The Journal of Molecular Diagnostics, Vol. 22, No. 5, May 2020

ELSEVIER

the Journal of Molecular Diagnostics
jmd.amjpathol.org

Upgraded Standardized Minimal Residual Disease Detection by Next-Generation Sequencing in Multiple Myeloma

Qiumei Yao,* Yinlei Bai,† Alberto Orfao,‡ Shaji Kumar,§ and Chor S. Chim*

From the Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Pokfulam, Hong Kong; the Institute for Immunology and School of Medicine,† Tsinghua University, Beijing, People's Republic of China; the Department of Medicine and Cytometry Service (Nucleus),‡ Cancer Research Centre, Institute of Cancer Molecular and Cellular Biology, University of Salamanca-Consejo Superior de Investigaciones Científicas, and Centro de Investigación Biomédica en Red de Cáncer, Institute for Biomedical Research of Salamanca, University of Salamanca, Salamanca, Spain; and the Division of Hematology,§ Mayo Clinic, Rochester, Minnesota*

Accepted for publication February 7, 2020.

Address correspondence to Chor S. Chim, M.D., Ph.D., Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Pokfulam, Hong Kong. E-mail: jcschim@hku.hk.

Minimal residual disease (MRD) is one of the most powerful prognostic factors in multiple myeloma. Therefore, standardization and easy operation of MRD testing are crucial. Previously, we validated the sensitivity of 10^{-5} with spike in of plasmid controls for a standardized next-generation sequencing (NGS) approach based on triplicate measurements of bone marrow by LymphoTrack-MiSeq platform. To improve the technique, we replaced spike-in plasmid controls by genomic DNA from myeloma cells. A spike-in control of 0.001% was consistently detected in all 19 samples tested, confirming a uniform sensitivity of 10^{-5} of this upgraded protocol. MRD was detected in 14 of 19 patients (78%), with a significant ($P = 0.04$) impact on progression-free survival based on high versus low MRD levels. Reproducibility of detection was confirmed by the extremely small interrun variation tested in three patients. In nine patients, MRD was tested in parallel by allele-specific oligonucleotide real-time quantitative PCR. NGS showed an improved sensitivity and provided quantification of MRD for cases assigned positive but not quantifiable by real-time quantitative PCR, obviating the need of patient-specific probes/primers. In summary, the use of genomic DNA as spike-in control simplifies NGS detection of MRD while preserving the sensitivity of 10^{-5}. Validity and reproducibility of the standardized procedure were verified, and the prognostic impact of NGS-based MRD in myeloma was confirmed. *(J Mol Diagn 2020, 22: 679–684; https://doi.org/10.1016/j.jmoldx.2020.02.005)*

Minimal residual disease (MRD) has been established as a powerful predictor of survival in multiple myeloma (MM), with superior outcome in patients achieving MRD negativity.[1] Next-generation sequencing (NGS)–based MRD negativity, defined as the absence of myeloma plasma cells in 1,000,000 normal bone marrow (BM) cells ($<10^{-6}$), has been shown to be a major prognostic factor in multiple myeloma.[2] However, to achieve a sensitivity of 10^{-6} with 95% confidence, ≥2.4 million cells (equivalent to approximately 21.6 μg of DNA) are required by clonoSEQ version 2.0 (Adaptive Biotechnologies, Seattle, WA), and 3 million cells (approximately 27 μg DNA) are required by LymphoTrack,[3] which is technically challenging for library preparation for sequencing, and demanding in routine practice because relatively large volumes of BM are required for clinical testing.

Recently, we have applied a standardized experimental design using triplicate measurements of 1 μg DNA input and 1 million sequencing reads measured in the LymphoTrack-MiSeq platform, with a verified sensitivity of

Supported by Hong Kong Blood Cancer Foundation grant AR170007 (C.S.C.) and National Natural Science Foundation of China grant 81470369 (C.S.C.).

Q.Y. and Y.B. contributed equally to this work.

