Dynamic chromosomal rearrangements in Hodgkin's lymphoma are due to ongoing three-dimensional nuclear remodeling and breakage-bridge-fusion cycles

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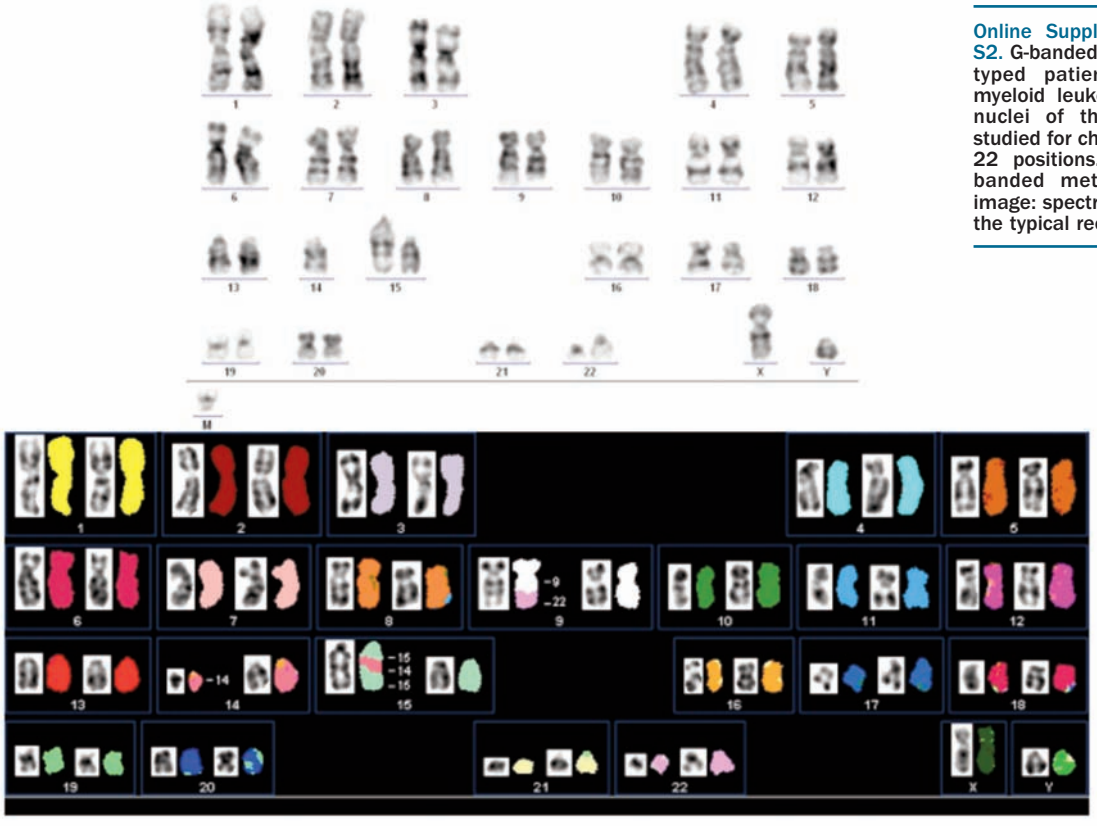
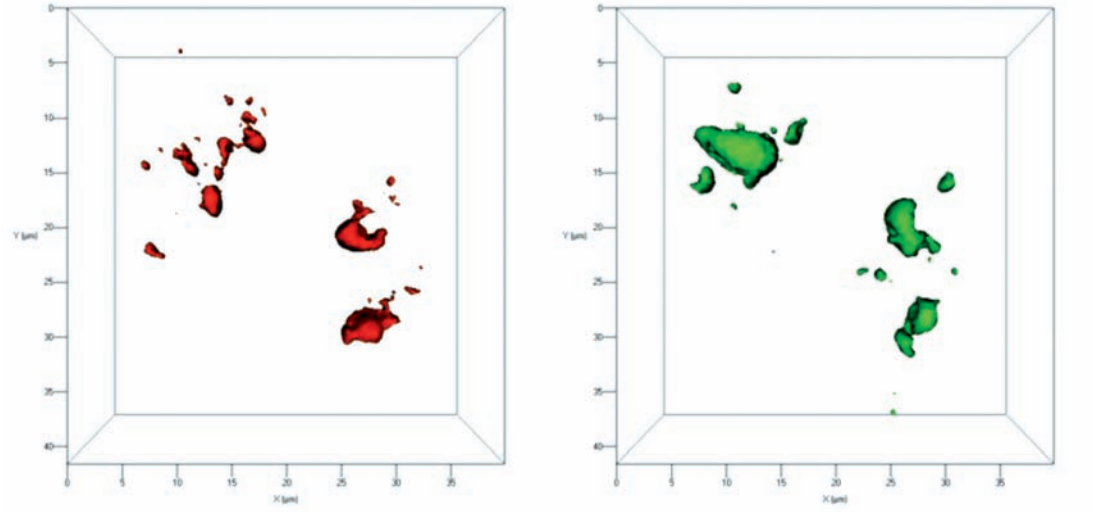
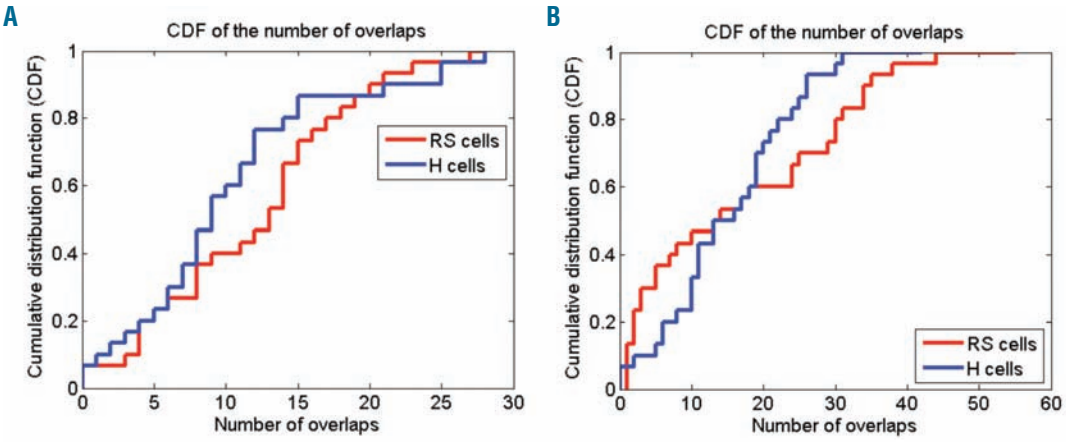
Online Supplementary Figure S1. Semi-transparent 3D surface renderings of (A) signals from the distinct chromosome channels (red and green) and (B) overlapping regions between the channels. (C) Overlap images were thresholded using parameters established from training data to exclude small overlaps that were not likely to represent translocations.



