**Supplementary Methods**

**MET mutational screening**

Using genomic DNA, multiplex PCR for the regions of interest was performed with an Amplicon length between 60 and 100 base pairs. After treatment with shrimp alkaline phosphatase to dephosphorylate any remaining dNTPs, reaction took place upon addition of a mixture of all four dNTPs to the extension primers already bound directly adjacent to the mutations. The product of reactions was desalted and loaded on a chip for MALDI-TOF mass spectrometry. Mass peaks for extended and unextended primers were analyzed in order to calculate allelic frequency of mutated variants. FFPE tissues for this approach were from 223 different patients: 191 primary tumors and 209 LNMs. 177 pairs of samples were patient-matched. The mutations screened and primer sequences used are shown in the table below.

|  |  |  |  |
| --- | --- | --- | --- |
| **CDS** | **Protein** | **CDS\_POS** | **Primer sequences** |
| c.504G>T | p.E168D | 504 | AGCAGCAAAGCCAATTTATCAGGAGGTGTTTGGAAAGATAACATCAACATGGCTCTAGTTGTCGACACCTACTATGATGATCAACTCATTAGCTGTGGCAGCGTCAACAGAGGGACCTGCCAGCGACATGTCTTTCCCCACAATCATACTGCTGACATACAGTCGGAGGTTCACTGCATATTCTCCCCACAGATAGAAGA[G/T]CCCAGCCAGTGTCCTGACTGTGTGGTGAGCGCCCTGGGAGCCAAAGTCCTTTCATCTGTAAAGGACCGGTTCATCAACTTCTTTGTAGGCAATACCATAAATTCTTCTTATTTCCCAGATCATCCATTGCATTCGATATCAGTGAGAAGGCTAAAGGAAACGAAAGATGGTTTTATGTTTTTGACGGACCAGTCCTACAT |
| c.503A>G | p.E168G | 503 | CAGCAGCAAAGCCAATTTATCAGGAGGTGTTTGGAAAGATAACATCAACATGGCTCTAGTTGTCGACACCTACTATGATGATCAACTCATTAGCTGTGGCAGCGTCAACAGAGGGACCTGCCAGCGACATGTCTTTCCCCACAATCATACTGCTGACATACAGTCGGAGGTTCACTGCATATTCTCCCCACAGATAGAAG[A/G]GCCCAGCCAGTGTCCTGACTGTGTGGTGAGCGCCCTGGGAGCCAAAGTCCTTTCATCTGTAAAGGACCGGTTCATCAACTTCTTTGTAGGCAATACCATAAATTCTTCTTATTTCCCAGATCATCCATTGCATTCGATATCAGTGAGAAGGCTAAAGGAAACGAAAGATGGTTTTATGTTTTTGACGGACCAGTCCTACA |
| c.1124A>G | p.N375S | 1124 | CACAAAGAAGGAAGTGTTTAATATACTTCAGGCTGCGTATGTCAGCAAGCCTGGGGCCCAGCTTGCTAGACAAATAGGAGCCAGCCTGAATGATGACATTCTTTTCGGGGTGTTCGCACAAAGCAAGCCAGATTCTGCCGAACCAATGGATCGATCTGCCATGTGTGCATTCCCTATCAAATATGTCAACGACTTCTTCA[A/G]CAAGATCGTCAACAAAAACAATGTGAGATGTCTCCAGCATTTTTACGGACCCAATCATGAGCACTGCTTTAATAGGGTAAGTCACATCAGTTCCCCACTTATAAACTGTGAGGTATAAATTAGAAATAAGTATCAGTCTCAAAAAGAATATCCAGGGCTTCTTTTGTGCTTTGTAAATGGTGTTTATCCAAAATAGTTGC |
| c.3804G>A | p.M1268I | 3804 | CACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGAT[G/A]GCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAATTCCAGTTTTCTTCATATGTAAAAATGGACTTGTCTGATACGTACAC |