Disclosures: None declared.

https://doi.org/10.1016/j.jmoldx.2020.02.005

10^{-5},[4] as required by the International Myeloma Working Group criteria for complete response (CR) by NGS.[5] However, only a limited number of samples ($n = 7$) were tested. Moreover, two plasmids containing unique Ig heavy-chain locus (*IGH*) sequences were added to each replicate, one at a concentration of 10^{-5} (copy number equivalent to 0.001% of all cells in a replicate) for validation of the sensitivity of 10^{-5}, and the other at 5×10^{-5} or 10^{-4} for obtaining an amplification factor to normalize for the percentage of tumor alleles per sequencing read.[4] To further validate this platform, in this article, a larger series of 19 MM patients was studied. Moreover, the previously reported protocol was modified by adding genomic DNA from purified $CD138^+$ plasma cells of MM patients with clonal *IGH*/Ig κ locus (*IGK*) rearrangements with highly diverse sequences[6] as spike-in controls instead of plasmids. Such modification has two clear advantages over the use of plasmid DNA spike-in controls. First, diluting plasmid DNA from the stored concentration to the appropriate one for use (generally from 10^9 to 6 copies per microliter) is too tedious and prone to pipetting errors. Second, the concentration of spike-in controls is more accurate when genomic DNA (versus plasmid DNA) is used, because in the former situation cell numbers of both the spike-in controls and BM MRD samples are measured by real-time PCR with a standard curve, whereas calculation of the number of copies of plasmid DNA is based on the assumption that the average weight of a bp is 650 Daltons.

Materials and Methods

Patients and Samples

Nineteen Chinese MM patients were included in this study. All 19 patients received autologous stem cell transplantation after bortezomib-based induction[7] or the staged approach,[8] a response-adapted approach in which all patients had upfront vincristine-doxorubicin-dexamethasone chemotherapy induction, followed by reinduction with bortezomib-thalidomide-dexamethasone for those patients with suboptimal response after vincristine-doxorubicin-dexamethasone, autologous stem cell transplantation, and, subsequently, thalidomide maintenance (thalidomide, 50 mg daily) until disease progression. Bone marrow aspiration was performed at diagnosis and subsequently at follow-up. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 16-111) and included appropriate individual informed consents. Patient and sample characteristics are shown in Supplemental Table S1.

MRD Measurements by NGS

Identification of clonality in MM diagnostic samples was performed as previously described.[4] A myeloma clonal *IG* rearrangement was defined as an identical sequence with a frequency of >2.5% in samples in which >100,000 total sequencing reads had been obtained.[9]

For MRD detection in follow-up samples, triplicate measurements of 1 μg DNA input and 1 million sequencing reads were applied using a single LymphoTrack *IGH* framework region (FR) 1, FR2, FR3, or *IGK* assay, according to the (clone identification) results obtained at diagnosis. The number of cells contained in 1 μg of each sample was validated by the real-time PCR standard curve method using plasmids, in which the albumin gene was cloned. Two spike-in controls generated from genomic DNA of purified $CD138^+$ plasma cells of MM patients were added to each replicate, one at a concentration of 10^{-5} for validation of the sensitivity of 10^{-5} and the other at 10^{-4} for obtaining an amplification factor (ie, percentage of tumor alleles per sequence read). The sequences corresponding to the clonal *IGH/IGK* rearrangements of spike-in controls are shown in Supplemental Table S2. MRD level for each replicate were calculated from the corresponding reads of the myeloma-specific sequence and the amplification factor. The final MRD level of a sample was defined as the mean MRD levels of the corresponding triplicates.

ASO qPCR–Based MRD Analysis

Clonality detection and subsequent MRD assessment by allele-specific oligonucleotide (ASO) real-time quantitative PCR (qPCR) were performed as previously described.[10] Clonality was identified by sequential PCR of the *IGH* VDJ, *IGH* DJ, and *IGK* VJ rearrangements, followed by Sanger sequencing. For MRD assessment, ASO forward primers with/without patient-specific reverse primer or probe were designed. qPCR was performed and interpreted according to the EuroMRD guidelines.[11]

Statistical Analysis

Progression was defined according to the International Myeloma Working Group criteria.[5] Progression-free survival (PFS) was calculated as survival from the moment of starting treatment until the date of disease progression, last follow-up, or death. Survival functions were estimated by the Kaplan-Meier method and compared by the log-rank test. Analyses were performed by SPSS software version 19.0 (IBM, Chicago, IL).

