Therapeutic Potential of Ralimetinib, a p38/MAPK14 Inhibitor, in Anaplastic Thyroid Cancer

Muthusamy Kunnimalaiyaan[1], Ali Dadbin[1], Ying Henderson[2],Elena McBeath Fujiwara[1], Clifford Stephan[3], Roza Nurieva[4], Naifa L. Busaidy[1], Jennifer R Wang[2], Stephen Lai[2], Maria Cabanillas[1], Marc Zafereo[2], Ramona Dadu[1], and Marie-Claude Hofmann[1].

[1]Department of Endocrine Neoplasia & HD, MDA; [2]Department of Head & Neck Surgery, MDA; [3]Texas A&M Institute for Biosciences and Technology, [4]Department of Immunology, MDA

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center Making Cancer History®

Purpose

To identify effective drug combination strategies targeting multiple oncogenic pathways in aggressive thyroid cancers.

Background

Treatments with BRAF inhibitors such as dabrafenib induce significant tumor size reduction in $BRAF^{V600E}$-mutated aggressive papillary (PTC) and anaplastic thyroid carcinoma (ATC) patients. However, these drugs are ultimately ineffective due to the development of resistance, probably because the cells reactivate multiple oncogenic pathways used to bypass the treatment. Therefore, novel therapeutic strategies are still urgently needed. We used reverse-phase protein arrays (RPPA), TCGA analysis, and RNA-Seq (GEO datasets) analyses of PTC and ATC patient samples to identify components of the p38/MAPK pathway as possible targets. This study reports the in vitro and in vivo effects of pharmacological inhibitors of p38/MAPK used alone or in combination with BRAF inhibitors on pre-clinical samples.

Pre-Clinical Models

1) ATC mouse model. We established *TPO-CreER^{T2}*; *BRAF$^{Ca/+}$; p53$^{fl/fl}$* mice that develop ATC tumors when treated with tamoxifen (Figures 1A +1B). These tumors show the same histology as human tumors (Figure 1C)

2) Mouse cell lines. From these tumors, we established several cell lines, including MCH2.2 and PPA6 (Figure 1D)

3) Human cell lines. We used the human cell lines MDA-T85 (PTC) and SW1736 (ATC)

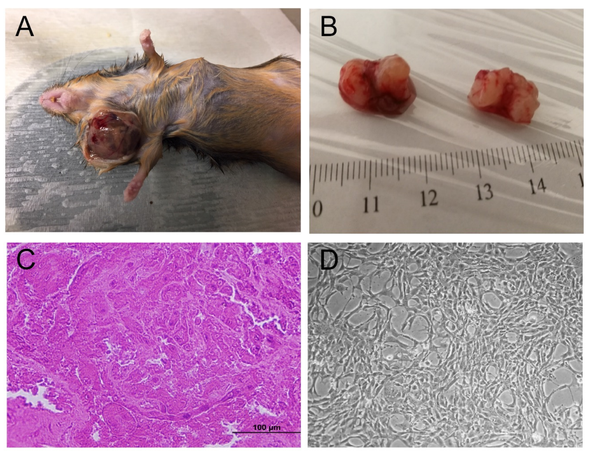


Figure 1: ATC mouse model and derived cell lines. A, B: Tumors develop about 3-4 months after tamoxifen injection. **C**: Histology (A&E) shows typical lack of organization and high cellular de-differentiation. **D**: PPA6 cell line.

Workflow

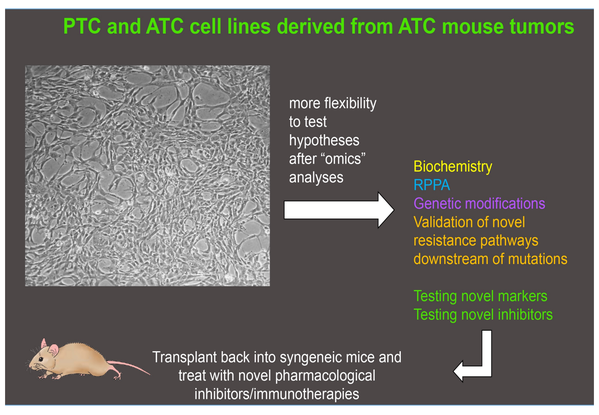
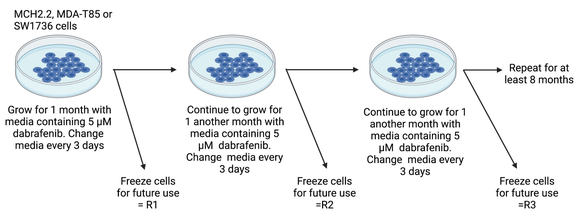


Figure 2: Mice bearing tumors can be directly treated with combinations of inhibitors. Alternatively, tumor cells can be manipulated in vitro (e.g. shRNA) to test pathways, then implanted into syngeneic mice. Because the mice are immunocompetent, this also allows combination treatments with immune checkpoint inhibitors.

