**Minimal residual disease detection by next-generation sequencing in multiple myeloma: A comparison with real-time quantitative PCR**

Running title: Residual disease detection in myeloma

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**Table S1. Results of clonality identification by Sanger sequencing and next-generation sequencing.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Patient ID | Clonality detected by Sanger sequencing  |  | Clonality detected by NGS | Type of clonal sequence |
| Clonal sequence | PCR |  | V-gene | J-gene | CDR3 identical to Sanger sequencing |
| Clonality done by both Sanger sequencing and NGS  |
| 1 | VH3.9(0)-1-7-(2)D5.12(4)-2-JH6 | IGH VDJ |  | IGHV3-9\_01 | IGHJ6\_03 | Yes | Productive |
|  | VH3.13(10)-5-(7)D3.22(10)-11-(1)JH3 | IGH VDJ |  | IGHV3-13\_01 | IGHJ3\_02 | Yes | Unproductive |
| 2 | VH2.5(2)-6-(2)D1.26(2)-3-(5)JH4 | IGH VDJ |  | IGHV2-5\_09 | IGHJ4\_02 | Yes | Productive |
| 3 | VH3.66(0)-5-(15)D3.16(7)-7-(14)JH6 | IGH VDJ |  | IGHV3-66\_02 | IGHJ6\_02 | Yes | Productive |
| 4 | VH3.21(1)-9-(8)D2.21(3)-3-(5)JH6 | IGH VDJ |  | IGHV3-21\_02 | IGHJ6\_03 | Yes | Productive |
| 5 | VH1.18(4)-9-(1)D3.10(10)-20-(3)JH4 | IGH VDJ |  | IGHV1-69\_02 | IGHJ4\_02 | Yes | Productive |
| 6 | VH3.11(1)-15-(5)D2.21(12)-8-(10)JH6 | IGH VDJ |  | IGHV3-11\_05 | IGHJ6\_03 | Yes | Productive |
| 7 | VH3.20(0)-10-(5)D1.26(3)-8-(4)JH4 | IGH VDJ |  | IGHV3-20\_01 | IGHJ4\_02 | Yes | Productive |
| 8 | VH1.24(2)-(7)D1.1(1)-12-(17)JH5 | IGH VDJ |  | IGHV1-24\_01 | IGHJ5\_02 | Yes | Productive |
| 9 | VH4.61(2)-4-(6)D2.2(12)-20-(8)JH3 | IGH VDJ |  | IGHV4-61\_02 | IGHJ3\_02 | Yes | Productive |
| 10 | VH3.66(0)-0-(0)D3.3(21)-12-(3)JH5 | IGH VDJ |  | IGHV3-66\_02 | IGHJ5\_02 | Yes | Productive |
| 11 | VH3.23(5)-10-(7)D4.23(0)-5-(8)JH5 | IGH VDJ |  | IGHV3-23\_04 | IGHJ5\_02 | Yes | Productive |
| 12 | VH2.5(2)-15-(0)D5.18(3)-2-(11)JH4 | IGH VDJ |  | IGHV2-5\_08 | IGHJ4\_02 | Yes | Productive |
| 13 | VH1.18(0)-1-6-(2)D5.24(2)-13-(19)JH6 | IGH VDJ |  | IGHV1-18\_01 | IGHJ6\_03 | Yes | Productive |
| 14 | VH4.34(3)-14-(16)D2.2(2)-25-(16)JH4 | IGH VDJ |  | IGHV3-33\_01 | IGHJ3\_02 | No | Unproductive (Sanger)Productive (NGS) |
| 15 | VH5.10(1)-15-(3)D3.22(11)-3-(3)JH4 | IGH VDJ |  | IGHV5-10\_01 | IGHJ4\_02 | Yes | Productive |
| 16 | VH1.2(2)-11-(6)D4.11(2)-5-1-(0)JH5 | IGH VDJ |  | IGHV1-2\_02 | IGHJ5\_02 | Yes | Productive |
| 17 | VH4.61(2)-4-(1)D4.23(2)-1-(8)JH4 | IGH VDJ |  | IGHV4-61\_01 | IGHJ4\_02 | Yes | Productive |
| 18 | VH1.3(2)-8-(0)D6.19(0)-3-(7)JH5 | IGH VDJ |  | IGHV1-3\_01 | IGHJ5\_02 | Yes | Productive |
