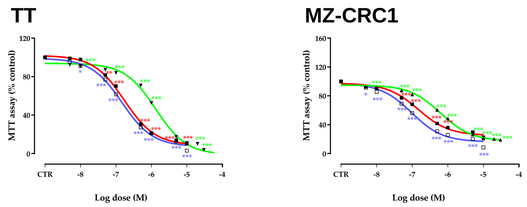
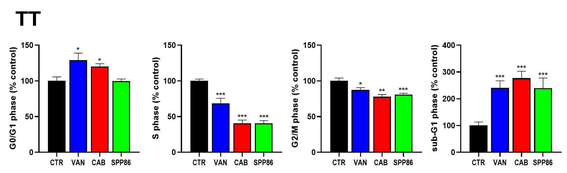
Due to the zebrafish embryo permeability to small molecules **VAN**, **CAB** and **SPP86** were directly dissolved into fish medium, at the concentration of 2.5 µM, and their anti-angiogenic effects on MTC cell grafted embryos were evaluated. As control of the pharmacological treatments, we considered embryos incubated with the vehicle (DMSO).

IN VITRO RESULTS

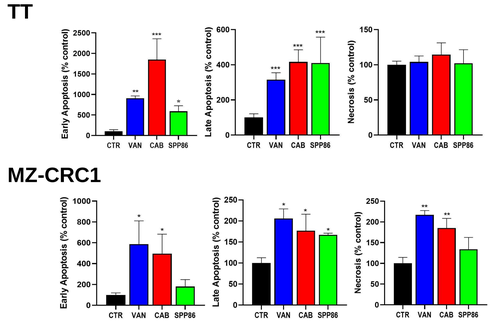
MTT assays showed a significant inhibition of cell viability in both MTC cell lines after incubation with all TKIs. In TT cells the maximal inhibition after **SPP86** (-100%) was significantly higher than that of **CAB** (-91.2%, p<0.001) and **VAN** (-92.7%, p<0.001), while in MZ-CRC-1 cells the maximal inhibition of **SPP86** (-82.5%) was higher than **CAB** (-74.9%, p<0.01) and comparable to **VAN** (-83.7%).



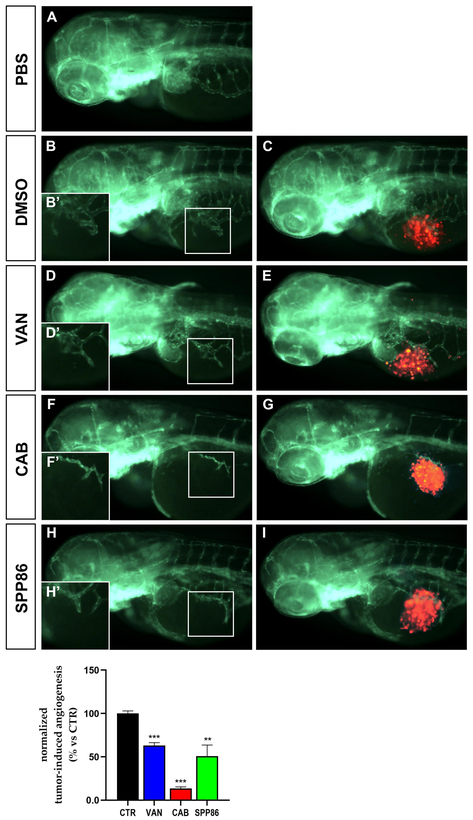
Cytofluorimetric analyses showed that **SPP86** and **CAB** similarly decreased the fraction of TT cells in the S and G2/M phases, while the effect of **VAN** was less prominent. **SPP86** e **VAN** significantly decreased the fractions of MZ-CRC-1 cells in the S and G2/M phases.



We observed a significant pro-apoptotic activity of all compounds in both cell lines.



IN VIVO RESULTS



In zebrafish model the impact of **SPP86** (-49%) in inhibiting TT-induced angiogenesis was comparable with that observed after **VAN** (-37%) and less potent than **CAB** (-86.4%, p<0.05).

CONCLUSIONS/NEXT STEPS

Our *in vitro*/*in vivo* platform resulted particularly reliable to easily test the effects of TKIs in MTC. This study revealed a significant anti-tumor activity exerted by SPP86, a new RET-specific inhibitor with potentially less adverse effects than multi-target TKIs, such as CAB and VAN.

Future studies should compare the anti-tumor effects of SPP86 with selpercatinib and pralsetinib, two RET-specific inhibitors recently approved by the FDA for the therapy of RET-mutant MTC. The possibility to implant a small number of cells makes the tumor xenograft/zebrafish model particularly suitable for cells derived from MTC patients, whose tumor cells availability is often limited, because of the small size of post-surgical samples. In this context, our platform may open a promising scenario in the field of precision medicine, promoting the identification of the most appropriate therapies in MTC patients.