| c.3803T>C | p.M1268T | 3803 | CCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGA[T/C]GGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAATTCCAGTTTTCTTCATATGTAAAAATGGACTTGTCTGATACGTACA |
| c.3802A>G | p.M1268V | 3802 | GCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGG[A/G]TGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAATTCCAGTTTTCTTCATATGTAAAAATGGACTTGTCTGATACGTAC |
| c.3785A>G | p.K1262R | 3785 | GGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAA[A/G]GCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAATTCCAGTTTTCTTCATATGTAAAAATGG |
| c.3784A>G | p.K1262E | 3784 | TGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCA[A/G]AGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAATTCCAGTTTTCTTCATATGTAAAAATG |
| c.3757T>G | p.Y1253D | 3757 | CAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATAC[T/G]ATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAA |
| c.3757T>C | p.Y1253H | 3757 | CAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATAC[T/C]ATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAA |
| c.3757T>A | p.Y1253N | 3757 | CAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATAC[T/A]ATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAATTCAACACCACCAA |
| c.3744T>C | p.Y1248Y | 3744 | AGTCAATAGAGGCCAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGTA[T/C]GATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAAT |
| c.3743A>G | p.Y1248C | 3743 | TAGTCAATAGAGGCCAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATGT[A/G]TGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAAA |
| c.3742T>C | p.Y1248H | 3742 | TTAGTCAATAGAGGCCAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGACATG[T/C]ATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAAAAAA |
| c.3738C>T | p.D1246D | 3738 | AGTGTTAGTCAATAGAGGCCAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAGA[C/T]ATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTAA |
| c.3737A>G | p.D1246G | 3737 | TAGTGTTAGTCAATAGAGGCCAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGAG[A/G]CATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTTA |
| c.3736G>C | p.D1246H | 3736 | TTAGTGTTAGTCAATAGAGGCCAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAGGTTGCTGATTTTGGTCTTGCCAGA[G/C]ACATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGAAGTTTTATCACTACTTAATTTTTT |