Results

For all 19 myeloma patients investigated, clonality was studied by LymphoTrack *IGH* (FR1, FR2, or FR3) and *IGK* assays in diagnostic BM. Clonal myeloma-specific sequences from all 19 cases were tumor specific as they were not detectable in normal BM controls or unrelated MM follow-up samples. Hence, they qualified for MRD monitoring (Table 1).

Table 1 Clonal *IGH* and *IGK* Rearranged Sequences Identified by NGS or Sanger Sequencing

	Clonality detected by NGS			
Patient ID	Rearrangement	Frequency, %	Type	Sanger sequencing
1	V_H5.51(3)-12-(4)D3.10(9)-4-(8)J_H4	25	Productive	V_H5.51(3)-12-(4)D3.10(9)-4-(8)J_H4
2	V_H4.39(0)-2-9-(2)D3.22(9)-8-(10)J_H4	9	Productive	V_H4.39(0)-2-9-(2)D3.22(9)-8-(10)J_H4
	V_K1.39(5)-2-(0)J_K1*	39	Productive	
3	V_H3.11(1)-15-(5)D2.21(12)-8-(10)J_H6	72	Productive	V_H3.11(1)-15-(5)D2.21(12)-8-(10)J_H6
4	V_H3.20(0)-10-(5)D1.26(3)-8-(4)J_H4*	79	Productive	V_H3.20(0)-10-(5)D1.26(3)-8-(4)J_H4
	V_K3.11(0)-0-(1)J_K4	89	Unproductive	
5	V_K1.39(0)-0-(0)J_K5*	46	Productive	V_K1.39(0)-0-(0)J_K5
	V_K1.33(1)-1-(3)J_K4	15	Unproductive	
6	V_H1.24(2)-0-(7)D1.1(1)-12-(17)J_H5	10	Productive	V_H1.24(2)-0-(7)D1.1(1)-12-(17)J_H5
	V_K2.40(0)-1-(5)J_K2*	59	Productive	
7	V_K2.29(0)-0-(3)J_K4*	13	Productive	D6.25(3)-18-(11)J_H4b
	V_K1.6(0)-0-(3)J_K1	10	Productive	
8	V_H3.33(0)-1-2-(6)D6.13(3)-4-(7)J_H3*	7	Productive	V_H4.34(3)-14-(16)D2.2(2)-25-(16)J_H4
9	V_H3.23(0)-2-(7)D3.3(12)-0-(5)J_H3	9	Productive	V_H3.23(0)-2-(7)D3.3(12)-0-(5)J_H3
	V_K5.2(0)-1-3-(0)J_K1*	10	Unproductive	
10	V_K2.40(0)-1-(6)J_K2	10	Unproductive	ND
11	V_H2.70(2)-6-(6)D3.22(3)-12-(14)J_H6	10	Productive	ND
12	V_K1.39(1)-2-(8)J_K1*	33	Unproductive	V_K1.39(1)-2-(8)J_K1
	V_K1.33(6)-4-(1)J_K1	28	Productive	
13	V_H1.3(0)-2-3-(1)D4.17(6)-9-(5)J_H3	26	Productive	D6.25(0)-5-(10)J_H4b
	V_K1.5(4)-0-(0)J_K2*	92	Productive	
14	V_H3.30(0)-4-(8)D5.12(9)-4-(6)J_H4	12	Productive	V_H3.30(0)-4-(8)D5.12(9)-4-(6)J_H4
	V_K4.1(6)-(12)J_K2*	83	Unproductive	
15	V_K4.1KDEL	15	NA	ND
16	V_H3.74(0)-0-(13)D1.26(0)-6-1-(0)J_H4	6	Productive	ND
17	V_H4.39(0)-8-1-(0)D4.23(5)-0-(0)J_H5*	87	Productive	ND
	V_K3.15(3)-1-1-(0)J_K1	79	Unproductive	
18	V_H3.21(0)-1-3-(3)D6.13(3)-3-(4)J_H4*	41	Productive	ND
	V_K2.18(4)-7-(3)J_K4	31	Unproductive	
19	V_H3.23(0)-4(5)D3.3(12)-22-(5)J_H6	7	Productive	ND

*Indicates the sequence used as minimal residual disease target in cases with more than one clonal rearranged sequence, as determined by sequence diversity and frequency in sequencing reads.

