The landscape of somatic structural rearrangements in RAS pathway genes

Angeliki Pantazi
Harvard GCC

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Whole genome sequencing pipeline

WGS (6-8X)
~50 bp reads
~300 insert size

Preprocessing
BWA, MarkDuplicates, Realign, Recalib

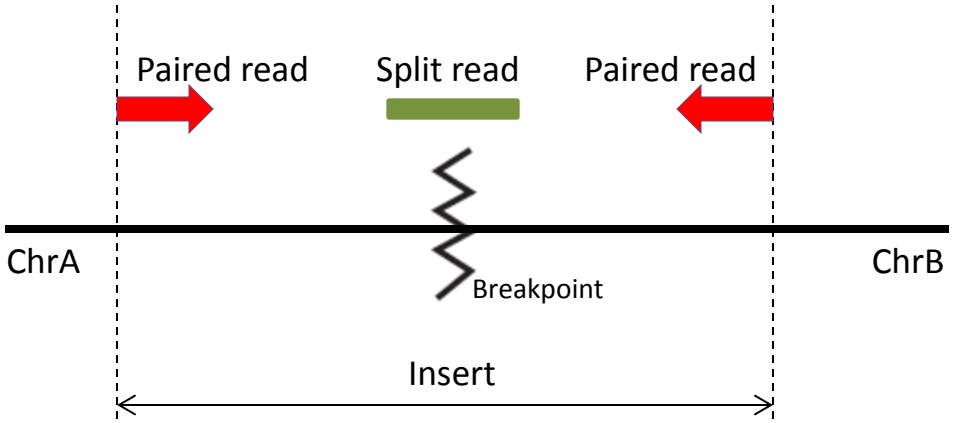
Detection of structural variants
Breakdancer (paired-end mapping)
Meerkat (paired-end mapping/split read)

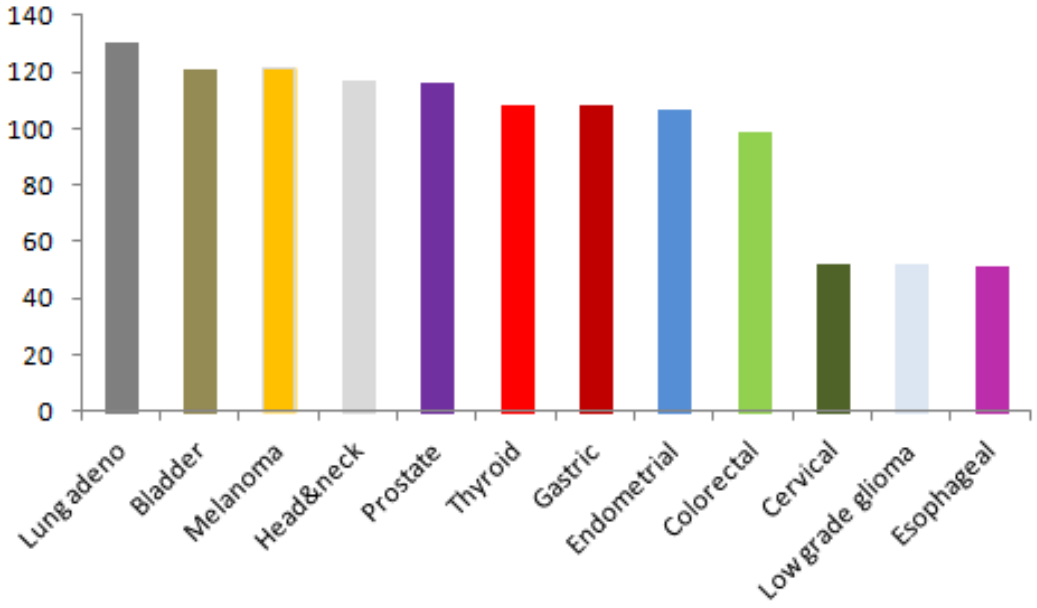
QC-Validation

Gene annotation

Filtering
(normal samples)

Data interpretation

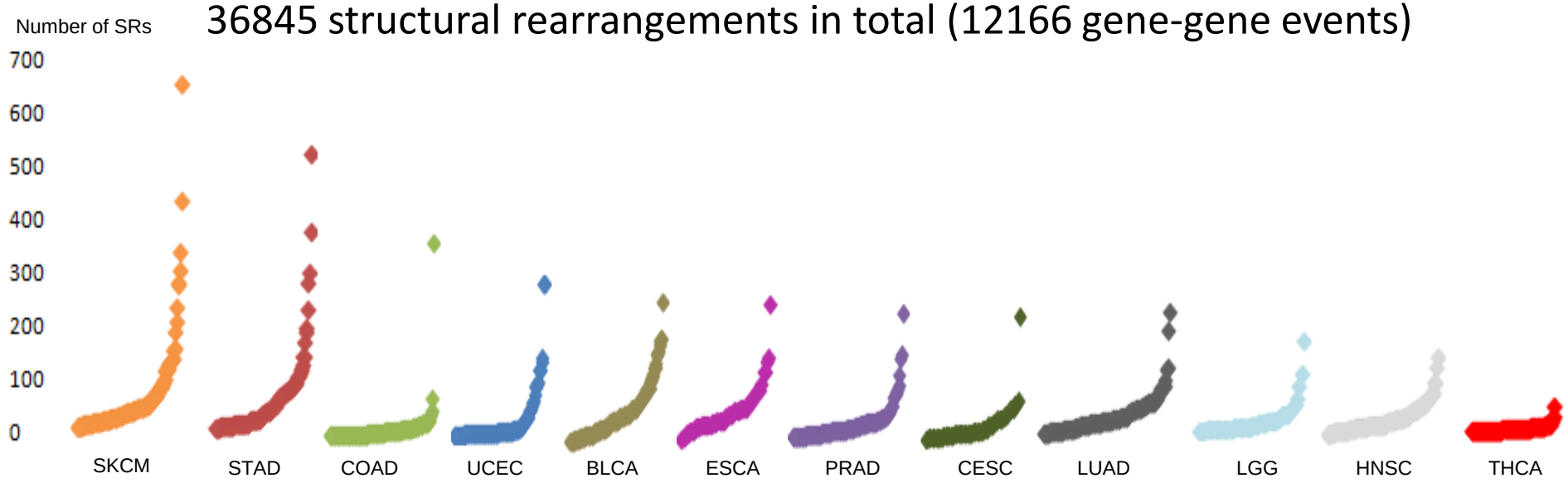


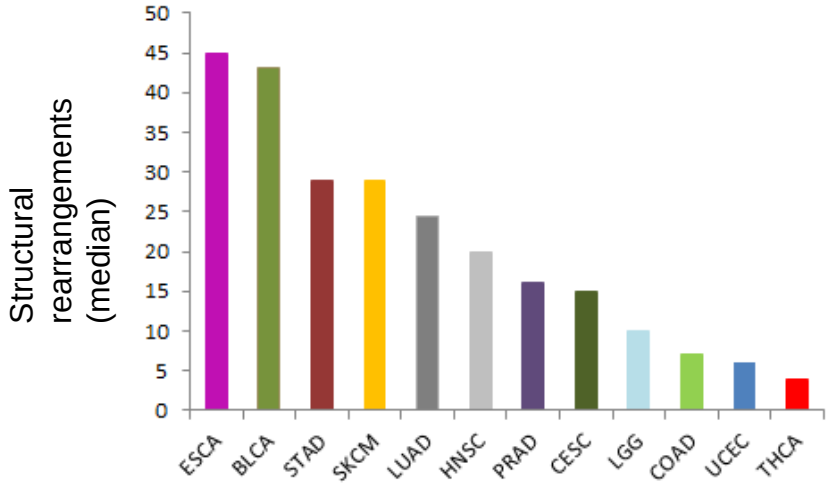


- 12 tumor types,
  1181 tumor and matched normal (tissue/blood) sample pairs

Incidence of structural rearrangements in 12 tumor types

36845 structural rearrangements in total (12166 gene-gene events)





Validation of gene-gene events:

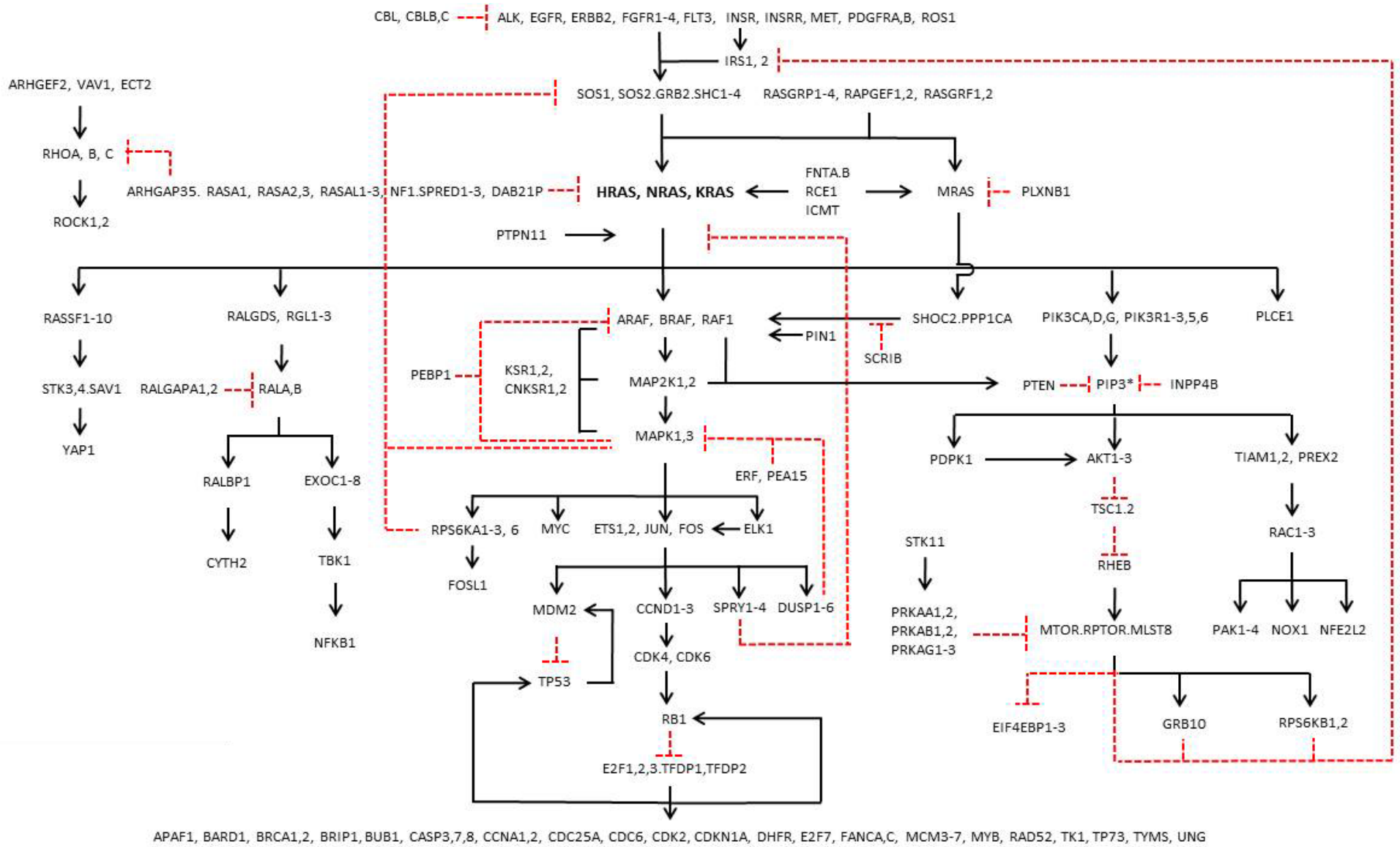
- *In silico:* rearrangements detected by both DNA callers/by RNAseq
- *In vitro:* PCR amplification of the junction fragment

✓ **Validation rate: 80%**

Pathways affected: RAS pathway

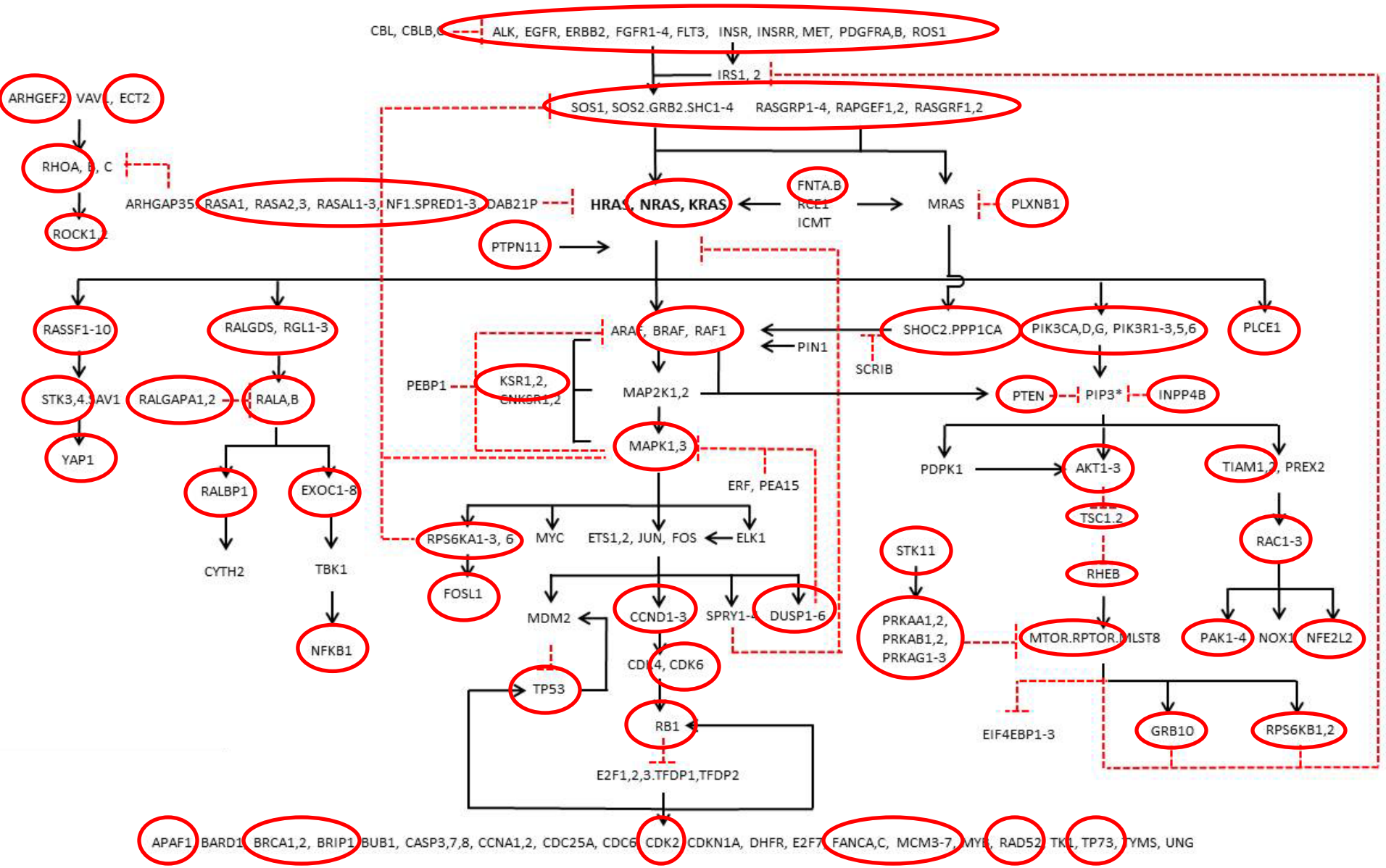
- ✓ RAS-MAPK pathway is known to have an important role in cancer.
- ✓ Mutations in KRAS, NRAS and BRAF have been found to activate RAS/MAPK pathway in many solid tumors.
- ✓ There is evidence to suggest that there may be alternative mechanisms of activation of the pathway.

- We examined the role of structural aberrations in the activation of the RAS-MAPK pathway in 12 tumor types.
- 199/1181 (17%) TCGA samples have somatic RAS rearrangements.



http://www.cancer.gov/researchandfunding/priorities/ras/advance-research/ras-blogs/ras-pathway-v2

Rearrangements in RAS pathway



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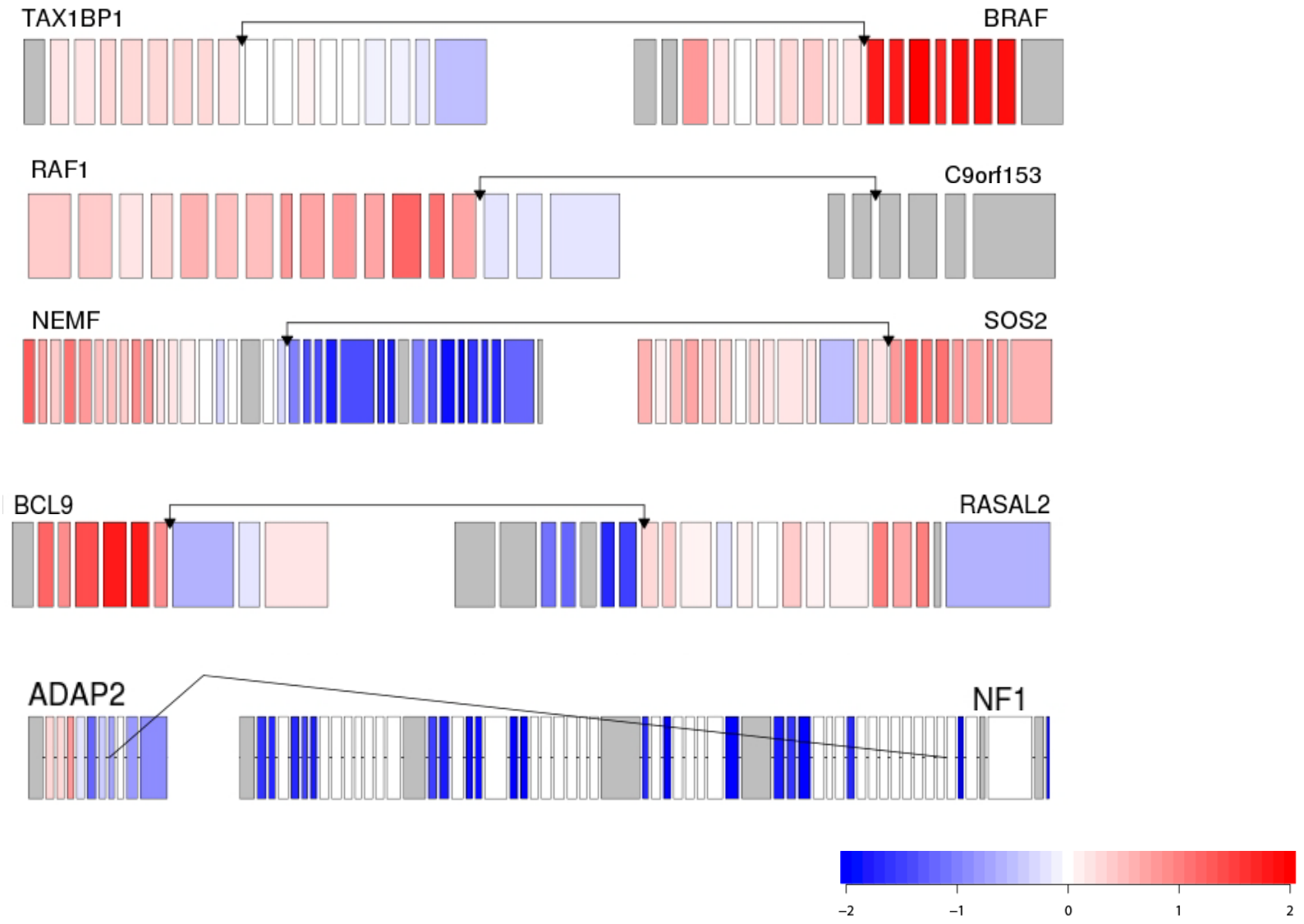
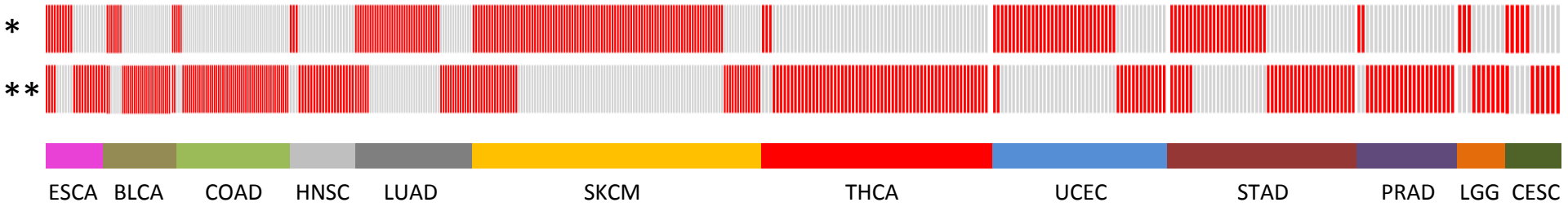
Rearrangements in RAS pathway



