Investigation of genetic abnormalities in plasma cell myeloma

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Doctoral (Ph.D.) thesis

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1. Introduction

Plasma cell myeloma (PCM) is currently an incurable oncohematological disorder characterized by unleashed clonal proliferation of terminally differentiated malignant B-cells. As the second most common hematological malignancy, it accounts for 1-2% of all cancers. Studies employing various molecular genetic technologies have unambiguously demonstrated that PCM typically associated with characteristic symptoms and end-organ damages always evolves from a pre-malignant condition called monoclonal gammopathy of undetermined significance (MGUS) through smouldering myeloma (SMM), a symptomless stage with elevated burden of clonal myeloma cells. At an advanced stage of disease progression, myeloma cells may become independent from the bone marrow microenvironment and by making transition into the peripheral blood, plasma cell leukemia or extramedullary disease can develop, with the latter one conferring functional damage in the infiltrated organ. The clinical presentation and course of PCM are very heterogeneous across patients in terms of response to various lines of therapy and expected survival. In the last two decades, introduction and sequential application of immunomodulatory drugs, proteasome inhibitors and monoclonal antibody therapies allowed to achieve deeper responses providing longer survival in a wide range of patients with PCM. Consequently, the proportion of patients with a survival of >10 years is increasing, especially among standard-risk patients undergoing autologous transplantation. However, approximately 20% of PCM patients still succumb to the disease within 2 years.

Improved performance of molecular biology techniques provided evidence that PCM is a highly heterogeneous and complex disorder both clinically and genetically. The diverse genetic background is characterized by various reciprocal chromosome translocations, unbalanced genetic aberrations with structural and numerical chromosome changes, as well as by point mutations. Specific reciprocal translocations involving the immunoglobulin heavy chain (*IGH)* gene, as well as hyperdiploidy typically associated with trisomies of odd number chromosomes are considered early, primary genetic events playing an essential role in the oncogenesis. Progression of the disease is mainly driven by secondary, randomly accumulated genetic events providing proliferative advantage under the selective pressure of bone marrow microenvironment and the applied treatment strategy (Figure 1.).

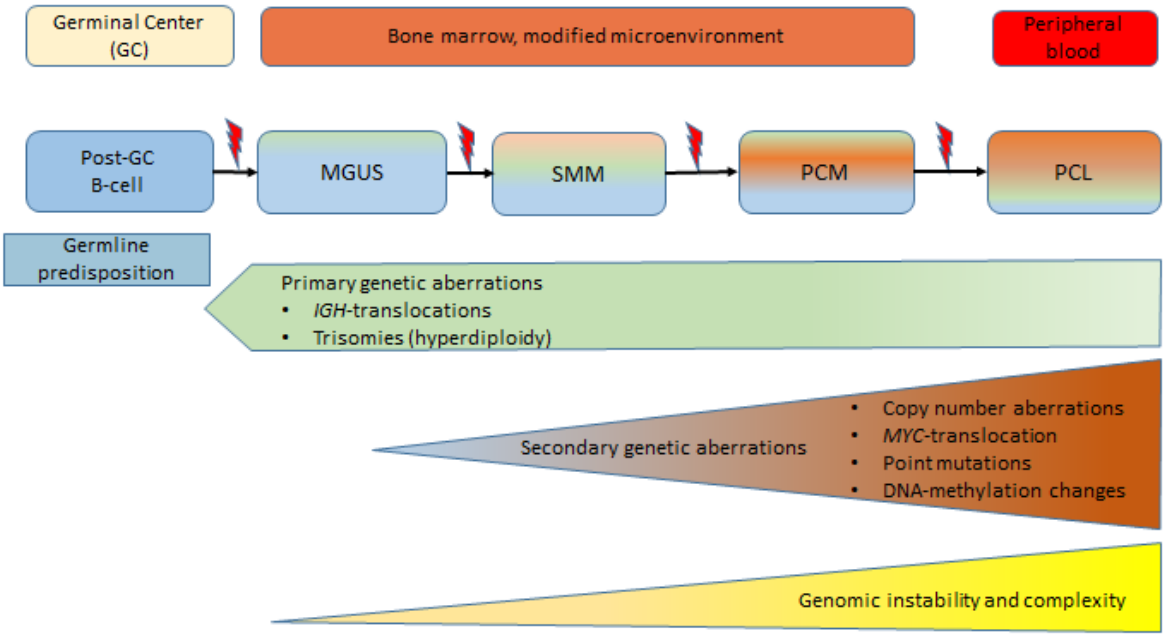


Figure 1. Development and progression of plasma cell myeloma.