Methods and Results

Reverse Protein Array (RPPA) analysis

To test which molecules/pathways are induced over time during BRAF inhibitors treatment, and if dedifferentiation of tumor cells occurs, we cultured BRAF-mutated PTC and ATC cells for 8 months with dabrafenib as follows:



Results (heatmap) indicate strong up-regulation/activation of specific pathways over 8 months treatment (arrows), including the MAPK/ERK1/2 (p44/42 MAPK) pathway (Figure 3).

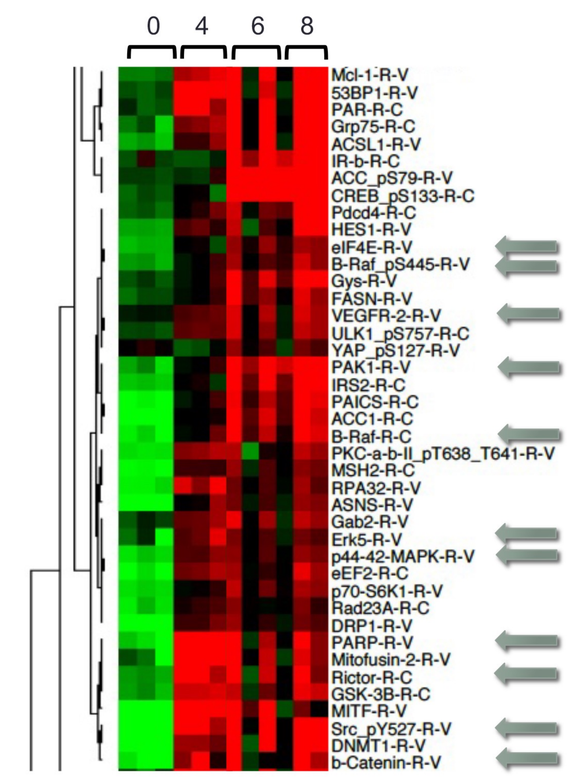


Figure 3: Part of a RPPA supervised analysis revealing potential oncoproteins upregulated/activated over 0, 4, 6, and 8 months treatment with dabrafenib in MDA-T85 cells (human PTC).

Western blots

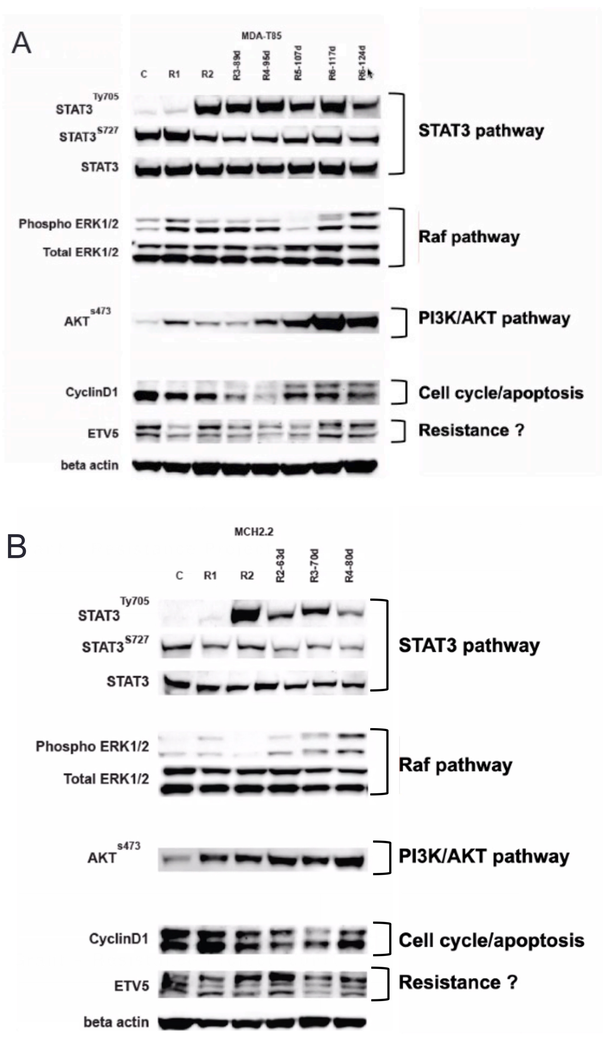


Figure 4: Western blots validating major pathways up-regulated over time. **A**: MDA-T85 human PTC cells. **B**: MCH2.2 mouse ATC cells

Methods and Results (cont.)