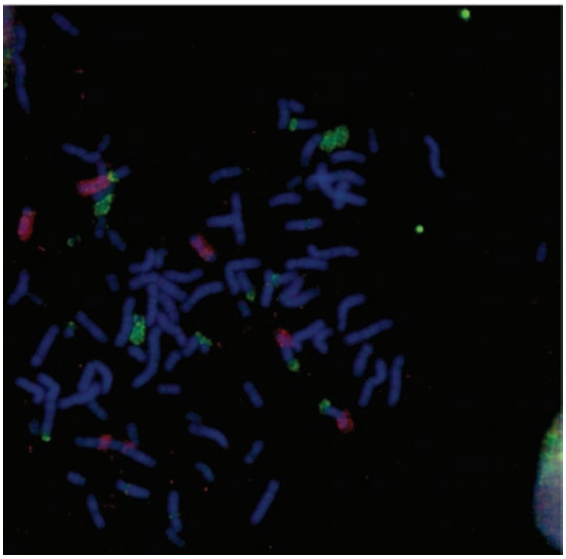
Online Supplementary Figure S2. G-banded and SKY karyotyped patient with chronic myeloid leukemia. Interphase nuclei of this patient were studied for chromosome 9 and 22 positions. Top image: G-banded metaphase; bottom image: spectral karyotype with the typical reciprocal t(9;22).



Online Supplementary Figure S3. Interphase chromosome positions of mononucleated H cells of HDLM-2. Separate 3D representation of chromosomes 9 (red) (left image) and 22 (green) (right image) of the H nuclei shown in Figure 2A of the article.