PCM evolves through distinct clinical stages. Tumor initiation associated with the acquisition of primary genetic aberrations emerges via malignant transformation of germinal center B-cells harboring somatic hypermutation and class switch recombination in their *IGH* genes. The presence of certain germline mutations is associated with a higher risk of malignant transformation. Primary aberrations remain clonal throughout the course of the disease. Progression is driven by the accumulation of randomly acquired secondary aberrations (red thunder) leading to a gradually increasing genomic instability and complexity. As a result, myeloma cells may become independent of the bone marrow microenvironment conferring plasma cell leukemia or extramedullary disease. The clonal architecture characterized by different subclones with distinct genetic compositions changes with the progression over time, illustrated by different color transitions of certain disease stages. *IGH:* immunoglobulin heavy-chain gene; MGUS: monoclonal gammopathy with undetermined significance; SMM: smouldering myeloma; PCM: plasma cell myeloma; PCL: plasma cell leukemia.

Besides patient and disease specific clinical parameters, genetic lesions were found to have profound influence on patient survival as confirmed by several clinical studies. Although, the prognostic relevance of some aberrations still remain to be uncovered, the adverse effect on survival has already been proven for a subset of recurrent chromosomal alterations. In 2014, The International Myeloma Working Group published the revised international staging system (R-ISS), which includes only 3 recurrent high-risk aberrations for patient classification, such as del(17p), t(4;14) and t(14;16). However, the genetic background of PCM is highly complex and the various combinations of co-seggregating aberrations may alter the expected survival in several different ways.

While genetic abnormalities can only be detected in 30-40% of patients by karyotyping, alternative methods with higher resolution and sensitivity (e.g. fluorescent *in situ* hybridization (FISH), gene expression profiling, DNA-microarray and next generation

sequencing) can identify aberrations in nearly 100% of patients with PCM. Numerous combinations of whole chromosome aberrations and subchromosomal alterations conferring specific translocations, copy number alterations (CNA) and somatic mutations in genes of certain signal transduction pathways may occur across the patients. Screening of these aberrations and identifying their combinations may facilitate the precise genetic characterization and accurate prognostic classification of patients with PCM.

In clinical practice, FISH performed on interphase nuclei (iFISH) is a widely used method for the targeted investigation of genetic abnormalities in myeloma cells. However, in routine diagnostics it allows the simultaneous analysis of only 2-3 chromosomal regions. In several cases, control FISH probes covering distant loci positioned on the same chromosome are also needed for the proper interpretation of the results, which requirement further reduces the number of regions that can be analyzed in a single reaction. Multiplex ligation-dependent probe amplification (MLPA) is a targeted method able to interrogate the copy number status of 55-60 chromosomal regions simultaneously. Although, MLPA cannot provide information at single-cell level and it requires a tumor cell purity of >25-30%, with its higher resolution exceeding the typical 100 kilobase - 1 megabase resolution of iFISH, it is able to provide data on relative copy number status at single-exon level within 24 hours. DigitalMLPA is a novel method combining the principal of MLPA with the power of next generation sequencing, thus increasing the number of simultaneously analyzable genomic loci by an order of magnitude as compared to MLPA. Despite their advantages, MLPA and digitalMLPA have not been widely implemented in the clinical practice in Hungary to date.

Among genetic alterations recurrently observed in PCM, certain *IGH* translocations such as t(4;14), t(11;14) and t(14;16), as well as abnormalities of chromosome 13 and deletions affecting the *TP53* gene are the most intensively investigated lesions. While the biological and prognostic significance of these lesions are well established, scientific knowledge about their localization and sequence on the pathogenetic timescale of PCM was highly limited at the time of our studies. Data available in the literature were mainly based on indirect conclusions drawn from frequency values of various abnormalities observed at different stages of the disease.

2. Aims

For a more precise prognostic classification of patients with PCM, comprehensive and advanced genetic characterization is required which can be achieved by the targeted interrogation of a large number of chromosomal regions. We aimed to test relevant techniques which can realistically be implemented in the clinical diagnostics in Hungary. In addition, clonal evolution processes associated with the pathogenesis of PCM were also scrutinized.