ID, identifier; NA, not applicable; ND, not done; NGS, next-generation sequencing.

Regarding sensitivity, *IGH* sequences of the 10^{-5} diluted control were detected in at least one of the triplicates of all 19 follow-up BM samples tested. Therefore, the sensitivity of 10^{-5} was consistently achieved (Supplemental Table S3). In parallel, on the basis of the standard curves of ASO qPCR constructed according to the EuroMRD guidelines[11] for the 12 cases tested by this approach, a sensitivity of between 10^{-4} and 10^{-5} was achieved (Supplemental Table S3). In this later approach, patient-specific primers were required in all 12 cases, and patient-specific probes were also needed in 1 of 12 cases, making this method far more labor intensive, time-consuming, and technically demanding.

Another feature of the experimental approach herein evaluated is that apart from the spike-in control used for sensitivity, a second spike-in control with a distinct complementarity determining region 3 sequence was also added at a concentration of 10^{-4} for normalization of MRD levels. Of note, a concentration of 10^{-4} was used herein for this second spike-in control because previous data comparing such normalization control at concentrations of 5×10^{-5} and 10^{-4} had shown that 10^{-4} is associated with lower variability in frequency of sequencing reads among triplicates.[4] Overall, the rationale to calculate MRD levels in NGS-based MRD assays using spike-in controls relies on the same principles of using the standard curve in ASO qPCR. According to the EuroMRD guidelines for ASO qPCR, dilution points included in the standard curve for quantitation of MRD in the follow-up samples should be within the quantitative range,[11] which requires the ΔCt of the two replicates to differ by <1.5 (equivalent to a 2.8-fold copy number difference). Indeed, variation of <2.8-fold in the frequency of sequencing reads among triplicates of the 10^{-4} spike-in control was achieved in this study for 18 of 19 samples tested (Supplemental Table S3). On the other hand, the fold difference in frequencies observed among triplicates of the remaining sample was four only, hence still within an acceptable range.

Among the 19 follow-up BM MM patient samples, 6 had very good partial response and 13 were in CR at the time of MRD testing. MRD was detected by NGS in 14 of 19 cases

