Optical genome mapping reveals complex cytogenetic abnormalities in multiple myeloma

Authors

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Supplementary Table S1. Detailed Results from Next Generation Sequencing, Karyotyping, Fluorescence *In Situ* Hybridization, and Optical Genome Mapping

MM Patient	NGS Gene Variant (VAF)	Karyotype	FISH*	OGM HR alterations/Classification of the cytogenetic profile‡ SVs/CNVs (ISCN 2024)†§
1	***TENT5C*** p.K128Nfs*22 (2%)	N/A	NORMAL	NO high-risk alterations identified **NORMAL** cytogenetic profile
2	N/A	No dividing cells	NORMAL	NO high-risk alterations identified. **NORMAL** cytogenetic profile
3	ND	NORMAL	NORMAL	NO high-risk alterations identified. **NORMAL** cytogenetic profile
4	***ZFHX4*** p.S2062Cfs*77 (23%) ***KRAS*** p.G13D (5%) ***NRAS*** p.Q61K (3%)	NORMAL	NORMAL	NO high-risk alterations identified. **NON-COMPLEX** cytogenetic profile with **hyperdiploidy** (ogm (9)x3[0.26],(15)x3[0.16],(19)x3[0.19])
5	***CYLD*** p.W847* (8%), ***ZFHX4*** p.A3245V (7%) ***FAT3*** p.E2048K (7%)	N/A	NORMAL	NO high-risk alterations identified. **NON-COMPLEX** cytogenetic profile. (ogm[GRCh38] 9q21.11q34.2(67018228_133526602)x3[0.19], 13q14.3q34.3(52185900_110867008)x1[0.2])
6	N/A	No dividing cells	1q21 gain	High-risk alterations identified: **1q21 gain.** **NON-COMPLEX** cytogenetic profile. (ogm[GRCh38] 1q21.1q44(144294282_248943333)x3[0.2],8p23.1p12(8158967_36285880)x1[0.2])
7	***DUSP2*** p.V224A (18%)	N/A	1q21 gain,	High-risk alterations identified: **1q21 gain.** **NON-COMPLEX** cytogenetic profile. (ogm[GRCh38] 1q21.1q44(144080779_248943333)x3[0.3],t(11;11)(q13.1;q13.3)(64406822;70013323)[0.21],t(11;11)(q13.1;q23.3)(65861642;120576312)[0.13])
8	***TRAF3*** p.K436Nfs*17 (5%) ***KRAS*** p.G12A (4%)	NORMAL	1p32 deletion 1q21 gain	High-risk alterations identified: **1p32 deletion, 1q21 gain.** **NON-COMPLEX** cytogenetic profile. (ogm[GRCh38] 1p33p32.3(49103054_50903109)x1[0.2],1q21.1q21.2(143278152_148928812)x3[0.3],13q21.1q33.3(57628403_107673930)x1[0.17])
9	***NRAS*** p.Q61R (13%)	No dividing cells	NORMAL	NO high-risk alterations identified. **COMPLEX** cytogenetic profile. (ogm[GRCh38] t(6;14)(p21.1;q32.33)(41973240;105710125)[0.36], 6p21.1p11.2(41974762_58445657)x1[0.3],9q33.1q34.11(117729225_130596749)x3[0.2], (13)x1[0.3],(14)x1[0.3],16q11.1q23.3(38277017_82690601)x1[0.3])
10	***NRAS*** p.Q61H (7%)	NORMAL	NORMAL	NO high-risk alterations identified. **COMPLEX** cytogenetic profile with **hyperdiploidy.** (ogm (5)x3[0.2],(9)x3[0.3],(11)x3[0.3],(15)x3[0.2],(19)x3[0.16],(21)x3[0.16])
11	ND	NORMAL	NORMAL	NO high-risk alterations identified. **COMPLEX** cytogenetic profile with **hyperdiploidy.** (ogm[GRCh38] t(X;3)(q27.3;p26.3)(12920;143474934)[0.56], t(1;8)(p22.2;p11.21)(90183393;41042182)[0.11],1p22.2p12(90469199_120191913)x1[0.3], t(1;22)(q23.3;q13.1)(161777800;38687685)[0.09],(3)x3[0.2],t(4;5)(p16.3;q14.3)(1901670;88835964)[0.13],5q14q35.3(88845196_181472714)x3[0.2], (7)x3[0.4],8p23.2p11.1(3935347_41156020)x1[0.25],9q21.11q34.2(65322076_133526602)x3[0.2],(11)x3[0.2],(14)x1[0.3],(15)x3[0.2], t(17;20)(q21.31;q13.13)(45283546;49126484)[0.08],(19)x3[0.2])
12	***TRAF3*** p.E271* (13%) ***LTB*** c.163-1G>A (11%)	55,XY,+2,+5,+7,+9,+11, -14,+15,+15,+19,+21, +mar[6]/46,XY[24]).	NORMAL	NO high-risk alterations identified. **COMPLEX** cytogenetic profile with **hyperdiploidy.** (ogm[GRCh38] (2)x3[0.3],(5)x3[0.7],6p25.3q27(76216_169967990)x3[0.3],t(6;14)(q21;q23.3)(106247425;65375083)[0.18],(7)x3[0.4],(9)x3[0.2],(11)x3[0.5], 14q23.3q32.31(65352855_102165030)x1[0.4],(15)x3[0.6],(19)x3[0.5],(21)x3[0.3])
13	***KRAS*** p.G12C (13%)	N/A	NORMAL	NO high-risk alterations identified. **COMPLEX** cytogenetic profile with **hyperdiploidy.** (ogm[GRCh38] Xq21.31q28(92477611_155695743)x2[0.6],(Y)x0[0.3],(3)x3[0.2],(5)x3[0.5],6p25.3q13(76216_69646176)x3[0.4], t(6;8)(p24.3;q24.21)(8750925;127741499)[0.17], t(6;21)(p24.3;q21.3)(8756616;29321555)[0.12],t(6;9)(q21;q31.1)(113958488;103806436)[0.14],(7)x3[0.2], 8p21.3p12(22305520_35035604)x1[0.3],(9)x3[0.2],(11)x3[0.2],(15)x3[0.2],(17)x3[0.3],(19)x3[0.2])