2.1. Screening for DNA copy number alterations by MLPA technique

Performance of MLPA was tested with a special probemix allowing for the simultaneous investigation of 42 chromosomal regions recurrently affected by CNAs in PCM. Power and limitations of the technique were explored by comparing the data with results of the gold-standard method iFISH, which was used for validation.

2.2. Detection of DNA copy number alterations by digitalMLPA technique

Copy number alterations in diagnostic samples were analyzed by digitalMLPA and data were compared with MLPA and iFISH results. Besides analyzing the power and limitations of digitalMLPA, the high throughput of the technique was also tested.

2.3. Detection of a specific point mutation by digitalMLPA technique

DigitalMLPA should theoretically be able to detect known point mutations; therefore, the performance of the method was tested for detecting $BRAF^{V600E}$, a therapeutically targetable mutation emerging in 4-10% of patients with PCM.

2.4. Molecular cytogenetic studies on PCM patients of Tolna and Baranya counties

We summarized the results of molecular cytogenetic investigations performed on diagnostic samples of PCM patients from Baranya and Tolna counties in the Department of Pathology, University of Pécs over the last decade. By comparing the data obtained by iFISH and MLPA, we investigated the added value of each method, with an ultimate goal to improve efficacy of the diagnostic workflow.

2.5. Investigation of clonal evolution at single-cell level

Clonal evolution associated with the pathogenesis of PCM was scrutinized by iFISH at single-cell level in order to unravel the temporal sequence of various abnormalities and to obtain information about the potential role of early onset aberrations in the initiation of the disease.

3. Materials and methods

3.1. Samples

Patients diagnosed with plasma cell dyscrasia at the Division of Hematology, 1st Department of Medicine, University of Pécs were included in our studies. Bone marrow samples were analyzed from most of the patients, while in some cases only peripheral blood was available. Samples from healthy donors or patients without malignancy were used as negative controls. Diagnostic workup was performed at the Department of Pathology, University of Pécs, with special genetic studies partially performed in collaboration with the 1st Department of Pathology and Experimental Cancer Research at Semmelweis University, Budapest, and with the Institute of Molecular Cell Biology at Leiden University Medical Center.

3.2. Fractional assessment and enrichment of plasma cells

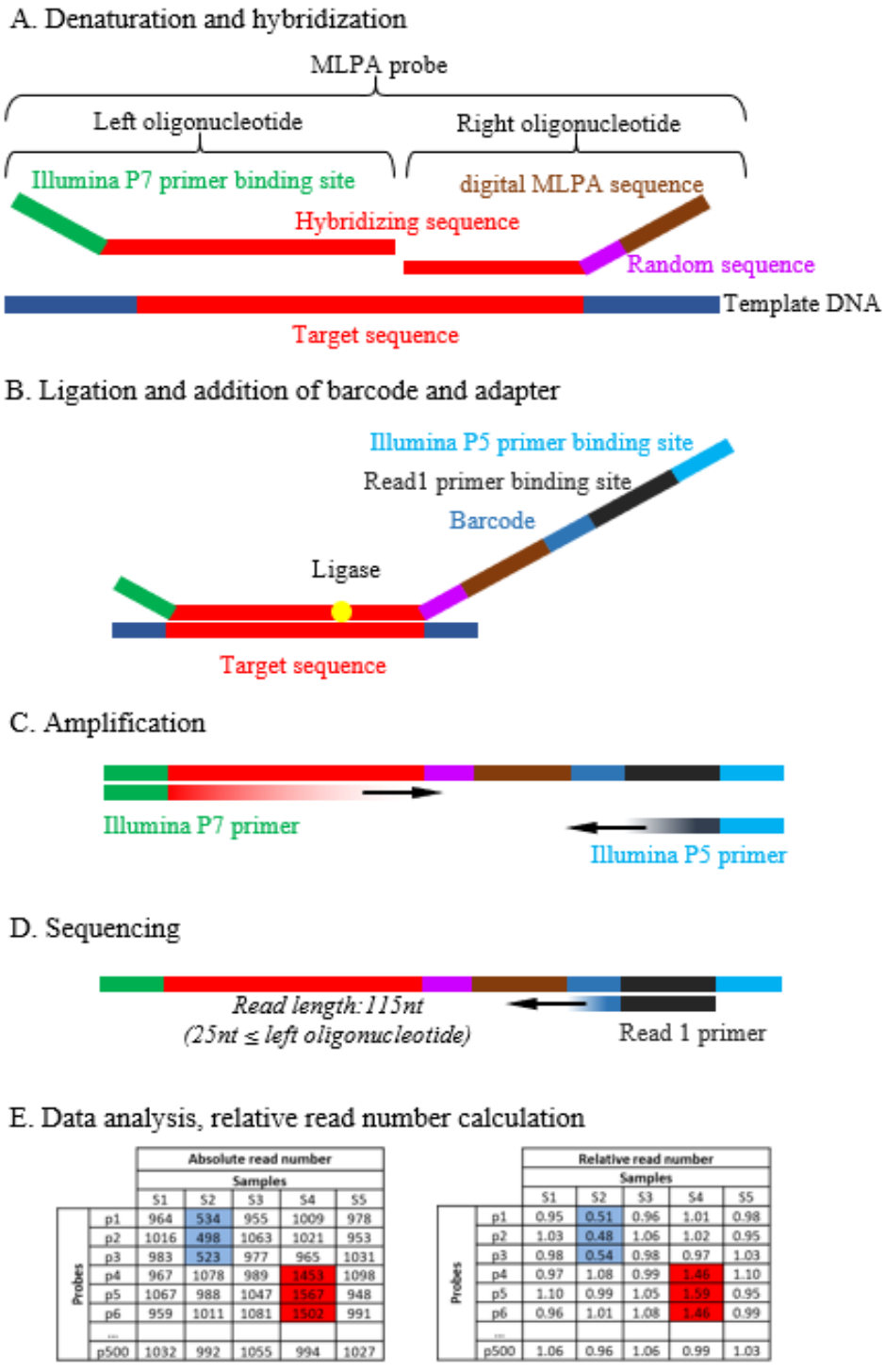
Immunophenotyping was performed by five-color flow cytometry using CD45-PerCP-Cy5.5, CD19-PE-Cy7, CD38-FITC, CD138-APC and CD56-PE markers. Immunomagnetic cell enrichment based on CD138 expression was applied at samples with a purity of <20%.