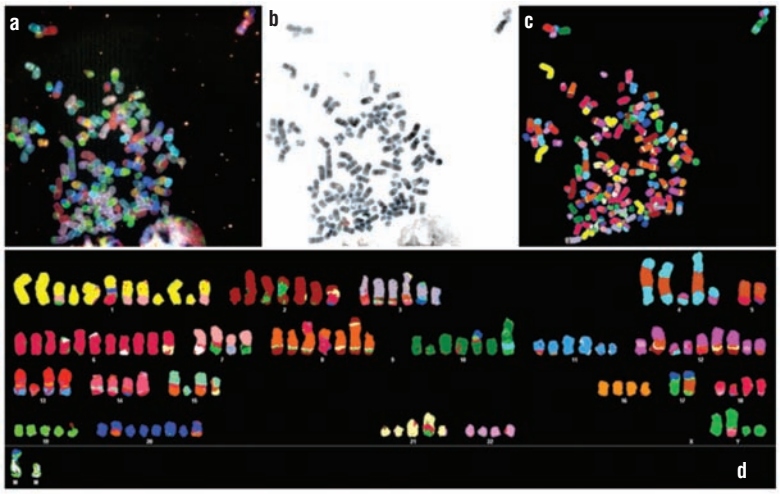


Online Supplementary Figure S4. Culmulative distribution functions of the number of overlaps for chromosomes 9 and 22. The curves illustrate the distribution of chromosomes 9 and 22 for L-428 (P=0.11), and L-1236 (P=0.34). The P values are obtained by the Kolmogorov-Smirnov test applied to the data shown in the figures. The red lines illustrate the overlaps of chromosomes 9 and 22 in RS cells, while the blue lines show this comparison for H cells. In each cell line, 30 nuclei were measured each for H and RS cells.

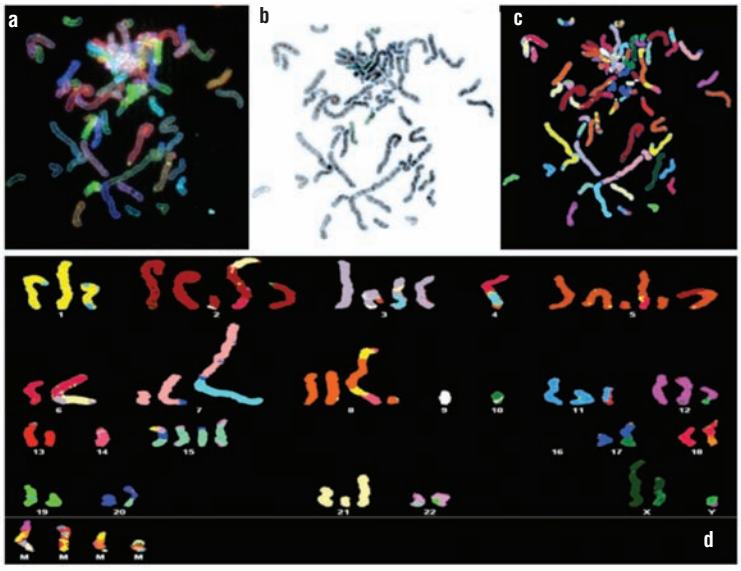


Online Supplementary Figure S5. Representative L-428 metaphase spread illustrating multiple rearrangements involving chromosomes 9 (red) and 22 (green). Note multiple insertions, translocations and fusions. For more details, see text and Table 1 summarizing the overall involvement of chromosomes 9 and 22 in HDLM-2, L-428 and L-1236.

A

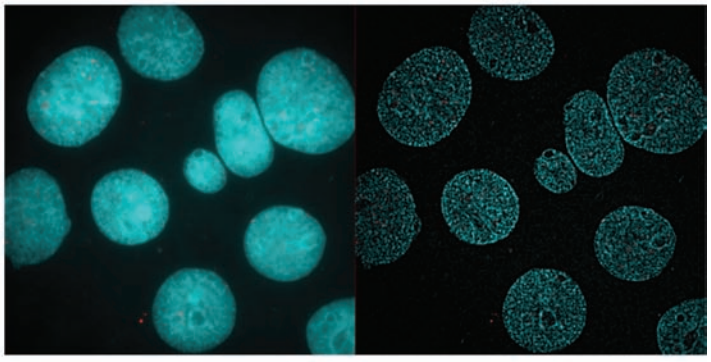


B

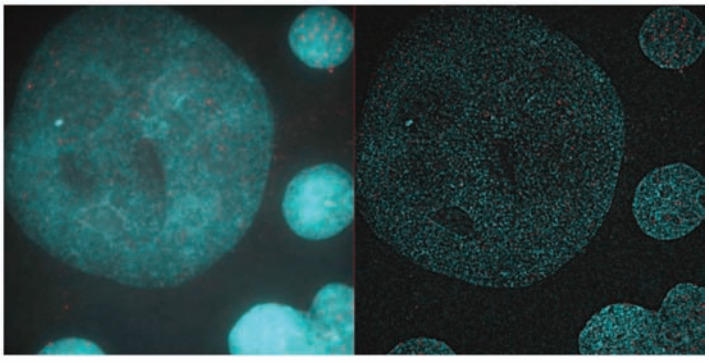


Online Supplementary Figure S6. Spectral karyotyping (SKY) of HDLM-2 (Panel A) and L-1236 (Panel B). Complex rearrangements in HDLM-2 and L-1236, indicative of BBF cycles, unequal chromosome segregation and centrosome duplication defects (Knecht *et al.*, 2009). (a) is the raw spectral image, (b) is the inverted DAPI image, (c) is the pseudo colour image and (d) is the karyotype table, which shows the classification of chromosomes. See also *Online Supplementary Table S1* for a summary of all aberrations detected.

A



B



Online Supplementary Figure S7. Super resolution imaging (3D-SIM) of H and RS cells. 3D-fixed nuclei of HDLM-2 were imaged using 3D-SIM. (A) Maximum projection images of wide field (left) and 3D-SIM (right) of mono-nucleated H cells. The nucleus is counterstained with blue (DAPI), telomeres are shown in red (cyanine 3). (B) Maximum projection wide field (left) and 3D-SIM (right) images showing RS cell next to H cells. The nuclei are blue (DAPI) and telomeres are red (cyanine 3).

Online Supplementary Table S1. Summary of numerical and structural aberrations detected by SKY in HDLM-2, L-1236, and L-428 cells. Twenty metaphases were analyzed for each of the cell lines. The copy number of each chromosome was recorded along with all structural aberrations. Translocations noted in red or green indicate the evolution of H to RS cell. (SEE EXCEL FILE)

Online Supplementary Table S2. Summary of telomere metaphase Q-FISH for HDLM-2, L-428, and L-1236 cells. Twenty metaphases were imaged and the presence *versus* absence of telomeric signals as well as of interstitial telomeric signals recorded.

HDLM-2	Telomere free end	Interstitial telomeric signal
image 1	3	9
image 2	2	6
image 3	7	3
image 4	4	5
image 5	5	2
image 6	3	4
image 7	3	5
image 8	2	3
image 9	7	4
image 10	3	3
image 11	1	1
image 12	0	1
image 13	0	1
image 14	2	0
image 15	4	1
image 16	1	1
image 17	4	0
image 18	2	0
image 19	2	1
image 20	2	0

L-1236	Telomere free end	Interstitial telomeric signal
image 1	3	1
image 2	8	4
image 3	2	3
image 4	1	0
image 5	0	1
image 6	2	3
image 7	0	2
image 8	0	1
image 9	1	1
image 10	0	2
image 11	1	2
image 12	2	1
image 13	2	1
image 14	0	1
image 15	1	0
image 16	3	1
image 17	2	1
image 18	3	1
image 19	0	0
image 20	1	1

L-428	Telomere free end	Interstitial telomeric signal
image 1	3	0
image 2	6	2
image 3	1	1
image 4	2	1
image 5	3	0
image 6	2	1
image 7	3	0
image 8	2	0
image 9	2	1
image 10	1	0
image 11	3	1
image 12	2	0
image 13	3	0
image 14	2	2
image 15	3	1
image 16	5	1
image 17	0	1
image 18	3	2
image 19	1	0
image 20	3	1