| c.3712G>A | p.V1238I | 3712 | TTTATTCAAGAATTCTGTTGTAATTTAGTGTTAGTCAATAGAGGCCAGATGAAATACTTCCTTCAGAAGTTATGGATTTCAAATACTGAAGCCACTTGTTTAATCTGTAGATATTCAGCATCATTGTAAATTATTCTATTTCAGCCACGGGTAATAATTTTTGTCCTTTCTGTAGGCTGGATGAAAAATTCACAGTCAAG[G/A]TTGCTGATTTTGGTCTTGCCAGAGACATGTATGATAAAGAATACTATAGTGTACACAACAAAACAGGTGCAAAGCTGCCAGTGAAGTGGATGGCTTTGGAAAGTCTGCAAACTCAAAAGTTTACCACCAAGTCAGATGTGGTAATGTATTGGTTATCTCTGAGTTTCTCCTCTTTTACTTTCATATCCAACTTTTTTTGA |
| c.3638T>C | p.L1213P | 3638 | ATTACAGGCTTGAGCCATTAAGACCAAACTAATTTTTGAGACAAGATAATTTTTTATAAATAAATATTTCAGAATTCTAAGGTCAAAATTAGAACAGTAGATGCTTAGTTTATGCTTTTCTAACTCTCTTTGACTGCAGAATCCAACTGTAAAAGATCTTATTGGCTTTGGTCTTCAAGTAGCCAAAGGCATGAAATATC[T/C]TGCAAGCAAAAAGTTTGTCCACAGAGACTTGGCTGCAAGAAACTGTATGTAAGTATCAGAATCTCTGTGCCACAATCCAAATTAAGTGACAAGGAGGAATCTGTTTCCCACTGTTCAATGCTAGTTAAGCTGTTTTCTCTTCTTATGCAAAAGTCCTTTATTTCTGTTACAATCTTAAATCGATGTGTAAGCCCTGGGGA |
| c.3637C>G | p.L1213V | 3637 | GATTACAGGCTTGAGCCATTAAGACCAAACTAATTTTTGAGACAAGATAATTTTTTATAAATAAATATTTCAGAATTCTAAGGTCAAAATTAGAACAGTAGATGCTTAGTTTATGCTTTTCTAACTCTCTTTGACTGCAGAATCCAACTGTAAAAGATCTTATTGGCTTTGGTCTTCAAGTAGCCAAAGGCATGAAATAT[C/G]TTGCAAGCAAAAAGTTTGTCCACAGAGACTTGGCTGCAAGAAACTGTATGTAAGTATCAGAATCTCTGTGCCACAATCCAAATTAAGTGACAAGGAGGAATCTGTTTCCCACTGTTCAATGCTAGTTAAGCTGTTTTCTCTTCTTATGCAAAAGTCCTTTATTTCTGTTACAATCTTAAATCGATGTGTAAGCCCTGGGG |
| c.3573T>C | p.T1191T | 3573 | TCTCCACCACTGGATTTCTCAGGAATCACTGACATAGGAGAAGTTTCCCAATTTCTGACCGAGGGAATCATCATGAAAGATTTTAGTCATCCCAATGTCCTCTCGCTCCTGGGAATCTGCCTGCGAAGTGAAGGGTCTCCGCTGGTGGTCCTACCATACATGAAACATGGAGATCTTCGAAATTTCATTCGAAATGAGAC[T/C]CATGTAAGTTGACTGCCAAGCTTACTAACTGGCAAACTAGCTGTAAGCCAGCCATCCCTTCAAAATAGGCCTGCTCTGAGTCTTTAAAAAGCTAGTAGCCAAAGATGCACATTTAAAATGTTAGCATCATTCAAATGCACCTCAAAGTCTTCTATCCTGGTGGGAAATAGTGACACCTGGAAGGGTTTCCTGGAGCAATG |
| c.3572C>T | p.T1191I | 3572 | GTCTCCACCACTGGATTTCTCAGGAATCACTGACATAGGAGAAGTTTCCCAATTTCTGACCGAGGGAATCATCATGAAAGATTTTAGTCATCCCAATGTCCTCTCGCTCCTGGGAATCTGCCTGCGAAGTGAAGGGTCTCCGCTGGTGGTCCTACCATACATGAAACATGGAGATCTTCGAAATTTCATTCGAAATGAGA[C/T]TCATGTAAGTTGACTGCCAAGCTTACTAACTGGCAAACTAGCTGTAAGCCAGCCATCCCTTCAAAATAGGCCTGCTCTGAGTCTTTAAAAAGCTAGTAGCCAAAGATGCACATTTAAAATGTTAGCATCATTCAAATGCACCTCAAAGTCTTCTATCCTGGTGGGAAATAGTGACACCTGGAAGGGTTTCCTGGAGCAAT |
| c.3446T>C | p.M1149T | 3446 | GTGATGTAAAAAGAACACTCTGCAGTCAAACCCTCAGGACAAGATGCTAACTGTGTGGTTTACCATTTCATTGCTCTTCCTATCTAAATTTGACAAAAGTATTCACTGTTCCATAATGAAGTTAATGTCTCCACCACTGGATTTCTCAGGAATCACTGACATAGGAGAAGTTTCCCAATTTCTGACCGAGGGAATCATCA[T/C]GAAAGATTTTAGTCATCCCAATGTCCTCTCGCTCCTGGGAATCTGCCTGCGAAGTGAAGGGTCTCCGCTGGTGGTCCTACCATACATGAAACATGGAGATCTTCGAAATTTCATTCGAAATGAGACTCATGTAAGTTGACTGCCAAGCTTACTAACTGGCAAACTAGCTGTAAGCCAGCCATCCCTTCAAAATAGGCCTG |