3.3. Multiplex ligation-dependent probe amplification

MLPA reactions were performed using the SALSA P425-A1 probemix (MRC Holland, Amszterdam) according to the manufacturer's recommendations. The probes covered 42 chromosomal regions recurrently altered in PCM: 1p32 (*FAF1*, *CDKN2C*, *PLPP3* and *DAB1* genes), 1p21, 1q21.3 (*CKS1B* gene), 1q23.3, 5q31.3, 12p13.31, 13q14 (*RB1* and *DLEU1*/*DLEU2* genes), 16q12 (*CYLD* gene), 16q23 (*WWOX* gene) and 17p13 (*TP53* gene).

3.4. Digital multiplex ligation-dependent probe amplification

The D006 probemix recently developed by MRC Holland was used for all digitalMLPA reactions. The probemix contained (*i*) 268 target probes primarily specific for subchromosomal regions recurrently altered by CNAs in PCM, (*ii*) one probe for the specific detection of the $BRAF^{V600E}$ mutation, (*iii*) 105 reference probes hybridizing to copy number stable regions, and (*iv*) 128 internal control probes for sample identification, as well as for quality assessment. Reference probes were used for data normalization and together with a subset of the target probes, for the identification of whole chromosome gains and losses by covering all chromosomes near the centromere, telomere or the middle of the arm. The workflow of digitalMLPA analysis is shown in Figure 2.



		Absolute read number				
		Samples				
		S1	S2	S3	S4	S5
Probes	p1	964	534	955	1009	978
	p2	1016	498	1063	1021	953
	p3	983	523	977	965	1031
	p4	967	1078	989	1453	1098
	p5	1067	988	1047	1567	948
	p6	959	1011	1081	1502	991
	...					
	p500	1032	992	1055	994	1027

		Relative read number				
		Samples				
		S1	S2	S3	S4	S5
Probes	p1	0.95	0.51	0.96	1.01	0.98
	p2	1.03	0.48	1.06	1.02	0.95
	p3	0.98	0.54	0.98	0.97	1.03
	p4	0.97	1.08	0.99	1.46	1.10
	p5	1.10	0.99	1.05	1.59	0.95
	p6	0.96	1.01	1.08	1.46	0.99
	...					
	p500	1.06	0.96	1.06	0.99	1.03

<u>Figure 2.</u> Workflow of digital multiplex ligation-dependent probe amplification

The method employs the principles of conventional MLPA with the difference that PCR products are not separated based on their lengths and are not quantified by capillary electrophoresis; rather, the nucleotide sequences of the probes are determined by next generation sequencing, and the relative copy number of different genomic regions represented by the specific probes are inferred from the absolute read numbers via a two-step, intra-sample (reference probe based) and inter-sample (reference sample based) normalization. The P7 and P5 primer binding sites located on both ends of all ligated probes allow for the amplification and sequencing of all generated PCR products on any Illumina sequencing platform.

