Understanding downstream signaling of the kinase RET WT and mutant.

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Study Design
We have used kinase activity profiling using PamChip array to compare the kinase activity of RET and the mutant RET V804M in vitro (figure 1). This is to investigate whether the membrane bound RET is able to directly phosphorylate and activate downstream kinase targets including FAK. Western analysis on lysates of three mutant RET transfected HEK293 cell lines compared to wild-type RET was performed to confirm the hypothesis that FAK plays a role downstream of RET in case of mutant RET (figure 2).

Key Findings
These studies on PamChip arrays as further described in the reference have shown that the FAK1-569-581 peptide, which is a direct downstream kinase of RET, is phosphorylated more profoundly by the RET V804M compared to the wildtype RET (figure 1). Western analysis of mutant RET in HEK293 confirmed our hypothesis that the mutants including the vandetanib resistant RET V804M shows increased phosphorylation of FAK on P-Tyr575/6-FAK.

FAK inhibitors may therefore provide a new therapeutic option for certain RET mutant forms of MEN2-patients.

“Author Quote”
Structural studies are currently underway to uncover the molecular bases of the RET-FAK interaction.

Background
RET oncogene (identified in 1993) gain of function mutations are responsible for hereditary medullary thyroid carcinoma or Multiple Endocrine Neoplasia 2A (MEN2A) and 2B (MEN2B). RET mutation cause gastrointestinal dysfunction.

Kinase inhibitors (including vandetanib, AstraZeneca) are currently in clinical evaluation to treat MEN. Studies have shown that the RET V804M mutant is resistant to vandetanib. Understanding the RET downstream signaling might provide a new treatment modality of MEN patients.

Conclusion
Comparison of the RET kinase and its oncogenic mutant RET V804M on PamChip arrays have identified a downstream kinase FAK which is overexpressed in the mutant RET V804M form which may be a novel target of MEN2 escape mutants.

References: