

Clonal analysis of NRAS activating mutations in KIT-D816V systemic mastocytosis

Todd M. Wilson,¹ Irina Maric,² Olga Simakova,² Yun Bai,¹ Eunice Ching Chan,¹ Nicolas Olivares,² Melody Carter,¹ Dragan Maric,³ Jamie Robyn,⁴ and Dean D. Metcalfe¹

¹Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases; ²Department of Laboratory Medicine, Clinical Center; ³Flow Cytometry Core Facility, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA; and ⁴Department of Veterans Affairs, Springfield, Ohio, USA

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Online Supplementary Table S1. Primers used in this study.

| Primer | Direction | Sequence, 5' → 3' | Annealing temperature (°C) | [MgCl ₂], mM | Length, bp |
|--------------------------------------|-----------|----------------------------|----------------------------|--------------------------|------------|
| Real Time PCR | | | | | |
| NRAS | Forward | CCTCTACAGGGAGCAGATTAAGCGA | 60 | * | 171 |
| | Reverse | CCCTGTCTGGTCTTGGCTGAGGT | | | |
| KRAS | Forward | GAGAGAGGCCTGCTGAAAATGACTGA | 60 | * | 200 |
| | Reverse | TGACCTGCTGTGTCGAGAATATCCA | | | |
| HRAS | Forward | AGAGGATTCCTACCGGAAGCAGGT | 60 | * | 189 |
| | Reverse | TGCTCCCTGTACTGGTGGATGT | | | |
| MRAS | Forward | TGGGCCATCTTGGACGTTCTGG | 60 | * | 172 |
| | Reverse | CCCTGTCTTTGACGCGCAGG | | | |
| GAPDH | Forward | CAGCCTCAAGATCATCAGCA | 60 | * | 106 |
| | Reverse | TGTGGTCATGAGTCCTTCCA | | | |
| ORF Amplification/ Sequencing | | | | | |
| NRAS | Forward | ATGACTGAGTACAACTGGTGGT | 55 | 2 | 570 |
| | Reverse | TTACATCACCACACATGGCAATC | | | |
| KRAS | Forward | ATGACTGAATATAAACTTGTGGT | 60 | 2 | 567 |
| | Reverse | TTACATAATTACACACTTGTCT | | | |
| HRAS | Forward | ATGACGGAATATAAGCTGGTGGT | 60 | 2 | 570 |
| | Reverse | TCAGGAGAGCACACACTTGCAGC | | | |
| MRAS | Forward | ATGGCAACCAGCGCCGTCCCA | 60 | 2 | 627 |
| | Reverse | TCACAAGATCACACATTGCAGTTTG | | | |
| ORF Internal Sequencing | | | | | |
| NRAS | Forward | AACCTCAGCCAAGACCAGAC | | | |
| | Reverse | CCAGCTGTATCCAGTATGTC | | | |
| KRAS | Forward | GACAAGACAGGGTGTGATG | | | |
| | Reverse | CTGCTGTGTCGAGAATATCC | | | |
| HRAS | Forward | ATCGAGACCTCGCCAAGAC | | | |
| | Reverse | ATGTCCAACAGGCACGTCTC | | | |
| MRAS | Forward | TGGGCCATCTTGGACGTTCTGG | | | |
| NRAS Nested PCR | | | | | |
| External | Forward | ATGGAGCTTGAGGTTCTTG | 55 | 2 | 653 |
| | Reverse | GTCAGTGCAGCTTGAAAGTG | | | |
| Internal | Forward | ATGACTGAGTACAACTGGTGGT | 55 | 2 | 570 |
| | Reverse | TTACATCACCACACATGGCAATC | | | |
| NRAS Genomic | | | | | |
| | Forward | GAACCAATGGAAGGTCACA | 55 | 2 | 301 |
| | Reverse | TGGGTAAGATGATCCGACA | | | |

* RT² SYBR® Green qPCR Master Mixes.