3.5. Pyrosequencing

Presence of the *BRAF*^{V600E} mutation was validated by pyrosequencing using PyroMark Q24 system (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instruction. Sequencing was performed in reverse direction, producing a CAC wild-type sequence in normal samples and a CTC genotype in case of the V600E mutation. Raw data was analyzed using the PyroMark Q24 software version 2.0.8.

3.6. Droplet digital PCR

High-sensitivity validation of the *BRAF*^{V600E} mutation was performed by droplet digital PCR (ddPCR) using *BRAF* assays specific for the wild-type and mutant alleles. Droplets were generated by QX200 Droplet Generator and reading was completed with the QX200 Droplet Digital PCR system (Bio-Rad, Hercules, USA). Results were evaluated using the Bio-Rad QuantaSoft software.

3.7. Fluorescent *in situ* hybridization

Dual-color *in situ* labeling was applied to visualize specific genetic alterations, such as -13/del(13q), del(17p) and the disruption of the *IGH* gene. In case of *IGH* positivity, three reciprocal translocations (t(4;14)(p16;q32), t(11;14)(q13;q32) and t(14;16)(q32;q23)) most commonly emerging in PCM were specifically analyzed. Abnormalities of chromosome 1 were investigated by commercially available probes, as well as by home-brew probes specially designed for regions 1p32.2, 1p21 and 1q21. IFISH signal patterns were evaluated according to the recommendations of the European Myeloma Network using Zeiss AxioImager A1 microscope and/or Zeiss Axioplan2ie MOT motorized cytometer system.

3.8. Motorized microscopy

Clonal evolutionary studies by combined iFISH analyses were performed on samples with at least two identified genetic aberrations. After visualizing the first aberration, slides were scanned and digitalized by a motorized microscope (Zeiss Axioplan2ie MOT). Removal of the first staining was followed by the re-hybridization of nuclei using probes specific for the second genetic aberration. Individual cells were backtracked based on their recorded coordinates allowing for the analysis of different aberrations in exactly the same nuclei.

3.9. Statistical analysis

Congruency between results obtained by iFISH, MLPA and digitalMLPA was evaluated with Fisher's exact test using the SPSS 15.0 software (SPSS Inc., Chicago, IL). In clonal evolutionary studies, incidences of different subclonal *IGH* translocations detected only in a subset of the cells were compared using the Kruskall-Wallis test.

4. Results

4.1. Screening for DNA copy number alterations by MLPA

CNAs were identified in 91% of the 81 patients examined by MLPA. Aberration of chromosome 13 proved to be the most frequent lesion followed by 1q gain, 1p deletion, 5q gain, 12p deletion, 16q deletion and 17p deletion. More than one CNA per sample were detected in 33 different combinations across the patients. To assess the performance of MLPA as a candidate technique to detect CNAs in routine diagnostics of PCM, results were compared with data obtained by iFISH, the most widely used gold-standard approach. Direct comparison of the two methods revealed concordant results at 368/405 (81 samples x 5 aberrations) test data points, corresponding to a congruency of 90.8%. The underlying reasons for discrepancies were the slightly different probe-binding sites within some target regions, the presence of focal deletions with small extension, and the low proportion of abnormal cells. IFISH and MLPA allowed for the simultaneous investigation of numerous balanced and unbalanced genetic abnormalities in individual patients. When used in combination, iFISH and MLPA revealed 53 different combinations of abnormalities in 66 patients, from which 22 were not detected by iFISH or MLPA alone. Graphical display of pairwise associations between cytogenetic abnormalities observed in our patient cohort is presented in Figure 3.