| 19 | VH4.61(4)-8-4-D5.18(7)-11-(6)JH4 | IGH VDJ |  | IGHV4-61\_03 | IGHJ4\_02 | Yes | Productive |
| 20 | VH3.30(3)-3-(0)D3.10(9)-4-(3)JH4 | IGH VDJ |  | IGHV3-30\_04 | IGHJ4\_02 | Yes | Productive |
| 21 | VH4.4(2)-13-(7)D6.6(0)-3-(1)JH3 | IGH VDJ |  | IGHV4-4\_02 | IGHJ3\_01 | Yes | Productive |
| 22 | VH4.39(0)-11-(2)D3.22(9)-8-(10)JH4 | IGH VDJ |  | IGHV4-39\_07 | IGHJ4\_02 | Yes | Productive |
| 23 | VH5.51(3)-12-(4)D3.10(9)-4-(8)JH4 | IGH VDJ |  | IGHV5-51\_03 | IGHJ4\_02 | Yes | Productive |
| 24 | VH3.23(0)-2-(7)D3.3(12)-0-(5)JH3 | IGH VDJ |  | IGHV3-23\_04 | IGHJ3\_01 | Yes | Productive |
| 25 | VH1.68(6)-4-(5)D6.13(2)-11-(14)JH6 | IGH VDJ |  | IGHV1-68\_02 | IGHJ6\_02 | Yes | Unproductive |
|  |  |  |  | IGHV3-20\_01 | IGHJ4\_02 |  | Productive |
| 26 | VH3.30(2)-7-(2)D3.3(15)-11-(8)JH4 | IGH VDJ |  | IGHV3-30-3\_01 | IGHJ4\_02 | Yes | productive |
| 27 | VH3.53(2)-3-(9)D3.9(12)-5-(11)JH6 | IGH VDJ |  | IGHV3-53\_02 | IGHJ6\_02 | Yes | Productive |
| 28 | VH4.39 (1)-(7)D5.12(5)-19-(12)JH6 | IGH VDJ |  | IGHV4-39\_07 | IGHJ6\_02 | Yes | Productive |
| 29 | VH3.30(0)-4-(8)D5.12(9)-4-(6)JH4 | IGH VDJ |  | IGHV3-30\_04 | IGHJ4\_02 | Yes | Productive |
| 30 | VH2.70(5)-9-(12)D3.16(14)-3-(15)JH6 | IGH VDJ |  | IGHV2-70\_12 | IGHJ6\_02 | Yes | Productive |
| 31 | D6.25(0)-5-(10)JH4b | IGH DJ |  | IGKV1-5\_03 | IGKJ2\_01 | N/A | N/A (Sanger) Productive (NGS) |
| 32 | D4.17(3)-6-(0)JH4b | IGH DJ |  | IGKV1-5\_03 | IGKJ1\_01 | N/A | N/A (Sanger) Productive (NGS) |
| 33 | D2.2(11)- 12-(6)JH5b | IGH DJ |  | IGKV3-11\_01 | IGKJ4\_01 | N/A | N/A (Sanger) Productive (NGS) |
| 34 | D6.25(3)-18-(11)JH4b | IGH DJ |  | IGKV2-29\_02 | IGKJ4\_01 | N/A | N/A (Sanger) Productive (NGS) |
|  |  |  |  | IGKV1-6\_01 | IGKJ1\_01 |  | N/A (Sanger) Productive (NGS) |
| 35 | D2.2(19)-9-(6)JH4b | IGH DJ |  | IGKV2D-29\_01 | IGKJ2\_03 | N/A | N/A (Sanger) Unproductive (NGS) |
| 36 | D2.2(6)-6-(2)JH5b | IGH DJ |  | No clonality |  |  |  |
| 37 | D6.25(3)-20-(12)JH4b | IGH DJ |  | No clonality |  |  |  |
| 38 | D1.26(4)-7-(0)JH3b | IGH DJ |  | No clonality |  |  |  |
| 39 | D2.2(4)-8-(15)JH6b | IGH DJ |  | No clonality |  |  |  |
| 40 | D6.13(2)-0-(9)JH4b | IGH DJ |  | No clonality |  |  |  |
| 41 | D3.9(17)-12-(2)JH5b | IGH DJ |  | No clonality |  |  |  |
| 42 | D1.26(2)-11-(5)JH5b | IGH DJ |  | No clonality |  |  |  |
| 43 | VK1.39(1)-2-(8)JK1 | IGK |  | IGKV1D-39\_01 | IGKJ1\_01 | Yes | Unproductive |
|  |  |  |  | IGKV1D-33\_01 | IGKJ4\_01 |  | Productive |
| 44 | VK1.39(0)-0-(0)JK5 | IGK |  | IGKV1D-39\_01 | IGKJ5\_01 | Yes | Productive |
|  |  |  |  | IGKV1D-33\_01 | IGKJ4\_02 |  | Unproductive |