| c.3411A>G | p.G1137G | 3411 | AAGGCACAGTGCACGGTGGCATCATTCACTCAGATGTGATGTAAAAAGAACACTCTGCAGTCAAACCCTCAGGACAAGATGCTAACTGTGTGGTTTACCATTTCATTGCTCTTCCTATCTAAATTTGACAAAAGTATTCACTGTTCCATAATGAAGTTAATGTCTCCACCACTGGATTTCTCAGGAATCACTGACATAGG[A/G]GAAGTTTCCCAATTTCTGACCGAGGGAATCATCATGAAAGATTTTAGTCATCCCAATGTCCTCTCGCTCCTGGGAATCTGCCTGCGAAGTGAAGGGTCTCCGCTGGTGGTCCTACCATACATGAAACATGGAGATCTTCGAAATTTCATTCGAAATGAGACTCATGTAAGTTGACTGCCAAGCTTACTAACTGGCAAACT |
| c.3410G>T | p.G1137V | 3410 | GAAGGCACAGTGCACGGTGGCATCATTCACTCAGATGTGATGTAAAAAGAACACTCTGCAGTCAAACCCTCAGGACAAGATGCTAACTGTGTGGTTTACCATTTCATTGCTCTTCCTATCTAAATTTGACAAAAGTATTCACTGTTCCATAATGAAGTTAATGTCTCCACCACTGGATTTCTCAGGAATCACTGACATAG[G/T]AGAAGTTTCCCAATTTCTGACCGAGGGAATCATCATGAAAGATTTTAGTCATCCCAATGTCCTCTCGCTCCTGGGAATCTGCCTGCGAAGTGAAGGGTCTCCGCTGGTGGTCCTACCATACATGAAACATGGAGATCTTCGAAATTTCATTCGAAATGAGACTCATGTAAGTTGACTGCCAAGCTTACTAACTGGCAAAC |
| c.3371A>G | p.H1124R | 3371 | TATTGTTAAAAGTATTTTTTAAATGTACTCTTTTGCTGTATAGAAAGAAGAAAGAATAAAATGAAGCTCATAAAGGGTTTGATAAATAATTATTTCATAATTAAATGTTACGCAGTGCTAACCAAGTTCTTTCTTTTGCACAGGGCATTTTGGTTGTGTATATCATGGGACTTTGTTGGACAATGATGGCAAGAAAATTC[A/G]CTGTGCTGTGAAATCCTTGAACAGTAAGTGGCATTTTATTTAACCATGGAGTATACTTTTGTGGTTTGCAACCTAATAAATAGCTTATAATAAAACGTTGATTTACACTTTCCCCTTGTGGAAAAATCAGCTACCACTGAAATTATGGGCCTAATCCTGAAAATTTGTTTTGTTCTAGACTTTTATTGAACTACTTCCCC |
| c.3370C>T | p.H1124Y | 3370 | GTATTGTTAAAAGTATTTTTTAAATGTACTCTTTTGCTGTATAGAAAGAAGAAAGAATAAAATGAAGCTCATAAAGGGTTTGATAAATAATTATTTCATAATTAAATGTTACGCAGTGCTAACCAAGTTCTTTCTTTTGCACAGGGCATTTTGGTTGTGTATATCATGGGACTTTGTTGGACAATGATGGCAAGAAAATT[C/T]ACTGTGCTGTGAAATCCTTGAACAGTAAGTGGCATTTTATTTAACCATGGAGTATACTTTTGTGGTTTGCAACCTAATAAATAGCTTATAATAAAACGTTGATTTACACTTTCCCCTTGTGGAAAAATCAGCTACCACTGAAATTATGGGCCTAATCCTGAAAATTTGTTTTGTTCTAGACTTTTATTGAACTACTTCCC |
| c.3370C>G | p.H1124D | 3370 | GTATTGTTAAAAGTATTTTTTAAATGTACTCTTTTGCTGTATAGAAAGAAGAAAGAATAAAATGAAGCTCATAAAGGGTTTGATAAATAATTATTTCATAATTAAATGTTACGCAGTGCTAACCAAGTTCTTTCTTTTGCACAGGGCATTTTGGTTGTGTATATCATGGGACTTTGTTGGACAATGATGGCAAGAAAATT[C/G]ACTGTGCTGTGAAATCCTTGAACAGTAAGTGGCATTTTATTTAACCATGGAGTATACTTTTGTGGTTTGCAACCTAATAAATAGCTTATAATAAAACGTTGATTTACACTTTCCCCTTGTGGAAAAATCAGCTACCACTGAAATTATGGGCCTAATCCTGAAAATTTGTTTTGTTCTAGACTTTTATTGAACTACTTCCC |