The expression of ETV5, a transcription factor driven by the MAPK pathway in these cells, was not downregulated by dabrafenib over time (Figure 4). Therefore, we searched for additional molecules/pathways co-expressed with ETV5 (TCGA and NIH GEO databases) in BRAF-mutated human PTC and ATC samples (Figures 5 and 6):

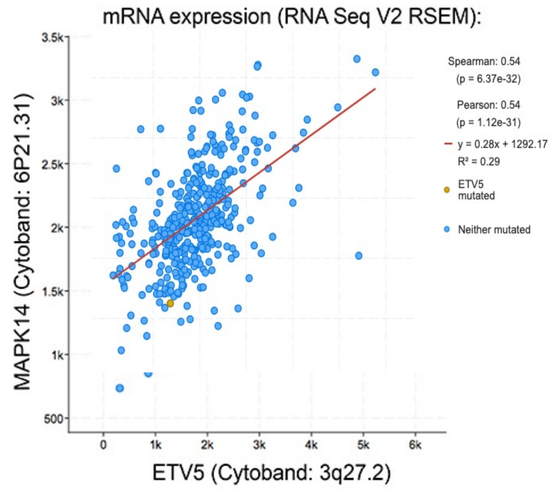


Figure 5: TCGA analysis through cBioportal showed strong association between ETV5 and p38/MAPK14 expression in 497 PTC patients.

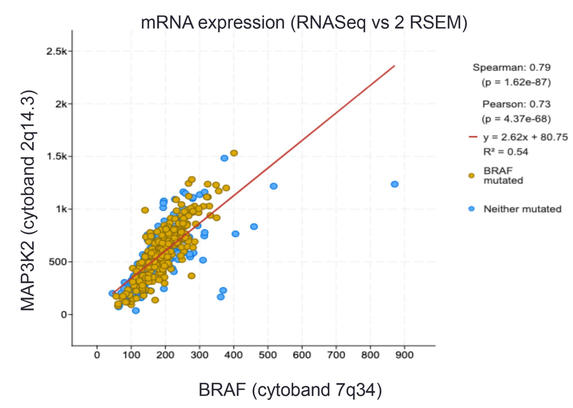


Figure 6: TCGA analysis through cBioportal showed strong association between mutated BRAF (yellow dots) and MAP3K2 (MKK3), a kinase that specifically phosphorylates and activates p38MAPK in 397 samples of PTCs

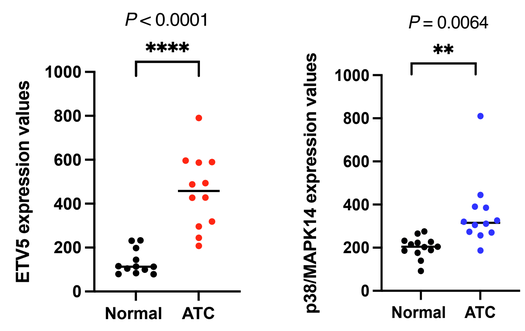
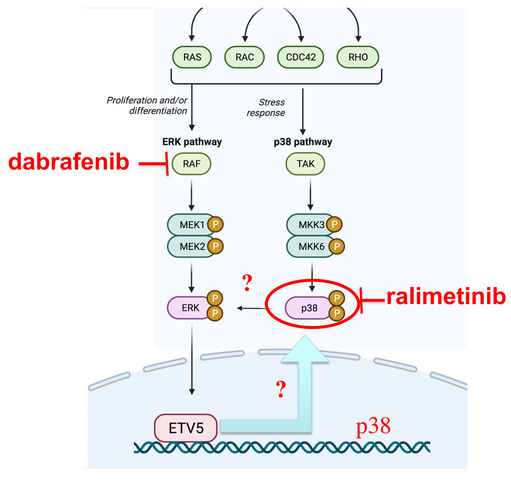


Figure 7: Similar to PTCs, expression of ETV5 and p38/MAPK14 is significantly higher in ATC patient samples than in normal samples. **A:** ETV5 expression, **B**: p38/MAPK14 expression. From NCBI GEO GSE65144.