| 45 | VK1.39(4)-0-(1)JK1 | IGK |  | IGKV1D-39\_01 | IGKJ1\_01 | Yes | Unproductive |
| 46 | VK1.12(1)-0-(2)JK3 | IGK |  | IGKV3D-20\_01 | Kdel | No | NA (Sanger)N/A (NGS) |
| 47 | VK1.5(8)-6-(2)JK2 | IGK |  | no clonality |  |  | NA (Sanger) |
| 48 | No clonality |  |  | No clonality |  |  |  |
| 49 | No clonality |  |  | No clonality |  |  |  |
| 50 | No clonality |  |  | No clonality |  |  |  |
| 51 | No clonality |  |  | No clonality |  |  |  |
| 52 | No clonality |  |  | No clonality |  |  |  |
| 53 | No clonality |  |  | No clonality |  |  |  |
| Clonality done by Sanger sequencing only |
| 54 | VH3.9(1)-5-(2)D6.6-(0)-13-(15)JH6 | IGH VDJ |  |  |  |  | NA (Sanger) |
| 55 | D2.15(2)-15-(0)JH6  | IGH DJ |  |  |  |  |  |
| Clonality done by NGS only |
| 56 |  |  |  | IGHV3-7\_01 | IGHJ4\_02 |  | Unproductive |
|  |  |  |  | IGHV3-64\_05 | IGHJ4\_02 |  | Productive |
| 57 |  |  |  | IGHV2-70\_01 | IGHJ6\_02 |  | Productive |
| 58 |  |  |  | IGKV4-1\_01 | Kdel |  | N/A |
| 59 |  |  |  | IGHV3-21\_02 | IGHJ4\_02 |  | Productive |
| 60 |  |  |  | IGHV3-23\_04 | IGHJ6\_02 |  | Productive |
| 61 |  |  |  | IGHV3-74\_01 | IGHJ4\_02 |  | Productive |
| 62 |  |  |  | IGHV4-39\_01 | IGHJ5\_02 |  | Productive |
| 63 |  |  |  | IGKV2D-40\_01 | IGKJ4\_01 |  | Unproductive |
| 64 |  |  |  | IGKV2D-26\_01 | Kdel |  | N/A |
| 65 |  |  |  | IGKV1D-33\_01 | IGKJ2\_01 |  | Productive |
| 66 |  |  |  | IGHV4-b\_02 | IGHJ4\_02 |  | Productive |
| 67 |  |  |  | IGHV3-48\_01 | IGHJ6\_02 |  | Productive |
| 68 |  |  |  | IGKV2D-28\_01 | Kdel |  | N/A |
| 69 |  |  |  | IGHV3-30\_07 | IGHJ1\_01 |  | Productive |
| 70 |  |  |  | IGHV2-5\_10 | IGHJ4\_02 |  | Productive |
|  |  |  |  | IGHV4-30-4\_06 | IGHJ6\_03 |  | Unproductive |
| 71 |  |  |  | IGHV4-59\_07 | IGHJ4\_02 |  | Productive |
| 72 |  |  |  | IGHV3-48\_03 | IGHJ4\_02 |  | Productive |
| 73 |  |  |  | IGHV4-39\_03 | IGHJ5\_02 |  | Productive |
| 74 |  |  |  | IGHV2-26\_01 | IGHJ6\_02 |  | Productive |
| 75 |  |  |  | IGHV4-59\_01 | IGHJ6\_02 |  | Productive |
| 76 |  |  |  | IGHV3-74\_03 | IGHJ4\_02 |  | Productive |
| 77 |  |  |  | IGKV5-2\_01 | IGKJ1\_01 |  | Unproductive |
| 78 |  |  |  | IGHV6-1\_02 | IGHJ5\_02 |  | Productive |
| 79 |  |  |  | No clonality |  |  |  |
| 80 |  |  |  | No clonality |  |  |  |

Abbreviations: NGS: next-generation sequencing; Kdel: Kappa deleting element; N/A: not applicable; NA: not available; IGH VDJ: IGH complete VDJ rearrangement; IGH DJ: IGH incomplete DJ rearrangement; IGK: IGK VJ rearrangement in Sanger sequencing, IGK VJ and V-Kdel rearrangement in NGS PCR.