Supplementary Table S1. Detailed Results from Next Generation Sequencing, Karyotyping, Fluorescence *In Situ* Hybridization, and Optical Genome Mapping (continued)

MM Patient	NGS Gene Variant (VAF)	Karyotype	FISH*	OGM HR alterations/Classification of the cytogenetic profile‡ SVs/CNVs (ISCN 2024)†§
14	***KRAS*** p.G12C (13%)	N/A	NORMAL	NO high-risk alterations identified. **COMPLEX** cytogenetic profile with **hyperdiploidy.** (ogm[GRCh38] Xq21.31q28(92477611_155695743)x2[0.6], (Y)x0[0.3],(3)x3[0.2],(5)x3[0.5],6p25.3q13(76216_69646176)x3[0.4], t(6;8)(p24.3;q24.21)(8750925;127741499)[0.17], t(6;21)(p24.3;q21.3)(8756616;29321555)[0.12],t(6;9)(q21;q31.1)(113958488;103806436)[0.14], (7)x3[0.2],8p21.3p12(22305520_35035604)x1[0.3],(9)x3[0.2],(11)x3[0.2],(15)x3[0.2],(17)x3[0.3],(19)x3[0.2])
15	N/A	NORMAL	1p32 deletion	High-risk alterations identified: **1p32 deletion.** **COMPLEX** cytogenetic profile. (ogm[GRCh38] 1p33p21.1(47726928_105547900)x1[0.16],6p11.1q27(59282244_167047482)x1[0.16],t(6;20)(q14.1;q11.1)(76543631;29866072)[0.05],(15)x3[0.25])
16	***TP53*** Y220C (24%) ***CCND1*** p.Y44H (31%)	N/A	1q21 gain *CCND1::IGH*	High-risk alterations identified: **1q21 gain.** **COMPLEX** cytogenetic profile. (ogm[GRCh38] 1q21.1q44(145439805_248943333)x3[0.3],3p24.3p11(19114300_89780698)x1[0.3],t(5;8)(q33.3;q24.21)(156787070;127808529)[0.24], 8p23.2p12(2696469_34845069)x1[0.4],t(8;11)(p12;q12.3)(34857598;63447000)[0.11],t(11;14)(q13.3;q32.33)(69453684;105887565)[0.4], (13)x1[0.4],14q22.2q24.3(54824875_74983142)x1[0.4],16q11.1q24.1(38277017_85265163)x1[0.4])
17	ND	N/A	1p32 deletion 1q21 gain	High-risk alterations identified: **1p32 deletion, 1q21 gain.** **COMPLEX** cytogenetic profile. (ogm[GRCh38] Xq21.3q28(26047969_155695743)x2[0.1],(Y)x0[0.3],1p32.3(50750291_50991589)x1[0.1],1q21.1q23.3(144349979_161410556)x3[0.2], (4)x1[0.25],5p15.32p11(4889453_46400789)x3[0.25],13p11.1q14.3(17542017_53293428)x3[0.16],(19)x3[0.25])
18	***KRAS*** p.G13D (19%)	N/A	1q21 gain *CCND1::IGH*	High-risk alteration identified: **1q21 gain.** **HIGHLY COMPLEX** cytogenetic profile with **chromoanagenesis** affecting chromosomes X, 1, 3, 6, 8, 10, 11, 12, 14, 15, 17, 19, 20 and 22. The t(11;14) (*CCND1::IGH*) translocation is detected, along with numerous CNVs (gains) associated with the rearrangement breakpoints and trisomies of chromosomes 9 and 18.