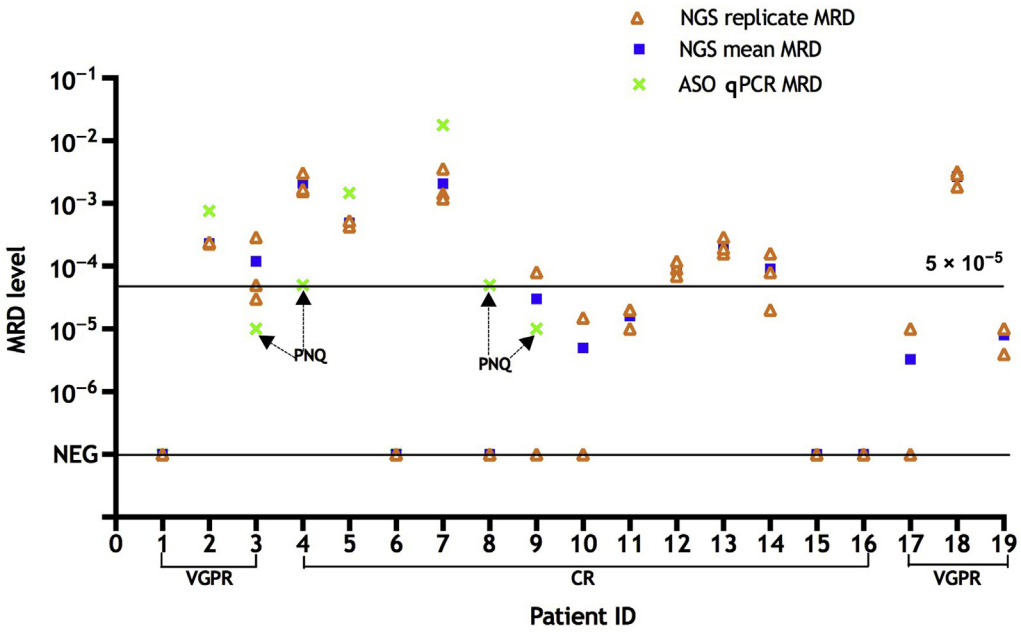

Figure 1 Minimal residual disease (MRD) results measured by next-generation sequencing (NGS) and allele-specific oligonucleotide (ASO) real-time quantitative PCR (qPCR). NGS-based MRD of triplicates and mean MRD are shown for individual patients. MRD positivity was identified in 9 of 13 patients (69%) who achieved complete response (CR) at the time of MRD testing. ASO qPCR–based MRD was available in Patients 1 to 9. For comparison purposes, cases that were defined as positive not quantifiable (PNQ) by ASO qPCR were placed to the sensitivity level reached for that patient (10^{-5} for Patients 3 and 9; 5×10^{-5} for Patients 4 and 8). NGS yielded MRD-positive or MRD-negative results concordant with ASO qPCR in 8 of 9 patients, whereas in Case 8, ASO qPCR yielded a positive result that was not confirmed by NGS. In Case 4, ASO qPCR MRD was assigned positive but not quantifiable because ΔCt values of the three replicates differed by >1.5; however, NGS provided quantification of MRD with a variation <2.8-fold in frequency among triplicates. The two **solid black lines** across the image correspond to MRD at negative (NEG; **bottom line**) and 5×10^{-5} level (**top line**). $n = 1$ to 19. ID, identifier; VGPR, very good partial response.

(78%) (Figure 1 and Supplemental Table S3). Thus, MRD was positive in 5 of 6 very good partial response cases (83%). The remaining MRD-negative case also achieved serologic CR after 3 months and remains relapse free after 6 years of follow-up, consistent with a delayed complete clearance of the circulating paraprotein after complete eradication of myeloma plasma cells in the bone marrow.[3] In turn, MRD was positive by NGS in 9 of 13 CR cases (69%), indicating that this NGS-based platform with a sensitivity of 10^{-5} is sensitive and feasible enough for MRD measurements in routine practice, as most CR patients were MRD positive, in line with previous findings based on both NGS and next-generation flow techniques.[2,12] With a median follow-up of 28 months (range, 15 to 92 months), nine progressions and four deaths were observed among the 19 patients. PFS in patients with low MRD levels (defined as $<10^{-4}$) was superior to that of those with high MRD levels ($\geq 10^{-4}$) (median PFS, 77 versus 32 months) (Figure 2). With a sensitivity of 10^{-5}, the median PFS was not yet reached in those MRD negative ($n = 5$), compared with a median PFS of 59 months in those MRD positive ($n = 14$) (Supplemental Figure S1).

To evaluate the reproducibility of this NGS-based approach for measuring MRD, DNA of BM MRD samples from three patients (Patients 1, 14, and 19) was submitted to amplification and sequencing for a second MRD detection. Measured MRD levels of these three samples were negative, 0.009%, and 0.0008% in the first run (Supplemental Table S3) and negative, 0.009%, and 0.001% in the second run (Supplemental Table S4). The small interrun variation supports this is a highly reliable approach that provides reproducible MRD data.

In 9 of the 19 patients studied herein by NGS, MRD was also analyzed by ASO qPCR (Figure 1 and Supplemental Table S3). NGS yielded MRD-positive or MRD-negative results concordant with ASO qPCR in 8 of 9 of these patients (89%). In the only discrepant case (Patient 8), ASO qPCR yielded a positive MRD result that could not be confirmed by NGS. This discordance

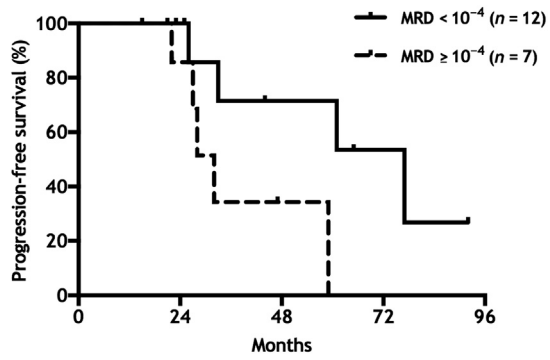

Figure 2 Progression-free survival for patients according to minimal residual disease (MRD) level determined by next-generation sequencing.