**Table S2. Results of clonality detection by Sanger sequencing in 16 patients who failed clonality identification by next-generation sequencing.**

|  |  |
| --- | --- |
| Sanger sequencing | Number of patients (n) |
| Unsuccessful | 6 |
| Successful | 8 |
| Not assessed | 2 |
| Total | 16 |

**Table S3. Minimal residual disease (MRD) levels measured by next-generation sequencing and allele-specific oligonucleotide real-time quantitative-PCR (ASO RQ-PCR) in follow-up bone marrow samples.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patient ID | Replicate | Sequencing reads (million) | 10-5 control reads | 10-4 control reads (frequency) | MRD reads |  Normalized MRD | Mean MRD | ASO RQ-PCR MRD |
| 1 (FU1) | -1 | 0.8 | 0 | 13128 (1.5x10-2) | 1737 | 0.0013% | 0.0004% | PNQ<10 |
| -2 | 0.9 | 1279 | 8216 (0.9x10-2) | 0 | 0 |
| -3 | 0.5 | 858 | 9344 (2.0x10-2) | 0 | 0 |
| 1 (FU2) | -1 | 2.0 | 11 | 702 (3.5x10-4) | 1021 | 0.016% | 0.010% | PNQ<50 |
| -2 | 1.2 | 250 | 616 (5.2x10-4) | 807 | 0.013% |
| -3 | 1.7 | 0 | 407 (2.4x10-4) | 141 | 0.003% |
| 2 (FU1) | -1 | 1.2 | 0 | 65 (5.5x10-5) | 0 | 0 | Negative | Negative |
| -2 | 1.0 | 11 | 70 (5.5x10-5) | 0 | 0 |
| -3 | 0.9 | 1 | 43 (4.8x10-5) | 0 | 0 |
| 2 (FU2) | -1 | 1.1 | 0 | 369 (3.4x10-4) | 0 | 0 | Negative | Negative |
| -2 | 0.5 | 5 | 0 | 0 | 0 |
| -3 | 0.8 | 48 | 516 (6.7x10-4) | 0 | 0 |
| 3 | -1 | 1.4 | 185 | 208 (1.4x10-4) | 3547 | 0.17% | 0.082% | PNQ<50 |
| -2 | 2.0 | 8 | 730 (3.7x10-4) | 1962 | 0.027% |
| -3 | 1.1 | 5 | 340 (3.2x10-4) | 1674 | 0.049% |
| 4 | -1 | 2.9 | 99 | 111 (3.8x10-5) | 687 | 0.061% | 0.042% | 30 |
| -2 | 1.3 | 9 | 120 (9.3x10-5) | 280 | 0.023% |
| -3 | 2.1 | 7 | 5 (2.3x10-6) | 311 | N/A |
| 6 | -1 | 1.4 | 298 | 729 (5.1x10-4) | 242 | 0.003% | 0.012% | PNQ<10 |
| -2 | 1.8 | 0 | 612 (3.4x10-4) | 1790 | 0.029% |
| -3 | 1.4 | 856 | 1206 (8.9x10-4) | 562 | 0.005% |
| 7 | -1 | 1.5 | 0 | 601 (4.0x10-4) | 18207 | 0.303% | 0.209% | 128/105cells |
| -2 | 1.3 | 0 | 1399 (1.1x10-3) | 21781 | 0.156% |
| -3 | 1.2 | 144 | 1243 (1.0x10-3) | 20949 | 0.169% |
| 8 | -1 | 1.5 | 184 | 101 (6.7x10-5) | 0 | 0 | Negative | Negative |
| -2 | 1.0 | 0 | 280 (2.9x10-4) | 0 | 0 |
| -3 | 1.2 | 83 | 154 (1.3x10-4) | 0 | 0 |
| 9 | -1 | 0.4 | 0 | 12123 (3.4x10-2) | 8757 | 0.007% | 0.005% | 16/105 cells |