19	***TP53*** p.V216M (8%)	No dividing cells	N/A	High-risk alterations identified: **17p deletion.** **HIGHLY COMPLEX** cytogenetic profile with **chromoanagenesis** affecting chromosomes 9, 12 and 15. (ogm[GRCh38] Xq23q28(113114252_155695743)x2[0.3],3p26.3q29(12920_195582326)x3[0.3],t(3;13)(p24.1;q21.1)(29318793;58877056), 4p16.1p13(7777952_42154587)x1[0.2],8p23.1q12.1(7685458_58265497)x3[0.2],8q24.13q24.3(123105050_141829244)x3[0.2],9p24.3p13.1(3997523_38885219)x3[0.2],t(9;9)(q21.11;q22.32)(69050405;94579639)[0.11],t(9;9)(q22.33;q34.12)(97250381;130692700)[0.13],(11)x3[0.3],t(12;12)(p13.1;q12)(13082454;41026558)[0.1],t(12;12)(q12 ;q13.13)(40964700;51695910)[0.1],t(15;15)(q12;q14)(26477223;36411226)[0.05],t(15;15)(q14;q21.3)(34258611;56714294)[0.12],17p13.1(7562929_7865216)x1[0.14],22q 11.23q12.3(25306236_33779120)x1[0.2])
20	***TP53*** p.R273C (86%) ***ZFHX4*** p.Y240* (44%)	NORMAL	1p32 deletion 1q21 gain 17p deletion	High-risk alterations identified: **1p32 deletion, 1q21 gain, 17p deletion.** **HIGHLY COMPLEX** cytogenetic profile with **hyperdiploidy** and **chromoanagenesis** affecting chromosomes 2 and 4. (ogm[GRCh38] Xq22.1q28(101912108_ 153085624)x2[0.2],t(1;17)(p36.21;q22)(14970717;59155199)[0.25],1p34.3p11.2(39493048_121608073)x1, 1q21.1q44(143278152_248943333)x3,t(1;2)(q43;p23.2)(243471142;29317751)[0.39], t(2;14)(p23.2;q32.11)(29222051;91322093)[0.47], t(2;2)(p23.1;p21)(30104027;47489559)[0.46],(3)x3[0.7],(4)x3[0.3],t(4;4)(q13.1;q21.1)(63907330;75894412)[0.06], t(4;4)(q21.1;q26)(76997597;116133956)[0.1],(5)x3[0.8],(6)x3[0.8],(7)x3[0.8],9q21.11q34.2(68310629_133526602)x3[0.3],(11)x3[0.8],(15)x3[0.8], 16p13.3(3051463_ 35562558)x3[0.8],17p13.1p11.2(9531696_16197684)x1[0.2], (19)x3[0.7],(21)x3[0.9])
21[a]	***NRAS*** p.Q61H (3.7%) ***RB1*** p.F336Sfs* (3.2%)	N/A	NORMAL	NO high-risk alterations identified. **NON-COMPLEX** cytogenetic profile. (ogm[GRCh38] t(X;5)(p22.12;q11.2)(55805207;19402072)[0.42],t(2;8)(p11.2;q24.21)(88623796;128150396)[0.06],t(8;11)(q21.3;q25)(90787268;135061119)[0.06])
21[b]	***NRAS*** p.Q61H (27%) ***RB1*** p.F336Sfs* (33%)	48,XY,t(2;8)(p13;q24), +3,del(3)(p13),-4,+6, add(6)(q27),del(6)(q23), +7,+9,der(9)t(1;9) (q12;p22),-13, dic(16;17)(q21;p13), -17,+mar[20]	1q21 gain 17p deletion	High-risk alterations identified: **1q21 gain, 17p deletion.** **HIGHLY COMPLEX** cytogenetic profile. (ogm[GRCh38] t(X;5)(p22.12;q11.2)(55805207;19403196)[0.5],1q21.1q44(144117875_248633312)x3[0.5],2p25.3p11.2(15924_88827954)x3[0.5], t(2;8)(p11.2;q24.21)(88623796;128139663)[0.16],t(3;3)(p12.3;p12.1)(78395759;83866294)[0.23],3q11.1q29(93819201_198230596)x3[0.5],(4)x1[0.6], 5p15.33p11(2186408_46118384)x3[0.5],6p25.3q26(76216_163053979)x3[0.5],t(6;6)(q26;q26)(161311088;161681393)[0.24], t(6;8)(q26;q11.1)(163057787;46376653)[0.21],(7)x3[0.5],8q11.1q24.1(45972483_127958936)x3[0.5],(9)x3[0.4],(13)x1[0.45],16q21q24.2(62505702_88143333)x1[0.5], t(16;17)(q21;p12)(62587688;14289017)[0.26],17p13.3p12(1315080_14305528)x1[0.5],19p13.2p11(9090781_ 24420459)x3[0.6])