could not be accounted for by a difference in sensitivity because the sensitivity reached with NGS for that case was of 10^{-5}, whereas for ASO qPCR it was lower (5×10^{-5}). In a previous report, it has been shown that false-positive MRD results might occur with ASO qPCR, as MRD amplification may arise from non-specific primer binding to similar VDJ sequences in normal lymphocytes.[13] In line with this possibility, this case remains disease free, after 31 months of follow-up, suggesting that the MRD status by NGS was probably more specific to that identified by ASO qPCR. Of note, in another patient (Case 4), MRD levels were assigned as being positive but not quantifiable with ASO qPCR, because ΔCt for the three replicates differed by >1.5. By contrast, NGS provided reliable quantification of MRD in this same patient sample with a variation <2.8-fold in the frequency of MRD levels among triplicates.

Discussion

Conventionally, the sensitivity of an MRD assay is determined by serial dilution of the diagnostic sample into normal peripheral blood or peripheral blood mononuclear cell DNA, assuming that an equivalent sensitivity in follow-up BM samples would be achieved whenever a similar DNA input and sequencing depth are used.[14,15] However, the proportion of B cells in follow-up BM samples is (most likely) different from that in normal peripheral blood; thereby, the sensitivity derived from this serial dilution experiment may not be guaranteed and applicable to every follow-up sample. Moreover, it is difficult to measure the exact tumor burden of the diagnostic sample used for the dilution experiment. To overcome these technical issues, we previously applied a sensitivity control in follow-up BM sample fully in parallel with the MRD measurement and verified the sensitivity of 10^{-5} of LymphoTrack-MiSeq platform by applying a standardized experimental design using triplicate measurements of 1 μg DNA input and 1 million sequencing reads.[4] This study further validated the upgraded platform, of which the adding of spike-in controls was simplified by genomic DNA. This is the only NGS technique that validates the sensitivity, 10^{-5} herein, in each and every follow-up MRD sample.

Furthermore, prognostic significance of MRD measured by this standardized NGS-based protocol was observed. Among the 19 MM patients with follow-up BM samples, MRD was detected by NGS in 14 (78%). When MRD at 10^{-4} was set as threshold for MRD low/high, PFS in patients with low MRD levels (defined as $<10^{-4}$) was superior to those with high MRD levels ($\geq 10^{-4}$) (median PFS, 77 versus 32 months; $P = 0.04$). Further studies with larger numbers of patients are warranted to confirm the clinical value of this newly modified NGS-based MRD approach. With a sensitivity of 10^{-5}, PFS of patients with undetectable MRD was not significantly different from those with detectable MRD despite a more promising separation of PFS curves due to the smaller number of cases in the MRD-negative group.

In summary, the validity and reproducibility of the upgraded version herein proposed of a standardized NGS-based protocol for MRD testing with a (uniform) sensitivity of 10^{-5}, based on the use of triplicates of 1 μg DNA input and 1 million sequencing reads measured by the LymphoTrack-Miseq platform, were verified. Compared with ASO qPCR, this NGS approach showed a higher sensitivity and enabled quantification of lower levels of MRD, which would otherwise be declared positive not quantifiable by ASO qPCR, without the need for patient-specific probe/primers, thereby translating into a much less labor-intensive approach with a faster turnaround time. Finally, the prognostic impact of NGS-based MRD in MM was confirmed. With these promising results, we have started a prospective, collaborative interhospital study of MRD in MM patients treated by a uniform protocol.

Supplemental Data

Supplemental material for this article can be found at *https://doi.org/10.1016/j.jmoldx.2020.02.005*.