RAS rearrangements and other RAS somatic aberrations across 12 tumor types

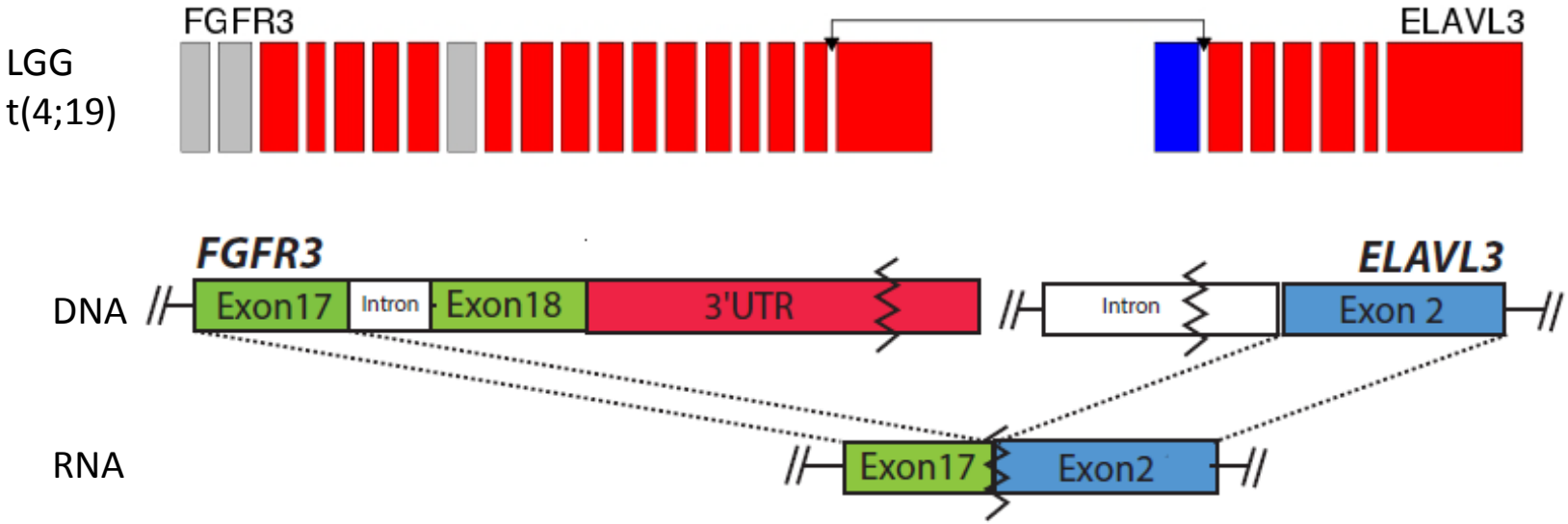
*RAS structural rearrangement

**KRAS/NRAS/BRAF mutation +/ amplification



- Rearrangements in genes of the RAS pathway are mutually exclusive to most of RAS/RAF somatic aberrations (DNA-level).