| c.3336T>C | p.H1112H | 3336 | ATATATTTACAAACACACCTACGTACCTATAGTGGTATTGTTAAAAGTATTTTTTAAATGTACTCTTTTGCTGTATAGAAAGAAGAAAGAATAAAATGAAGCTCATAAAGGGTTTGATAAATAATTATTTCATAATTAAATGTTACGCAGTGCTAACCAAGTTCTTTCTTTTGCACAGGGCATTTTGGTTGTGTATATCA[T/C]GGGACTTTGTTGGACAATGATGGCAAGAAAATTCACTGTGCTGTGAAATCCTTGAACAGTAAGTGGCATTTTATTTAACCATGGAGTATACTTTTGTGGTTTGCAACCTAATAAATAGCTTATAATAAAACGTTGATTTACACTTTCCCCTTGTGGAAAAATCAGCTACCACTGAAATTATGGGCCTAATCCTGAAAATT |
| c.3335A>T | p.H1112L | 3335 | TATATATTTACAAACACACCTACGTACCTATAGTGGTATTGTTAAAAGTATTTTTTAAATGTACTCTTTTGCTGTATAGAAAGAAGAAAGAATAAAATGAAGCTCATAAAGGGTTTGATAAATAATTATTTCATAATTAAATGTTACGCAGTGCTAACCAAGTTCTTTCTTTTGCACAGGGCATTTTGGTTGTGTATATC[A/T]TGGGACTTTGTTGGACAATGATGGCAAGAAAATTCACTGTGCTGTGAAATCCTTGAACAGTAAGTGGCATTTTATTTAACCATGGAGTATACTTTTGTGGTTTGCAACCTAATAAATAGCTTATAATAAAACGTTGATTTACACTTTCCCCTTGTGGAAAAATCAGCTACCACTGAAATTATGGGCCTAATCCTGAAAAT |
| c.3335A>G | p.H1112R | 3335 | TATATATTTACAAACACACCTACGTACCTATAGTGGTATTGTTAAAAGTATTTTTTAAATGTACTCTTTTGCTGTATAGAAAGAAGAAAGAATAAAATGAAGCTCATAAAGGGTTTGATAAATAATTATTTCATAATTAAATGTTACGCAGTGCTAACCAAGTTCTTTCTTTTGCACAGGGCATTTTGGTTGTGTATATC[A/G]TGGGACTTTGTTGGACAATGATGGCAAGAAAATTCACTGTGCTGTGAAATCCTTGAACAGTAAGTGGCATTTTATTTAACCATGGAGTATACTTTTGTGGTTTGCAACCTAATAAATAGCTTATAATAAAACGTTGATTTACACTTTCCCCTTGTGGAAAAATCAGCTACCACTGAAATTATGGGCCTAATCCTGAAAAT |
| c.3334C>T | p.H1112Y | 3334 | ATATATATTTACAAACACACCTACGTACCTATAGTGGTATTGTTAAAAGTATTTTTTAAATGTACTCTTTTGCTGTATAGAAAGAAGAAAGAATAAAATGAAGCTCATAAAGGGTTTGATAAATAATTATTTCATAATTAAATGTTACGCAGTGCTAACCAAGTTCTTTCTTTTGCACAGGGCATTTTGGTTGTGTATAT[C/T]ATGGGACTTTGTTGGACAATGATGGCAAGAAAATTCACTGTGCTGTGAAATCCTTGAACAGTAAGTGGCATTTTATTTAACCATGGAGTATACTTTTGTGGTTTGCAACCTAATAAATAGCTTATAATAAAACGTTGATTTACACTTTCCCCTTGTGGAAAAATCAGCTACCACTGAAATTATGGGCCTAATCCTGAAAA |
| c.3029C>T | p.T1010I | 3029 | GTGCTGTCTTATATGTAGTCCATAAAACCCATGAGTTCTGGGCACTGGGTCAAAGTCTCCTGGGGCCCATGATAGCCGTCTTTAACAAGCTCTTTCTTTCTCTCTGTTTTAAGATCTGGGCAGTGAATTAGTTCGCTACGATGCAAGAGTACACACTCCTCATTTGGATAGGCTTGTAAGTGCCCGAAGTGTAAGCCCAA[C/T]TACAGAAATGGTTTCAAATGAATCTGTAGACTACCGAGCTACTTTTCCAGAAGGTATATTTCAGTTTATTGTTCTGAGAAATACCTATACATATACCTCAGTGGGTTGTGACATTGTTGTTTATTTTTGGTTTTGCATTTATATTTTTATAAAAACCTAAAGGAAGTATTTACCTCTGCCAAGTAAGTATTTGACACAAA |
| c.2962C>T | p.R988C | 2962 | CTGTTCATTTTTAGAAGTTACCTTAAGAACACAGTCATTACAGTTTAAGATTGTCGTCGATTCTTGTGTGCTGTCTTATATGTAGTCCATAAAACCCATGAGTTCTGGGCACTGGGTCAAAGTCTCCTGGGGCCCATGATAGCCGTCTTTAACAAGCTCTTTCTTTCTCTCTGTTTTAAGATCTGGGCAGTGAATTAGTT[C/T]GCTACGATGCAAGAGTACACACTCCTCATTTGGATAGGCTTGTAAGTGCCCGAAGTGTAAGCCCAACTACAGAAATGGTTTCAAATGAATCTGTAGACTACCGAGCTACTTTTCCAGAAGGTATATTTCAGTTTATTGTTCTGAGAAATACCTATACATATACCTCAGTGGGTTGTGACATTGTTGTTTATTTTTGGTTT |