| -2 | 0.4 | 464 | 15234 (4.3x10-2) | 10007 | 0.007% |
| -3 | 0.5 | 2187 | 21140 (4.4x10-2) | 4541 | 0.002% |
| 12 | -1 | 1.2 | 108 | 1500 (1.4x10-3) | 1076 | 0.007% | 0.013% | 12/105 cells |
| -2 | 1.0 | 100 | 569 (0.6x10-3) | 658 | 0.012% |
| -3 | 1.3 | 221 | 579 (0.5x10-3) | 1155 | 0.020% |
| 13 | -1 | 1.7 | 0 | 1548 (1.0x10-3) | 9883 | 0.064% | 0.067% | 122/105 cells |
| -2 | 1.4 | 187 | 1462 (1.0x10-3) | 10616 | 0.073% |
| -3 | 0.8 | 17 | 934 (1.1x10-3) | 6294 | 0.067% |
| 14 | -1 | 1.0 | 54 | 219 (2.3x10-4) | 0 | 0 | Negative | PNQ<10 |
| -2 | 0.9 | 0 | 84 (9.2x10-5) | 0 | 0 |
| -3 | 0.9 | 188 | 149 (1.6x10-4) | 0 | 0 |
| 16 | -1 | 1.2 | 35 | 233 (1.9x10-4) | 20 | 0.0009% | 0.0006% | 75/105 cells |
| -2 | 1.4 | 146 | 346 (2.4x10-4) | 18 | 0.0005% |
| -3 | 1.2 | 0 | 313 (2.6x10-4) | 13 | 0.0005% |
| 17 (FU1) | -1 | 1.3 | 377 | 2125 (1.6x10-3) | 64 | 0.0005% | 0.0005% | PNQ<50 |
| -2 | 1.3 | 72 | 2101 (1.6x10-3) | 82 | 0.0005% |
| -3 | 1.4 | 174 | 6587 (4.6x10-3) | 99 | 0.0005% |
| 17 (FU2) | -1 | 1.1 | 2054 | 6751 (6.1x10-3) | 46 | 0.0005% | 0.0003% | Negative |
| -2 | 1.3 | 1397 | 8120 (6.1x10-3) | 36 | 0.0005% |
| -3 | 1.2 | 1802 | 11728 (9.7x10-3) | 0 | 0 |
| 18 | -1 | 1.3 | 682 | 6827 (1.6x10-3) | 4097 | 0.008% | 0.011% | 503/105 cells |
| -2 | 1.2 | 1224 | 1892 (1.6x10-3) | 4269 | 0.022% |
| -3 | 1.5 | 1695 | 7078 (1.6x10-3) | 4322 | 0.006% |
| 19 | -1 | 1.2 | 47 | 1456 (1.2x10-3) | 3080 | 0.02% | 0.02% | 30/105 cells |
| -2 | 1.2 | 0 | 1129 (0.9x10-3) | 2506 | 0.02% |
| -3 | 1.3 | 0 | 1508 (1.2x10-3) | 2612 | 0.02% |
| 20 | -1 | 1.2 | 177 | 3066 (2.6x10-3) | 0 | 0 | 0.0008% | PNQ<10 |
| -2 | 1.6 | 405 | 7442 (4.6x10-3) | 870 | 0.0012% |
| -3 | 1.2 | 0 | 4085 (3.4x10-3) | 609 | 0.0015% |
| 21 | -1 | 0.9 | 118 | 1632 (1.8x10-3) | 5 | 0.0005% | 0.0005% | 3.6/105 cells |
| -2 | 0.8 | 115 | 979 (1.2x10-3) | 52 | 0.0005% |
| -3 | 1.0 | 0 | 1624 (1.7x10-3) | 21 | 0.0005% |
| 22 | -1 | 1.4 | 0 | 19143 (1.4x10-2) | 44946 | 0.023% | 0.023% | 76/105 cells |
| -2 | 1.2 | 0 | 19897 (1.6x10-2) | 45450 | 0.023% |
| -3 | 1.3 | 10111 | 25462 (1.9x10-2) | 61598 | 0.024% |
| 23 | -1 | 0.7 | 2255 | 6005 (8.5x10-3) | 0 | 0 | Negative | Negative |
| -2 | 1.1 | 2924 | 7490 (6.9x10-3) | 0 | 0 |
| -3 | 1.2 | 826 | 4637 (3.8x10-3) | 0 | 0 |
