1148P: Identification and validation of non-canonical RET fusions in non-small cell lung cancer through DNA and RNA sequencing

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Background:

- Oncogenic RET rearrangements are reported in 1-2% patients with non-small-cell lung cancer (NSCLC) of which KIF5B-RET and CCDC6-RET are known as the most common forms of RET fusions.
- Some RET inhibitors have been approved by FDA as they showed remarkable responses and efficiencies in advanced RET-fusion positive NSCLC.
- DNA-based next-generation sequencing (DNA-seq) is able to detect RET fusions with novel partners, but further information on the effective transcripts of chimeric fusion remains unknown.
- Our study performed in-depth characterization on non-canonical RET fusions through DNA- and RNA-seq.

Methods:

- This retrospective study involved 149 NSCLCs patients harboring RET rearrangements identified by DNA-seq;
- Non-canonical RET fusions were defined as:
  1) rearrangement with a rare partner gene in addition to KIF5B and CCDC6;
  2) rearrangement with an unreported partner gene;
  3) rearrangement fused with an intergenic space;
  4) presence of more than one RET fusions.
- A total of 54 patients with non-canonical RET fusions were subjected to RNA-seq panel of 115 genes. After quality control, 44 patients with paired DNA-seq and RNA-seq results were eligible for subsequent comparisons and analyses.

DNA-seq demonstrated a high positive predictive value of 93.2% in detecting RET fusions, including those with a rare partner, prioritizing it as a reliable upfront screening method over other assays.

Combining RNA-seq with DNA-seq enables to depict a more clear-cut picture of molecular pathogenesis mediating the complex RET rearrangements emerging in the tumor genome.

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Results:

- In 44 patients with non-canonical RET fusions, DNA-seq identified **(Figure. 1A)**:
  - 27 patients with concurrent canonical RET-fusions, including 23 KIF5B-RET, fusions and four CCDC6-RET fusions.
  - 17 patients with non-canonical RET-fusions alone.

Figure.1A

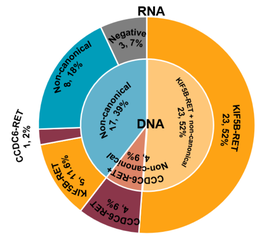
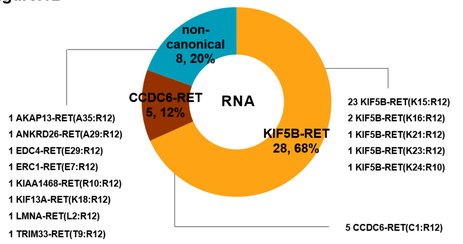
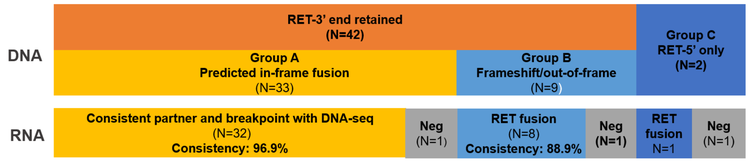


Figure.1B



- At RNA level, 41 of 44 patients (93.2%) were positive for RET-fusions **(Figure. 1A & 1B)**.
- Patients were classified into group A (75%), group B (20.5%) and group C (4.5%) based on the type of RET fusions identified by DNA-seq **(Figure 2)**.
  - In group A, 96.9% patients were validated by RNA-seq including 25 canonical and seven non-canonical RET fusions.
  - In group B, 88.9% patients were validated by RNA-seq including seven canonical and one non-canonical RET fusions.

Figure 2



- In eight patients, DNA-seq identified out-of-frame fusions while RNA-seq detected functional transcripts. The discordant RET fusions at DNA and RNA levels might mediated by four types of complex genomic rearrangement events **(Figure 3A-3D)**.

Figure. 3A Double inversions

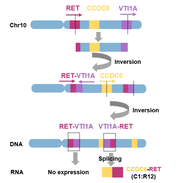


Figure. 3B Inversion & insertion

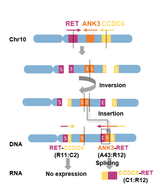


Figure. 3C Inversion & translocation

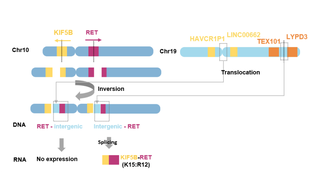


Figure. 3D Multichromosomal chromothripsis