Rearrangements affecting 3'UTR in *FGFR* genes



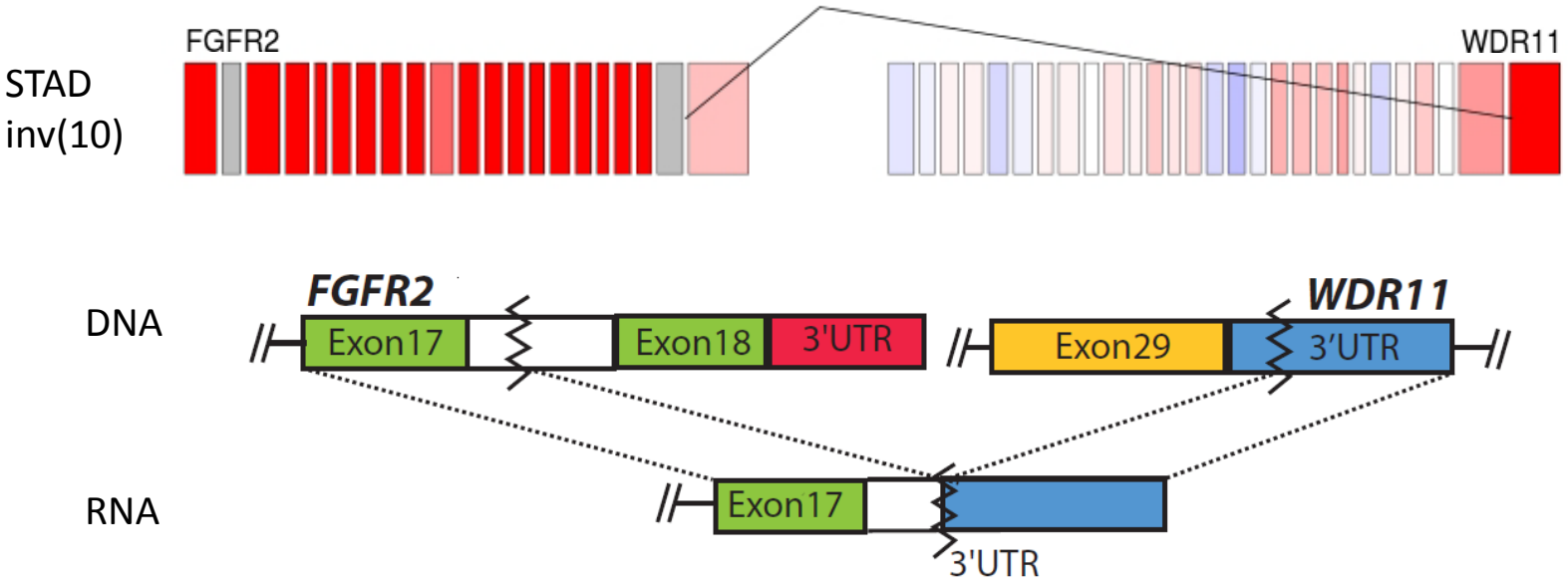
- The FGFR3 genomic breakpoint lies within its 3'UTR.
- Due to alternative splicing, the fusion transcript *FGFR3 -ELAV3* is missing exon 18 and the *3'UTR of FGFR3*.

J Clin Invest. 2013 Feb;123(2):855-65. doi: 10.1172/JCI67144. Epub 2013 Jan 9.

The tumorigenic FGFR3-TACC3 gene fusion escapes miR-99a regulation in glioblastoma.

Parker BC[1], Annala MJ, Cogdell DE, Granberg KJ, Sun Y, Ji P, Li X, Gumin J, Zheng H, Hu L, Yli-Harja O, Haapasalo H, Visakorpi T, Liu X, Liu CG, Sawaya R, Fuller GN, Chen K, Lang FF, Nykter M, Zhang W.