Abbreviations:CDS – Coding DNA Sequence; CDS-POS – Coding DNA Sequence Position.

**Cell lines**

The following table provides detailed information on the cell lines used in the present study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Origin** | **Age/Gender patient** | **Characteristics** | **Remarks** |
| FaDu | Pharynx | 56/Male | Adherent |  |
| Detroit-562 | Metastatic pleural effusion from a pharyngeal carcinoma | NA/Female | Adherent |  |
| SQ-20B | Larynx | NA/NA | Adherent | Derived following radiotherapy failure |
| SCC-61 | Oral cavity (tongue) | NA/Male | Adherent |  |
| HN5 | Oral cavity (tongue) | 73/Male | Adherent |  |
| UM-SCC-10A | Larynx (true vocal cord) | NA/Male | Adherent | Patient-matched with 10B |
| UM-SCC-10B | Lymph node metastasis | NA/Male | Adherent | Patient-matched with 10A |
| UM-SCC-81A | Larynx (false vocal cord) | NA/Male | Adherent | Patient-matched with 81B |
| UM-SCC-81B | Oropharynx (tonsil) | NA/Male | Adherent | Patient-matched with 81A |

Abbreviations: NA-not available

**Phospho-histone H3 immunohistochemistry**

Sections of 3-4 µm of FFPE tissue were mounted on positively charged SuperFrost®Plus slides (Menzel-Gläser, Braunschweig, Germany). The immunohistochemical staining procedure starting with the deparaffinization of sections was done with the staining instrument Discovery® XT (Ventana Medical Systems, Inc., Tucson, USA). After deparaffinization sections were heated for epitope retrieval in Tris-EDTA buffer pH 8.0. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide (part of detection kit, Ventana Medical Systems). Sections were incubated with in PBS diluted primary antibody pHistone H3 (Ser10) clone E173, Abcam). Finally, the horseradish peroxidase (HRP) conjugated polymers of the detection kit catalyses the 3,3’-diaminobenzidine tetrahydorchloride (DAB)/H2O2 reaction to produce an insoluble dark brown precipitate that can be visualized. Sections were counterstained with hematoxylin. Slides were washed in tap water, dehydrated, and mounted with glass coverslips in permanent mounting media Entellan® Neu (VWR, Germany).

**Preparation and culture of organotypic tissue cultures (OTCs) and TMA construction**

For the purposes of this study we considered only carcinomas of the oral cavity, the oropharynx, and the larynx. Slices were generated with a Vibratome VT1200 (Leica Microsystems) within 30 minutes following surgical resection (1). Slices were maintained in DMEM-F12 medium supplemented with 10% FCS, and antibiotic/antimycotic. OTCs were incubated in a humidified 5% CO2 atmosphere at 37°C. After overnight culture, the medium of the wells was replaced with fresh medium containing either tepotinib (1 µM) or vehicle (DMSO), and irradiation (6 Gy) took place after 6 hours. Medium with drug was replaced daily.

Owing to the small size of OTCs, tissue arrays were manually generated using the Arraymold Kit E (cores of 1.5 mm, IHCWORLD). A fragment of uncultured tissue (baseline reference) and the treated OTCs were fixed in 4% buffered formalin overnight and then vertically embedded in paraffin. At least two punches per sample were obtained. IHC was performed as described in Material and Methods in the main manuscript body.

**Single cell mass cytometry analysis**

To gain further insights into potential mechanisms which dictate MET inhibition (METi) radiosensitization, we selected the METi radiosensitized cell line UM-SCC-10B and the non METi sensitized cell line HN5. These cell lines were cultured and left to attach overnight. After 16 hours of treatment with either vehicle or tepotinib (100 nM), cells were irradiated (6 Gy) and lysed after 2 hours as previously described in order to perform single cell mass cytometry analysis (CyTOF)(2). Two biological replicates each containing three technical replicates were performed.