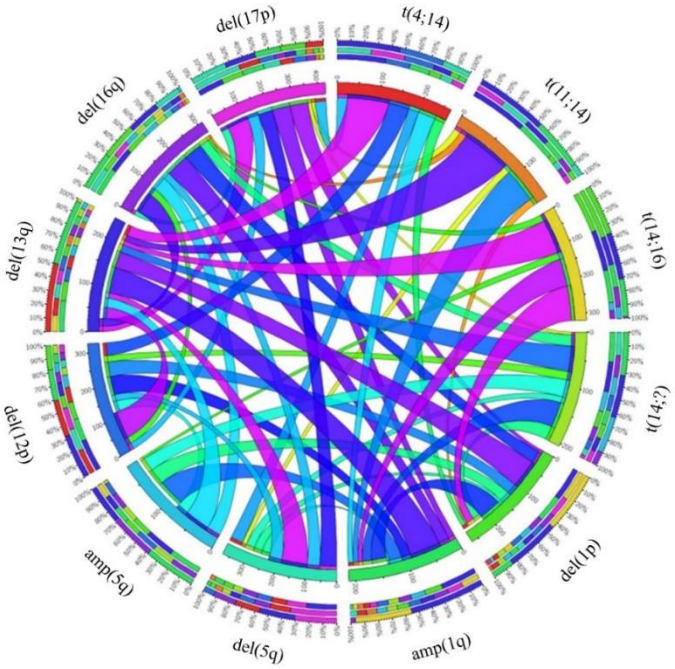


Figure 3. Pairwise co-seggregation frequency of individual cytogenetic abnormalities (circos plot).
Pairwise associations of aberrations identified by iFISH and/or MLPA are displayed. The width of each ribbon and the gradual transition of colors (from lilac to orange) are proportional to the frequency of the matching (paired) lesion observed within the group of patients harboring the particular aberration of interest.

4.2. Detection of DNA copy number alterations by digitalMLPA

DigitalMLPA was performed on 56 diagnostic patient samples and identified whole chromosome gains or losses in 84% of the cases, from which the most frequent aberration was monosomy 13. Most (94%) trisomies were detected in patients displaying hyperdiploid karyotype.

Across subchromosomal lesions, gain(1q) was the most commonly observed followed by loss(1p), loss(8p), loss(16q), loss(12p), loss(14q), gain(8q), gain(Xq), loss(13q), loss(6q), gain(14q), loss(17p), loss(20p), loss(22q), loss(5q), gain(6p) and gain(9q). The average number of subchromosomal CNAs per patient was 4.4 (range: 0 - 13), with 3.7 in the hyperdiploid subgroup and 4.8 in the non-hyperdiploid subgroup of patients. DigitalMLPA detected at least one subchromosomal CNA in 95% of the cases.

Forty-four digitalMLPA probes hybridized to different loci along chromosome 1 (19 to the short arm and 25 to the long arm) allowing for the detailed mapping of abrnomalities affecting this chromosome. Combined analysis of the various features of CNAs on chromosome 1 revealed 24 distinct patterns in 38 cases in terms of location, extension and allelic burden.

DigitalMLPA data was compared with conventional MLPA and iFISH results. The combined comparison of the three methods showed concordant results at 319/336 (56 samples x 6 aberrations) data points (congruency: 94.9%). Systematic pairwise comparison of these methods to detect individual aberrations also confirmed statistically high congruency (Fisher's exact test: $p<0.0001$). Concerning subchromosomal aberrations, digitalMLPA identified 156 lesions not detected by the other two techniques, in 45 hyperdiploid and non-hyperdiploid patients.

4.3. Detection of specific point mutation by digitalMLPA

BRAF^{V600E} mutation was detected in two patients by digitalMLPA. The abnormality was successfully validated by pyrosequencing in one of these patients, whereas droplet digital PCR providing higher sensitivity confirmed the presence of the mutation in both cases.

4.4. Molecular cytogenetic studies on PCM patients of Tolna and Baranya counties

Results of molecular cytogenetic analyses performed on 231 patients diagnosed with PCM at the Division of Hematology, University of Pécs and at the János Balassa Teaching Hospital of Tolna County between 2005 and 2018 were collected and evaluated.

Immunomagnetic cell enrichment was performed on 48 bone marrow samples with a plasma cell content of <20%. The procedure increased the purity to an average of 72% (range: 20 - 100%), corresponding to an 18-fold enrichment on average.

In our patient cohort, the prevalence of *IGH* translocations, 1q gain, chromosome 13 abnormalities and 17p deletion observed by iFISH were in accordance with previously published data. Eighty-nine unbalanced aberrations (gains or losses) were detected in 42 bone marrow samples analyzed by MLPA.