| 24 | -1 | 1.1 | 2544 | 6263 (6.0x10-3) | 0 | 0 | 0.0029% | PNQ<10 |
| -2 | 1.1 | 1563 | 3695 (3.5x10-3) | 0 | 0 |
| -3 | 0.9 | 0 | 3280 (3.6x10-3) | 2856 | 0.0087% |
| 29 | -1 | 1.2 | 10564 | 28172 (2.4x10-2) | 8882 | 0.0032% | 0.0089% | not done |
| -2 | 1.5 | 4 | 26516 (1.8x10-2) | 53399 | 0.021% |
| -3 | 1.3 | 6350 | 37562 (2.9x10-2) | 12676 | 0.0034% |
| 30 | -1 | 1.1 | 0 | 603 (5.7x10-4) | 3 | 0.0005% | 0.0002% | not done |
| -2 | 1.1 | 0 | 545 (5.1x10-4) | 0 | 0 |
| -3 | 1.5 | 0 | 581 (3.8x10-4) | 0 | 0 |
| 31 | -1 | 1.3 | 8 | 3608 (2.7x10-3) | 5732 | 0.016% | 0.021% | not done |
| -2 | 1.3 | 1673 | 2432 (1.9x10-3) | 4583 | 0.019% |
| -3 | 1.0 | 643 | 1750 (1.7x10-3) | 5122 | 0.029% |
| 32 | -1 | 1.1 | 0 | 229 (2.1x10-4) | 6926 | 0.3% | 0.300% | 184/105 cells |
| -2 | 1.1 | 131 | 281 (2.6x10-4) | 6909 | 0.2% |
| -3 | 1.2 | 0 | 228 (1.9x10-4) | 7945 | 0.3% |
| 34 | -1 | 1.0 | 43 | 167 (1.7x10-4) | 2401 | 0.144% | 0.207% | 1758/105cells |
| -2 | 1.0 | 50 | 66 (6.7x10-5) | 2352 | 0.357% |
| -3 | 0.6 | 57 | 113 (1.8x10-4) | 1349 | 0.119% |
| 43 | -1 | 0.9 | 48 | 79 (8.7x10-5) | 0 | 0 | Negative | not done |
| -2 | 1.1 | 41 | 157 (1.4x10-4) | 0 | 0 |
| -3 | 0.9 | 0 | 70 (7.5x10-5) | 0 | 0 |
| 44 | -1 | 0.9 | 5 | 1819 (2.1x10-3) | 9497 | 0.052% | 0.049% | 129/105cells |
| -2 | 0.7 | 0 | 2742 (3.7x10-3) | 11814 | 0.043% |
| -3 | 1.1 | 0 | 2907 (3.8x10-3) | 15268 | 0.053% |
| 45 | -1 | 1.3 | 35 | 81 (6.1x10-5) | 9595 | 1.2% | 1% | 584/105 cells |
| -2 | 1.7 | 102 | 114 (6.8x10-5) | 11574 | 1.0% |
| -3 | 1.4 | 0 | 128 (9.2x10-5) | 10030 | 0.8% |

Abbreviations: FU: follow-up; PNQ: positive but not quantifiable; N/A: not applicable.

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**Figure S1. Schematic diagram of clonality detection at diagnosis, sensitivity study and MRD detection at follow-up, by NGS and Sanger sequencing. NGS: next-generation sequencing; ASO RQ-PCR: allele-specific oligonucleotide real-time quantitative-PCR; MRD: minimal residual disease.**



**Figure S2. MRD measured by NGS and ASO RQ-PCR in 27 follow-up bone marrow samples from 24 MM patients. MM: multiple myeloma; NGS: next-generation sequencing; ASO RQ-PCR: allele-specific oligonucleotide real-time quantitative-PCR; MRD: minimal residual disease.**