**Drug-adapted RET mutations**

**RET** is a protein tyrosine kinase that is upregulated and activated in many diverse forms of cancer. Treatments using RET-specific inhibitors have been highly effective, but the threat of resistance to these drugs looms. For this reason, Dr. Jie Wu and Dr. Blaine Mooers from the University of Texas, together with Dr. Vivek Subbiah from the University of Oklahoma, decided to proactively identify mutations that could confer resistance in order to improve treatment against these RET-altered cancers.

**ABERRANT RET ACTIVATION**

RET is a transmembrane PTK which is important in fetal development of various areas of the body, such as the kidneys and the enteric nervous system. RET is normally activated by binding specific ligands, which induce a homodimerisation of the transmembrane protein and its co-receptor complex, where a protein complex is formed from two identical proteins or protein complexes. This allows the two RET PTKs to undergo autophosphorylation and subsequent activation of the signalling pathways. RET can activate several signalling pathways, particularly the RAS/RAF/MEK pathway, which is affected in many cancers. In normal circumstances, RET activation is regulated, but genetic changes can occur that lead to the protein becoming constantly active. RET can become altered either as a result of gene fusion or by point mutations, some of which are hereditary in families with predispositions to multiple endocrine neoplasia 2 (MEN2) – a condition affecting endocrine glands. Both of these changes lead to uncontrolled RET activation which, in turn, means that further reactions in the signalling pathway will also be switched on. Downstream effects of this are the increased proliferation and survival of cells, which causes tumours to form.

This aberrantly active RET protein has been seen in many types of cancer, including medullary thyroid carcinoma (MTC), papillary thyroid cancer (PTC), and non-small-cell lung cancer (NSCLC), amongst others. Laboratory experiments have verified that the genetically changed RET gene is the cause of these cancers and that abnormal RET kinase activity is required to keep the tumour cell alive. This makes RET a good target for cancer treatment.

**TARGETING PTKs**

Due to the oncogenic nature of many PTKs, they have been used as patient-specific targets in treating cancer. Many PTK inhibitors exist, such as EGFR inhibitors, and are used in clinical settings. They work by directly binding to the active site of the enzymes so that they can no longer phosphorylate proteins, thereby inactivating them, leading to anti-tumorigenic effects. Before RET-specific inhibitors could be established, multi-targeted tyrosine kinase inhibitors were developed for other kinases, such as vandetanib and lenvatinib. These inhibitors were used against RET-altered cancers with some success, however, they can lead to off-target effects, and resistance has been seen in some cases. Structural studies have revealed that these types of PTK inhibitors bind to the front and back of the drug-binding clefts of RET by going through the gate region between these two clefts. However, some cancer cells can adapt to these inhibitors by substituting an amino acid called the gatekeeper with a different amino acid. This substitution has been shown to confer resistance by interrupting the binding of the inhibitor to the RET molecule, allowing it to become activated again and the cancer to progress.

**ACQUIRED RESISTANCE TO RET**

Acquired resistance to these new drugs seems to be an inevitability based on repeated experiences with previous therapies. Certain mutations that conferred resistance to both selpercatinib and pralsetinib. These cell cultures were treated with increasing concentrations of the drugs which eventually led to the acquired resistance. Dr. Subbiah has been treating and following up patients with RET-positive cancers enrolled in these trials. He has seen resistance to these drugs, indicating that other inhibitors may be needed. Most patients respond to the therapy, which is indicated by tumour shrinkage in the scans and improved quality of life.

**FURTHER RESISTANCE BEING SEEN**

These new RET-specific drugs seem to be working much more effectively than the more general inhibitors, with response rates of 60–85% over 18 months. However, acquired resistance to these new drugs seems to be a certainty based on repeated experiences with previous therapies. It is likely to be inevitable that these inhibitors interact with several non-essential amino acid residues of the RET kinase. This makes pralsetinib and selpercatinib vulnerable to drug-resistant mutations in these amino acids.
However, after around two years, the drugs stop working. This is because cancer develops mechanisms that render the drugs ineffective. These are known as acquired resistance. The mechanisms of resistance seen in the structures by Dr Mooers and Dr Wu were also seen in the tumours that became resistant to therapy. In some cases, RET had been bypassed altogether by activating a downstream RET target. In other cases, there were direct drug-adapted mutations. As mentioned before, these inhibitors do not bind the gate region of RET, so they were able to overcome resistance by the gatekeeper mutations. It would seem, therefore, that non-gatekeeper mutations were responsible for the resistance seen in patients and cell cultures.

The resistant mutants from the cell cultures were sequenced, as was colony-free DNA from the patients who had acquired resistance. The mutations were determined to be in the solvent front, the hinge, and the $\beta_2$ strand areas of RET. All of these areas are involved in the binding between selpercatinib or pralsetinib with RET. The strongest resistance to both drugs was seen to be conferred by substitutions of the glycine 810 residue in the solvent front region, which implies that the RET with glycine 810 mutations will be the least inhibited by these drugs and particularly important for causing acquired resistance.

**Understanding how resistance is conferred is the key for the future of RET-specific treatment.**

### THE FUTURE OF RET THERAPY

Drug resistance is always a problem when developing a new target in cancer therapy. It is therefore important to take a proactive stance and predict what mutations could occur before they even develop in patients. This way, the next generation of drugs may be developed earlier to circumvent the resistance. RET is clearly an extremely good target as it is mutated in so many cancers, and inhibiting it has such promising results in patients. Being able to predict what could happen or understanding why resistance is occurring is vital to keeping treatments as effective as possible. More RET-specific drugs are in the works, and the possibility of using these in combination with others is promising. Understanding exactly how the drug works and how resistance is conferred is the key for the future of RET-specific treatment in order to keep one step ahead of cancer.

### References


### Personal Response

**Could combination therapy with RET inhibitors be used to overcome this resistance?**

In metastatic cancers, residual tumours that cannot be completely eliminated by a cancer treatment will evolve to become resistant tumours, leading to disease progression. Identification of drug-adapted mutations in protein tyrosine kinase-targeted cancer therapies is essential for developing the next generation of drugs to inhibit these mutants, in order to turn an incurable malignancy into a manageable chronic disease.