MLPA and iFISH showed concordant results in 96.2% of the cases. MLPA unveiled CNAs not detected by iFISH in 38% of the patients, while iFISH identified balanced aberrations which were undetectable by the applied MLPA probemix.

4.5. Investigation of clonal evolution at single-cell level

In our clonal evolutionary studies, correlated analysis of recurrent cytogenetic aberrations was performed on PCM patient samples at single-cell level. Initial screening with independent iFISH tests was carried out on 185 diagnostic samples included in the study. Sufficient amount of cells allowing for additional, combined iFISH analysis was available from 25 patients. In 21 cases, only one cell clone harboring all previously detected abnormalities was identified preventing the determination of the temporal sequence of cytogenetic evolution. In 4 out of 25 cases, two genetically distinct abnormal cell populations were observed. Our results suggest that among genetic aberrations investigated in this study, *IGH* translocations occur early in the course of the disease, while chromosome 13 aberrations and deletion of the *TP53* gene emerge later, providing selective advantage for the affected subclone. Across patients harboring *IGH* translocation, the aberration was detected in less than two-thirds of the plasma cells in 21.8% of the cases: *IGH*/*FGFR3* 20.0%, *IGH*/*CCND1* 16.7% and *IGH*/*MAF* 28.6%. Consequently, the initiating role of *IGH* translocations can be challenged, or even excluded in at least one-fifth of the affected patients.

5. Discussion

Plasma cell myeloma is a genetically heterogeneous hematological malignancy with diverse clinical outcome. The number of clinically relevant genetic aberrations is still showing an increasing tendency which should be reflected in the routine hematopathological diagnostics, also considering the therapeutical and financial conditions in Hungary. At the University of Pécs, this intention has led to the introduction of iFISH into the diagnostics of PCM some 15 years ago. The technique can rapidly and specifically detect the most frequent balanced translocations and unbalanced CNAs. However, the number of aberrations that can simultaneously be analyzed with the vast majority of commercial FISH probe kits is constrained to one or two, while the available amount of cell suspension/tissue sample is usually limited, which altogether restricts the application of the method. The limitation of sample availability is especially severe if plasma cell enrichment has to be performed on the bone marrow aspirate. Our results suggest that immunomagnetic cell enrichment can significantly increase the purity of the cell suspension, allowing to perform informative downstream genetic examinations. However, this procedure also reduces the amount of sample; hence, the introduction of alternative methods allowing for the simultaneous interrogation of several genomic loci can greatly facilitate the efficient characterization of patients.

Although conventional karyotyping gives an overview of the entire genome, its role in the diagnostics of PCM is limited by the low proliferation rate and *in vitro* vulnerability of the myeloma cells, and by the low resolution of the method. Array-based techniques and next generation sequencing can provide genome-wide information with higher resolution; however, the associated costs are currently hampering their widespread application in the molecular pathological diagnostics in Hungary.

Multiplex ligation-dependent probe amplification and digitalMLPA providing targeted analyses seem to be plausible alternatives due to their reasonable costs, relatively straightforward protocols and short turnaround time. Considering the current landscape of treatment options, these techniques allow for the simultaneous profiling of all unbalanced genetic aberrations with potential clinical relevance, thus could clearly contribute to a more comprehensive genetic characterization of Hungarian patients with PCM. In the majority of patients, MLPA and digitalMLPA unraveled aberrations which remained undetected by the applied iFISH tests. Furthermore, our results suggest that due to its exon-level resolution, MLPA and/or digitalMLPA might be capable of detecting small focal lesions undetectable by

iFISH, albeit with a higher sample-purity requirement. On the other hand, iFISH can detect balanced translocations with high clinical relevance in PCM and it provides data at single-cell level, facilitating the scrutiny of clonal heterogeneity and evolution of plasma cells in individual patients. The technical advantages and limitations mentioned above and our results obtained by the comparison of MLPA, digitalMLPA and iFISH support the conclusion that these methods are complementary, and by their combined application, at least one biologically and/or clinically relevant genetic aberrations can be detected in nearly all myeloma patients.