References

1. Munshi NC, Avet-Loiseau H, Rawstron AC, Owen RG, Child JA, Thakurta A, Sherrington P, Samur MK, Georgieva A, Anderson KC, Gregory WM: Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma: a meta-analysis. JAMA Oncol 2017, 3:28−35
2. Perrot A, Lauwers-Cances V, Corre J, Robillard N, Hulin C, Chretien ML, et al: Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. Blood 2018, 132:2456−2464
3. Landgren O, Rustad EH: Meeting report: advances in minimal residual disease testing in multiple myeloma 2018. Adv Cell Gene Ther 2019, 2:e26
4. Yao Q, Bai Y, Orfao A, Chim CS: Standardized minimal residual disease detection by next-generation sequencing in multiple myeloma. Front Oncol 2019, 9:449
5. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al: International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol 2016, 17:e328−e346
6. Moppett J, Wakeman S, Duez M, Bartram J, Wright G, Hubank M, Williamson H, Archer P, Hancock J: Immunoglobulin/T-cell receptor (Ig/TCR) allele usage in normal and on treatment bone marrow samples in childhood acute lymphoblastic leukaemia: implications for NGS based MRD analysis. Blood 2016, 128:4073
7. Chim CS, Lie AK, Chan EY, Liu HS, Lau CW, Yip SF, Sim J, Wan TS, Ma ES, Liang R, Tse E, Kwong YL: Treatment outcome and prognostic factor analysis in transplant-eligible Chinese myeloma patients receiving bortezomib-based induction regimens including the staged approach, PAD or VTD. J Hematol Oncol 2012, 5:28
8. Chim CS, Lie AK, Chan EY, Leung YY, Cheung SC, Chan SY, Liang R, Kwong YL: A staged approach with vincristine, adriamycin, and dexamethasone followed by bortezomib, thalidomide, and dexamethasone before autologous hematopoietic stem cell transplantation

in the treatment of newly diagnosed multiple myeloma. Ann Hematol 2010, 89:1019−1027
9. Arcila ME, Yu W, Syed M, Kim H, Maciag L, Yao J, Ho C, Petrova K, Moung C, Salazar P, Rijo I, Baldi T, Zehir A, Landgren O, Park J, Roshal M, Dogan A, Nafa K: Establishment of immunoglobulin heavy (IGH) chain clonality testing by next-generation sequencing for routine characterization of B-cell and plasma cell neoplasms. J Mol Diagn 2019, 21:330−342
10. Bai Y, Wong KY, Fung TK, Chim CS: High applicability of ASO-RQPCR for detection of minimal residual disease in multiple myeloma by entirely patient-specific primers/probes. J Hematol Oncol 2016, 9:107
11. van der Velden VH, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER, Flohr T, Sutton R, Cave H, Madsen HO, Cayuela JM, Trka J, Eckert C, Foroni L, Zur Stadt U, Beldjord K, Raff T, van der Schoot CE, van Dongen JJ: Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia 2007, 21:604−611
12. Flores-Montero J, Sanoja-Flores L, Paiva B, Puig N, Garcia-Sanchez O, Bottcher S, van der Velden VHJ, Perez-Moran JJ, Vidriales MB, Garcia-Sanz R, Jimenez C, Gonzalez M, Martinez-Lopez J, Corral-Mateos A, Grigore GE, Fluxa R, Pontes R, Caetano J, Sedek L, Del Canizo MC, Blade J, Lahuerta JJ, Aguilar C, Barez A, Garcia-Mateo A, Labrador J, Leoz P, Aguilera-Sanz C, San-Miguel J, Mateos MV, Durie B, van Dongen JJM, Orfao A: Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia 2017, 31:2094−2103
13. Kotrova M, van der Velden VHJ, van Dongen JJM, Formankova R, Sedlacek P, Bruggemann M, Zuna J, Stary J, Trka J, Fronkova E: Next-generation sequencing indicates false-positive MRD results and better predicts prognosis after SCT in patients with childhood ALL. Bone Marrow Transplant 2017, 52:962−968
14. Cheng S, Inghirami G, Cheng S, Tam W: Simple deep sequencing-based post-remission MRD surveillance predicts clinical relapse in B-ALL. J Hematol Oncol 2018, 11:105
15. Faham M, Zheng J, Moorhead M, Carlton VE, Stow P, Coustan-Smith E, Pui CH, Campana D: Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 2012, 120:5173−5180