Supplementary Table S1. Detailed Results from Next Generation Sequencing, Karyotyping, Fluorescence *In Situ* Hybridization, and Optical Genome Mapping (continued)

LCP Patient	NGS Gene Variant (VAF)	Karyotype	FISH*	OGM HR alterations†/Classification of the cytogenetic profile‡ SVs/CNVs (ISCN 2024)§
22	***DIS3*** p.D488N (39%) ***CYLD*** p.S409* (25%) ***KRAS*** p.G12R (67%) ***KRAS*** p.A59E (5%) ***BRAF*** p.G469A (4%)	N/A	1q21 gain	High-risk alterations identified: **1q21 gain.** **COMPLEX** cytogenetic profile. (ogm[GRCh38] t(X;10)(q27.3;p12.33)(18,215,572;145,450,166)[0.51],Xq27.3q28(145,473,969_155,695,747)x2[0.25],1q21.1q44(143278152_248642991)x3[0.2], 10p15.3p12.33(18514_6242295)x1[0.7],t(11;14)(q13.3;q32.33)(69511003;106340554)[0.44],11q13.3q25(68975206_135069565)x3[0.7])
23	ND	43,X,-X,+3, add(6)(q13), -11, -12,-13, add(16)(q24),-20, +mar[9]/43,sl, der(8)t(1;8)(q21;p23)[9] /43,sdl1,add(22)(p13)[2]/ 43,sdl2,der(17)t(1;17)(q2 1;p13)[3]/46,XX[2]	1q21 gain *FGFR3::IGH*	High-risk alterations identified: **1q21 gain, *FGFR3*::*IGH* (t(4;14)).** **COMPLEX** cytogenetic profile. (ogm[GRCh38] (X)x1[0.5],1q21.1q44(144293458_248304428)x3[0.4],(3)x3[0.5],t(4;14)(p16.3;q32.33)(1883975;106365267)[0.27], 6q12q27(68295166_167058007)x1[0.5],t(6;7)(q12;q21.12)(68295166;87457043)[0.23],7q11.23q36.3(77693781_158044213)x3[0.4], t(11;11)(q12.1;q22.3)(56028409;109141119)[0.21],11q14.1q22.3(85479021_104641634)x1[0.5],12p13.32q13.12(4271654_51023363)x1[0.6], t(12;20)(q13.12;p11.21)(51055371;22714750)[0.26],(13)x1[0.6],16q11.2q23.3(46478822_83057720)x1[0.5],20p13p11.21(3413731_22796609)x1[0.5])
24	***TP53*** p.R273H (64%) ***KRAS*** p.G12S (38%) ***TENT5C*** p.F177Lfs*34 (15%)	NORMAL	N/A	High-risk alterations identified**: 1p32 deletion, 17p deletion.** **HIGHLY COMPLEX** cytogenetic profile with **chromoanagenesis** events affecting chromosomes X, 3, 5, 8, 11, 13 and 22. Additional rearrangements, CNVs associated with translocation breakpoints, trisomies of chromosomes 9, 14, and 19, as well as monosomy of chromosome 13, are observed.