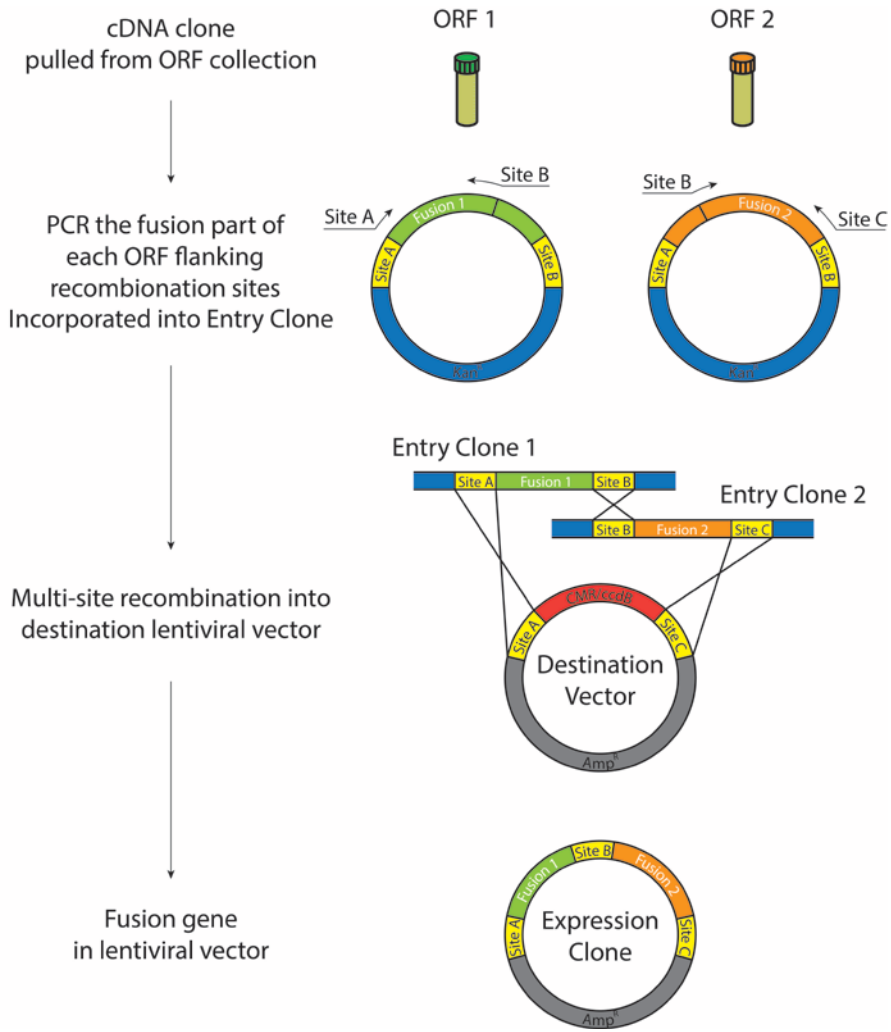
Rearrangements affecting 3'UTR in *FGFR* genes

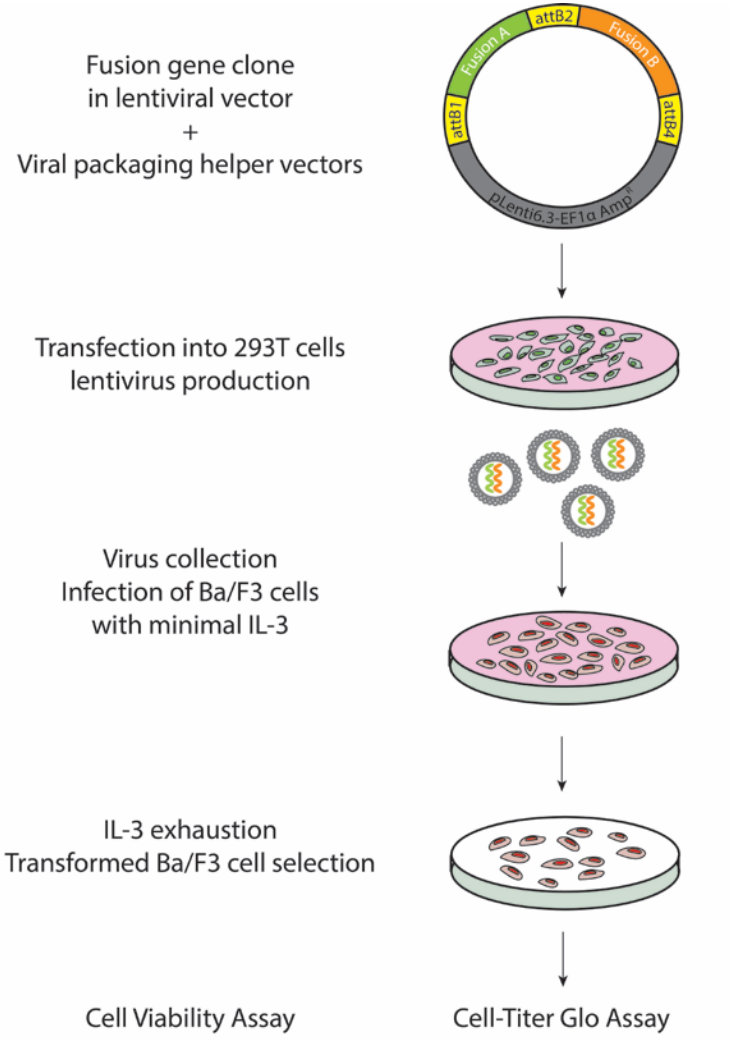


- The fusion transcript *FGFR2 –WDR11* is missing exon 18 and the *3'UTR of FGFR2*.
- The fusion transcript's 3'UTR is a hybrid of fragments of intron 17 of FGFR2 and of the 3'UTR of *WDR11.*

