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 BRAFV600E-mutated aggressive papillary thyroid cancer (PTC) 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-CreERT2; BRAFV600E;p53flox/flox 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).

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 upregulation/activation of specific pathways over 8 months treatment (arrows), including the MAPK/ERK1/2 (p44/42 MAPK) pathway (Figure 3).

Western blots
We next tested ralimetinib and other inhibitors of p38/MAPK4 for possible reduction of PTC and ATC cell growth in combination with dabrafenib.

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):

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).

Conclusions
• Many oncogenic molecules and pathways are activated by long-term culture with dabrafenib, a BRAFV600E 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 lervatnib and ralimetinib for BRAF wild type thyroid cancers.

• Test the interplay between p38/MAPK14 and TP53 mutations, also the role of STAT3 in ATCs.

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