NGS: Next Generation Sequencing; **VAF**: Variant Allele Frequency; **FISH**: Fluorescence *In Situ* Hybridization; **OGM**: Optical Genome Mapping; **HR:**High-Risk; **SV**: Structural Variant; **CNV**:Copy Number Variant; **ISCN**: International System for Human Cytogenetic Nomenclature 2024; **MM**: Multiple Myeloma; **PCL**: Plasma Cell Leukemia, **N/A**: Not Available; **ND:** Not Detected.

***** In the FISH study, probes were used to analyze recurrent and/or clinically relevant alterations: t(4;14), *FGFR3::IGH*; t(11;14) *CCND1::IGH*; and t(14;16), *IGH::MAF*; as well as 17p13 deletion (*TP53*), 1p32 deletion (*CDKN2C*), and 1q21 gain (*CKS1B*).

† HR alterations: t(4;14), t(14;16), t(14;20), 1q21 gain, 1p32 deletion, and 17p deletion.

‡ Classification of the cytogenetic profile: NORMAL (no significant SVs or CNVs detected), NON-COMPLEX (≤3 SVs/CNVs detected), COMPLEX (4-15 SVs/CNVs detected), HIGHLY COMPLEX (>15 SVs/CNVs detected with or without the presence of chromoanagenesis).

§ ISCN 2024 formula considering CNVs >5 Mb, SVs >1 Mb, and clinically relevant SVs <1 Mb, including interchromosomal and intrachromosomal translocations. In highly complex cases (patients #18 and #24) with numerous SVs, the chromosomal formula is not specified due to their great complexity

[a,b] Results from the same patient with Kappa IgA MM (patient #21) obtained at baseline (a) and at the time of progression six months later (b).