Functional validation of driver structural rearrangements

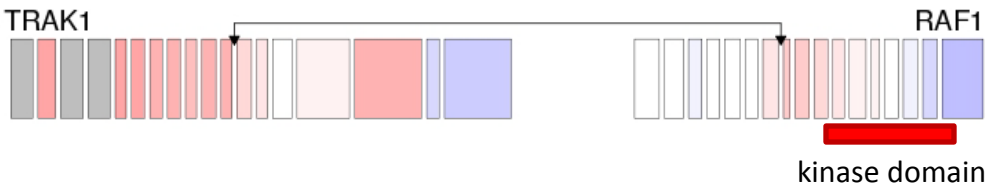
Gateway ORF cDNA clones

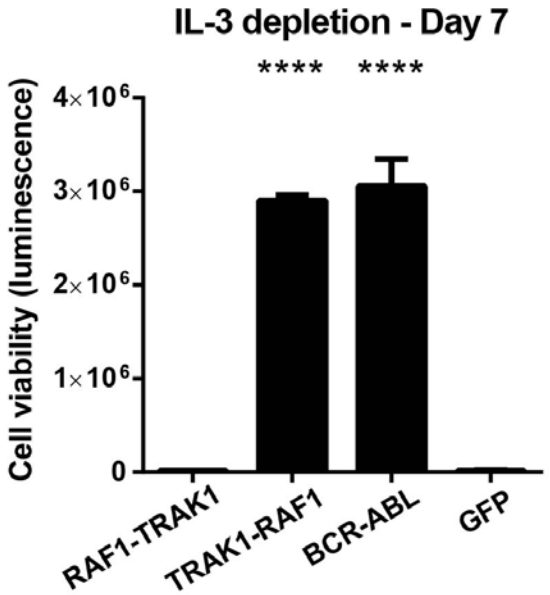




The *TRAK1-RAF1* rearrangement is a driver event in melanoma

SKCM, inv(3)





- An inversion in chr3 leads to both *TRAK1-RAF1* fusion, which retains the kinase domain, and its reciprocal partner *(RAF1-TRAK1).*
- Overexpression of TRAK1-RAF1 but not of TRAK-RAF1 fusion protein relieves mouse Ba/F3 cells from dependency on IL-3.

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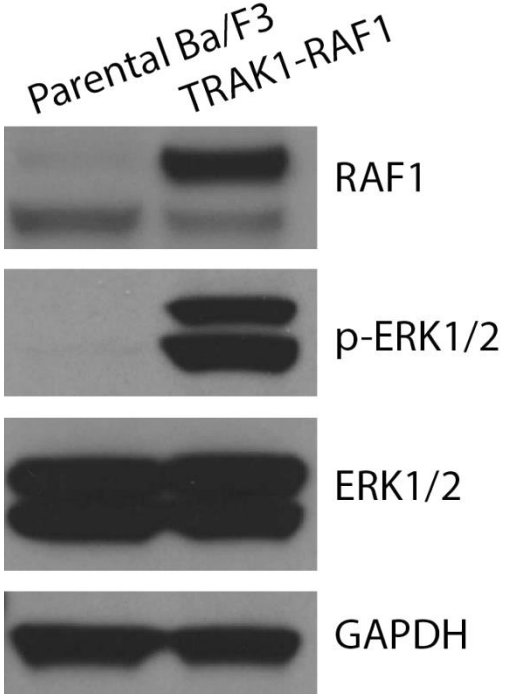
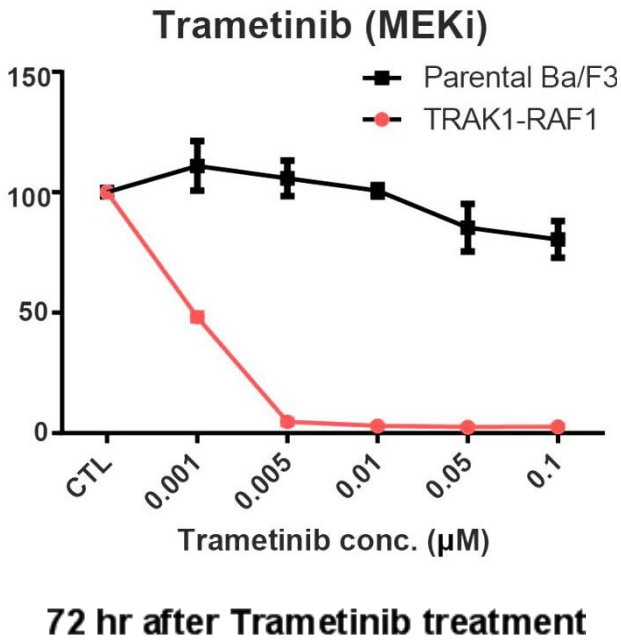
TRAK1-RAF1 activates the RAS pathway





• TRAK1-RAF1 transformed Ba/F3 cells show increased of levels of phosphorylated ERK1/2 protein and sensitivity to MEK inhibition.

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Summary

- RAS mutations are found at a high frequency in many cancers; structural rearrangements provide an alternative mechanism for activation of the pathway.
- RAS rearrangements are mutual exclusive to most RAS mutations/amplifications.
- Functional studies show the oncogenic potential of RAS rearrangements.

✓ Join me at poster #59!

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