A panel of relevant proteins was designed, including frequently altered proteins and pathways, based on the report of The Cancer Genome Atlas (3). Included in the panel were receptors tyrosine kinase commonly featuring gene amplification and some of which are currently targets in clinical practice (EGFR, pEGFR, HER2, EPHA2, and DDR2)(3, 4). Moreover, aberrant activation of and crosstalk between receptors tyrosine kinase have been shown to be a potential resistance mechanisms to both classical chemotherapy and targeted therapy (5-8). Amplification and mutations of members of the PI3K pathway were among the most common in HNSCC. We included phosphorylated AKT (pAKT) and S6 (pS6) as a surrogate marker of PI3K pathway activation status (3, 9, 10). Phosphorylated ERK (pERK) was used to evaluate MAPK activation. HES1 and YAP1 were used as readouts for NOTCH1 and Hippo pathway activation, respectively, since NOTCH1 inactivating mutations have been shown to be relatively common in HNSCC, as well as mutations affecting components of the Hippo pathway (3, 11, 12). Finally, phosphorylated p53 was used both to monitor response to IR and due to the fact that *TP53* mutations are the commonest in HNSCC (3).

Metal-labeled antibodies were used for target detection as previously described (13). The raw data obtained from the CyTOF analyzer was put in Cytobank (Cytobank, Inc.) and live cells were gated and checked for signal stability over time. Data robustness was assessed by comparing both experimental and technical replicates for both cell lines with viSNE plots made in Cytobank. Having confirmed data robustness, all measured CyTOF markers signals were transformed by arcsinh with a cofactor 5 according to common practice in the analysis of CyTOF data (14). The transformed expression values were combined with the results obtained from live-dead assays, using logistic regression to model the cell death probability. We finally report the death odds ratio (OR) between the 10th and 90th percentile of the transformed protein expression values. An OR value higher than 1 consequently indicates that the protein’s increased level is associated with a higher probability of cell death. Confidence intervals were obtained using nonparametric bootstrap of the input data with 2000 realizations.

**REFERENCES**

1. Vaira V, Fedele G, Pyne S, Fasoli E, Zadra G, Bailey D, et al. Preclinical model of organotypic culture for pharmacodynamic profiling of human tumors. Proceedings of the National Academy of Sciences of the United States of America. 2010;107:8352-6.

2. Brodie TM, Tosevski V. High-Dimensional Single-Cell Analysis with Mass Cytometry. Current protocols in immunology. 2017;118:5 11 1-5 25.

3. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517:576-82.

4. Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU, Sur RK, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. The Lancet Oncology. 2010;11:21-8.

5. Sun S, Wang Z. Head neck squamous cell carcinoma c-Met(+) cells display cancer stem cell properties and are responsible for cisplatin-resistance and metastasis. International journal of cancer Journal international du cancer. 2011;129:2337-48.

6. Seiwert TY, Jagadeeswaran R, Faoro L, Janamanchi V, Nallasura V, El Dinali M, et al. The MET receptor tyrosine kinase is a potential novel therapeutic target for head and neck squamous cell carcinoma. Cancer research. 2009;69:3021-31.

7. Singleton KR, Kim J, Hinz TK, Marek LA, Casas-Selves M, Hatheway C, et al. A receptor tyrosine kinase network composed of fibroblast growth factor receptors, epidermal growth factor receptor, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, and hepatocyte growth factor receptor drives growth and survival of head and neck squamous carcinoma cell lines. Molecular pharmacology. 2013;83:882-93.

8. Xu H, Stabile LP, Gubish CT, Gooding WE, Grandis JR, Siegfried JM. Dual blockade of EGFR and c-Met abrogates redundant signaling and proliferation in head and neck carcinoma cells. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011;17:4425-38.

9. Lui VW, Hedberg ML, Li H, Vangara BS, Pendleton K, Zeng Y, et al. Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. Cancer discovery. 2013;3:761-9.

10. Hammerman PS, Hayes DN, Grandis JR. Therapeutic insights from genomic studies of head and neck squamous cell carcinomas. Cancer discovery. 2015;5:239-44.

11. Sun W, Gaykalova DA, Ochs MF, Mambo E, Arnaoutakis D, Liu Y, et al. Activation of the NOTCH pathway in head and neck cancer. Cancer research. 2014;74:1091-104.

12. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333:1157-60.

13. Brodie TM, Tosevski V, Medova M. OMIP-045: Characterizing human head and neck tumors and cancer cell lines with mass cytometry. Cytometry Part A : the journal of the International Society for Analytical Cytology. 2018;93:406-10.

14. Bruggner RV, Bodenmiller B, Dill DL, Tibshirani RJ, Nolan GP. Automated identification of stratifying signatures in cellular subpopulations. Proceedings of the National Academy of Sciences of the United States of America. 2014;111:E2770-7.