SUPPLEMENTARY FIGURE 1

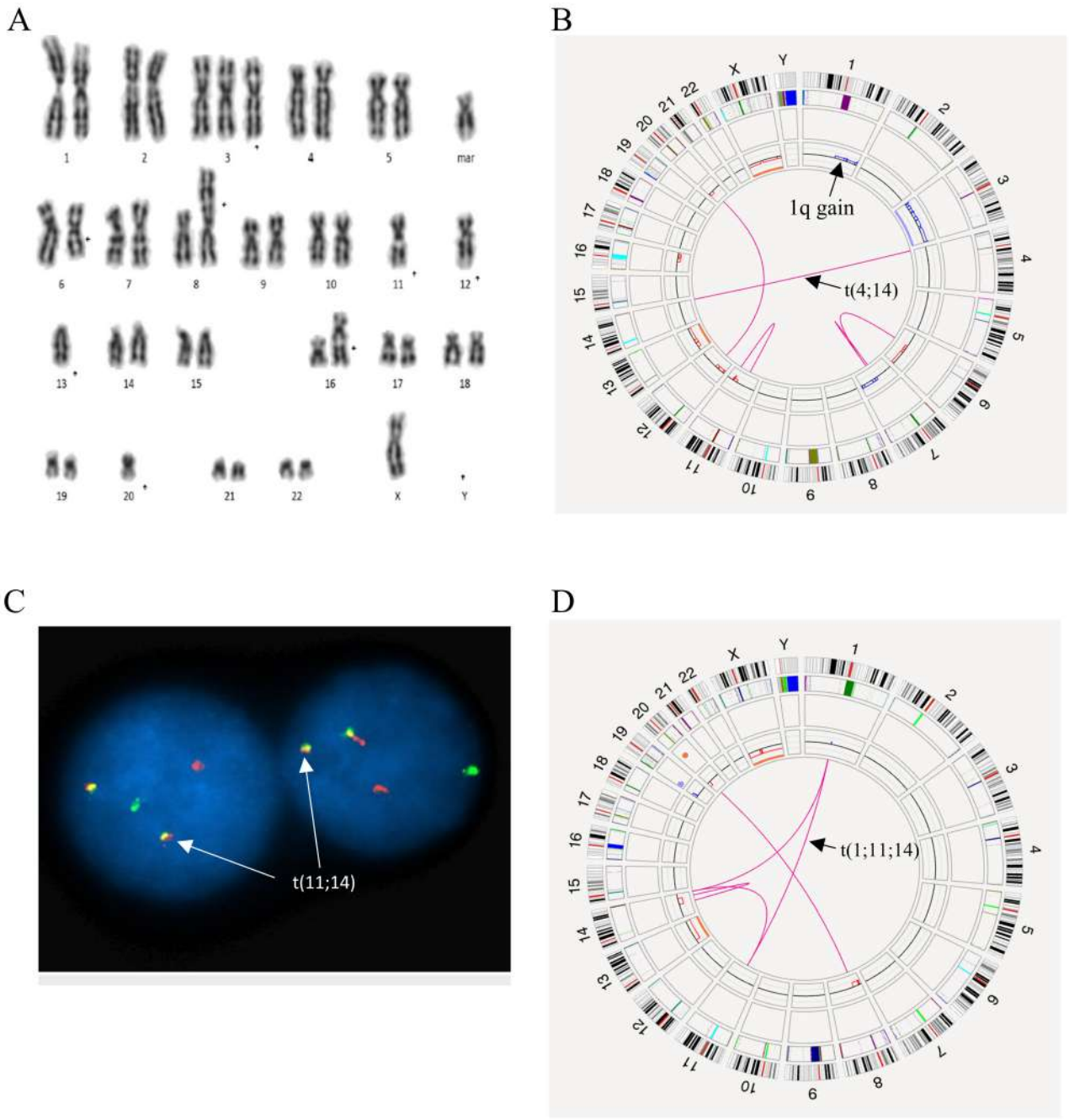

Figure 1. Chromosomal alterations identified by Optical Genome Mapping (OGM) compared to findings from conventional cytogenetic techniques. (A) Karyotype and **(B)** *Circos plot* of chromosomal alterations obtained using Bionano Access software for a patient with plasma cell leukemia (patient #23). The karyotype shows a complex hypodiploid profile (43 chromosomes), while OGM provides a more detailed and accurate representation of chromosomal abnormalities, including partial deletions in chromosomes 6, 11, 12, 16, and 20 as well as a gain on 7q and a t(12;20) translocation. Karyotyping identified two subclonal translocations (t(1;8) and t(1;17)) associated with the 1q gain, which were not detected by OGM because the breakpoints of the rearrangements involve regions of heterochromatin that are difficult to map. High-risk alteration relevant to disease prognosis, such as the t(4;14)(*FGFR3*::*IGH*) translocation was detected by OGM but not identified by karyotyping.
(C) FISH image showing the t(11;14)(*CCND1*::*IGH*) rearrangement using the XL MYEOV/IGH DF Dual Fusion probe (Metasystems) and **(D)** *Circos plot* of chromosomal alterations in patient #14 with multiple myeloma. Although FISH detects the t(11;14) translocation, OGM reveals that it is part of a more complex rearrangement involving chromosome 1, suggesting chromoplexy, a subtype of chromoanagenesis, among chromosomes 1, 11, and 14. OGM also identifies additional abnormalities, including monosomies of chromosomes X and 13, partial deletions in the long arm of chromosome 14 and in chromosomes 8 and 20, associated with a t(8;20) translocation, and a gain in the 19p region.
From the outer to the inner rings of the *Circos plot*, the following layers are displayed: (1) chromosomes and cytobands, (2) masked regions (heterochromatic, difficult to analyze), (3) structural variant (SV) region, (4) copy number variation (CNV) profile, and (5) in the center, inter- and intrachromosomal translocations represented as pink lines connecting the involved genomic loci. SVs<1 Mb were filtered out from the *Circos plot.*