We next tested ralimetinib and other inhibitors of p38/MAPK14 for possible reduction of PTC and ATC cell growth in combination with dabrafenib.



Methods and Results (cont.)

Our data demonstrate a good synergy between ralimetinib and dabrafenib using cell lines in vitro (Figure 8) and in vivo (Figure 9).

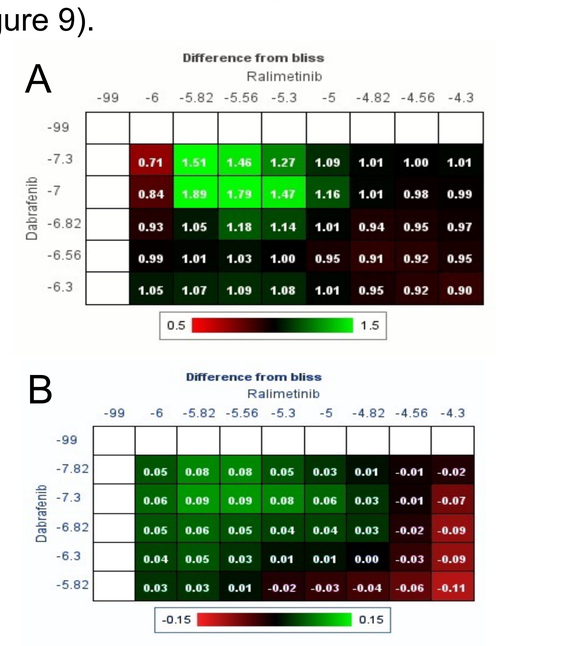


Figure 8: High-throughput screening to identify synergistic drug combinations. Log molar concentrations are indicated. Green = synergy, black = additivity, red = antagonism. **A**: MDA-T85 human PTC cells, **B**: SW1736 human ATC cells, **C**: MCH2.2 mouse ATC cells.

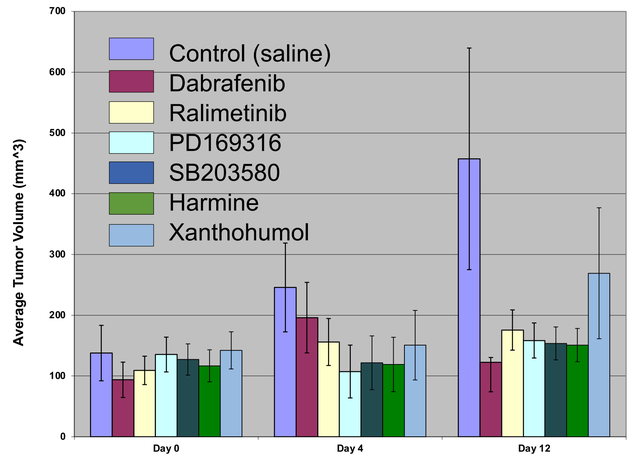


Figure 9: Growth of MCH2.2. ATC tumor cells in syngeneic mice in presence of dabrafenib or different p38/MAPK14 inhibitors. Harmine is a TWIST1 inhibitor. Xanthohumol is a NOTCH inhibitor and serves as negative control.

Conclusions

- Many oncogenic molecules and pathways are activated by long-term culture with dabrafenib, a $BRAF^{V600E}$ inhibitor
- Searching for downstream targets or parallel pathways through data mining revealed overexpression/activation of the p38/MAPK14 pathway in PTC and ATC patient samples
- The p38/MAPK14 inhibitor ralimetinib synergizes with dabrafenib, in particular in human PTC cells and mouse ATC cells
- Ralimetinib and other p38/MAPK inhibitors are efficient in mice bearing ATC tumors

Future work

- Test ETV5 as a marker of resistance in aggressive thyroid cancers
- Test combination therapies lenvatinib and ralimetinib for BRAF wild type thyroid cancers
- Test the interplay between p38/MAPK14 and TP53 mutations, also the role of STAT3 in ATCs

Acknowledgements: We thank the ATC Multidisciplinary Petrick Research Funds, the IBT Drug Discovery Program, and NIH P30 CA016672 MDA Core support grant.