Our combined iFISH analyses provided the first direct evidence on the temporal sequence of cytogenetic aberrations including structural abnormalities during the pathogenesis of PCM. These observations are consistent with the current developmental model of PCM, which considers *IGH* translocations as early events with chromosome 13 aberrations and *TP53* gene deletion emerging at a later stage of the disease. Our studies on clonal evolution have also elucidated that the screened *IGH* translocations are not present in all myeloma cells in at least one-fifth of the *IGH* positive patients, thus their tumor initiating role can be excluded.

In summary, we successfully established and tested molecular techniques (MLPA and digitalMLPA) which can reasonably improve the diagnostics of PCM patients in an economically sustainable way. Furthermore, with our single-cell studies, we managed to obtain internationally resounding novel data on the temporal sequence of cytogenetic aberrations and on their potential role in tumor initiation.

6. Summary of new results

1. First international publication on the application of MLPA to detect unbalanced genetic aberrations in PCM. Power and limitation of the technique have been described, and the results have been validated by iFISH which currently represents the most widely used gold-standard method.

2. First international publication on the application of digitalMLPA to the comprehensive profiling of unbalanced genetic aberrations in PCM. It has been demonstrated that besides detecting subchromosomal aberrations, the technique can reliably identify whole chromosome gains and losses, facilitating the robust identification of patients with hyperdiploid karyotype.

3. First international publication on the application of digitalMLPA to detect point mutations. The screened $BRAF^{V600E}$ mutation occurs in PCM with a frequency of 4-10% and allows for the application of targeted therapy. Mutations detected by digitalMLPA were validated by pyrosequencing and droplet digital PCR.

4. First comprehensive evaluation of molecular cytogenetic data obtained by the analysis of patients treated at the Clinical Center, University of Pécs and/or at the János Balassa Teaching Hospital of Tolna County over the past decade. It has been demonstrated that the combined application of MLPA and FISH could significantly improve the genetic profiling of Hungarian patients with PCM.

5. First international study on the clonal evolution of PCM at single-cell level using combined iFISH analysis. Our results confirmed that *IGH* translocations are early events during the oncogenesis of PCM; however, their initiating role can be challenged and even excluded in at least one-fifth of the *IGH* translocation positive cases.

7. Publications related to the thesis

Original articles

Nagy Z, Kajtár B, Jáksó P, Dávid M, **Kosztolányi S**, Hermesz J, Kereskai L, Pajor L, Alpár D. Evolutionary sequence of cytogenetic aberrations during the oncogenesis of plasma cell disorders. Direct evidence at single cell level. *Leuk Res*. 2011;35(8):1114-6. IF.: 2.923

Alpár D, de Jong D, Holczer-Nagy Z, Kajtár B, Savola S, Jákso P, Dávid M, **Kosztolányi S**, Kereskai L, Pajor L, Szuhai K. Multiplex ligation-dependent probe amplification and fluorescence in situ hybridization are complementary techniques to detect cytogenetic abnormalities in multiple myeloma. *Genes Chromosomes Cancer*. 2013;52(9):785-93. IF.: 3.836

Kosztolányi S, Kiss R, Atanesyan L, Gángo A, de Groot K, Steenkamer M, Jáksó P, Matolcsy A, Kajtár B, Pajor L, Szuhai K, Savola S, Bödör C, Alpár D. High-throughput copy number profiling by digital multiplex ligation-dependent probe amplification in multiple myeloma. *J Mol Diagn*. 2018;20(6):777-88. IF.: 4.426

Kosztolányi S, Horváth B, Hosnyánszki D, Kereskai L, Sziládi E, Jáksó P, Alizadeh H, Szuhai K, Alpár D, Kajtár B. Molecular cytogenetic analyses of patients with plasma cell myeloma in Tolna and Baranya counties in Hungary. *Orv Hetil*. 2019;160(24):944-51. [Hungarian] IF.: 0.564

Review paper

Kiss R, **Kosztolányi S**, Gángó A, Szuhai K, Bödör C, Alpár D. Multiplex ligation-dependent probe amplification in oncohematological diagnostics and research. *Orv Hetil*. 2018;159(15):583-92. [Hungarian] IF.: 0.322

Citable abstract

Kosztolányi S, Kiss R, Atanesyan L, Gángó A, De Groot K, Steenkamer M, Jáksó P, Matolcsy A, Kajtár B, Pajor L, Szuhai K, Savola S, Bödör C, Alpár D. Rapid and comprehensive screening for disease relevant copy number alterations in multiple myeloma using digital multiplex ligation dependent probe amplification. *Mol Cytogenet*. 2019;12(Suppl 1):43.

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