Optical genome mapping and fluorescence in situ hybridization is the best combination to identify genomic complexity and TP53 deletions in CLL patients

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Introduction

- Fluorescence in situ hybridization (FISH) is the gold-standard cytogenetic technique for the detection of the most frequent chromosomal alterations (CA) in chronic lymphocytic leukemia (CLL).
- In 2000, Döhner et al. established a hierarchical model for risk stratification including the following groups: isolated del13q, no abnormalities (normal), trisomy 12, del11q and del17p. Furthermore, complex karyotype (CK, ≥3 CA) identified by chromosome banding analysis (CBA) is also a poor prognostic and potentially predictive biomarker.
- Optical genome mapping (OGM) is a novel method that relies on the imaging of long DNA molecules (>250 Kb) labeled at specific sites.
- The unique pattern of labels throughout the genome allows their genomic location to be precisely mapped permitting the detection of both balanced and unbalanced abnormalities with high resolution.
- OGM has shown promising results for CK assessment in CLL (Puiggros et al, 2022).

Objective

To determine the ability of OGM to detect poor prognostic biomarkers in CLL by evaluating the concordance with FISH and CBA results to investigate the potential of OGM as a routine diagnostic test.

Patients and methods

- 102 patients from two European centers were selected: 86 CLL (59 Binet A, 17 Binet B/C) and 8 MBL; 77 men; 62 median age at diagnosis.
- FISH (13q, CEP12, *ATM* and *TP53*) and CBA data were available in all patients (Table1).
- FISH was performed on fixed peripheral blood (PB) cells (n=54) or PB (n=47) or bone marrow (BM) (n=1) after IL2+DSP30 cultures.
- CBA was performed on PB (n=101) or BM (n=1) cell cultures with IL2+DSP30 as mitogens, and in a second parallel PB cultures with TPA (n=54).
- For OGM analysis, DNA was extracted from whole PB (n=58), PB mononuclear cells (n=43) and BM (n=1). Samples were processed using the Saphyr System (Bionano Genomics) and analyses were performed using Bionano Access 1.7.2 with the Rare Variant Analysis pipeline. Clinically relevant CA (2p, 8q, 12 gains; 6q, 8p, 11q, 13q, 15q, 17p deletions; IGH, IGK, IGL, *BCL2* rearrangements), other translocations and CA ≥5Mb detected with OGM were compared with those obtained by conventional techniques (CBA and FISH).

Table 1. Comparison of chromosomal abnormalities detection and Döhner's hierarchical risk classification by FISH and OGM.

	FISH		OGM		
Genomic alterations	**Nº of patients (%) n=102**	**Median % of altered nuclei (range)**	**Nº of patients (%) n=102**	**Median % VAF (range)**	**Nº of FISH positive patients detected (%)**
del(13)(q14)	58 (57%)	76 (6-100)	51 (50%)	45 (2-92)	50/58 (86%)
Trisomy 12	19 (19%)	58 (11-88)	18 (18%)	37 (28-50)	18/19 (95%)
del(11)(q22q23)	29 (28%)	70 (7-99)	25 (24%)	43 (3-53)	24/29 (83%)
del(17)(p13)	20 (20%)	46 (13-98)	13 (13%)	39 (15-47)	13/20 (65%)
Risk group according to Döhner hierarquical classification	**Nº of patients (%) n=102**		**Nº of patients (%) n=102**		
13q deletion as sole abnormality	19 (19%)		22 (22%)		
Trisomy 12	17 (17%)		16 (16%)		
del(11)(q22q23)	28 (27%)		25 (25%)		
del(17)(p13)	20 (20%)		13 (13%)		
Normal FISH	18 (18%)		23 (23%)		

Results

1. Comparison of results between FISH and OGM

- In the 102 patients, 128 abnormalities were detected by FISH and/or OGM. Of these, 105 abnormalities were concordant (105/128, 82%) by both techniques (Figure1, Figure2A).
- Among the 23 discordant abnormalities (n=20 patients):
  - 2/23 were detected only by OGM (n=2 patients):
    - One case with a del13q of 7Kb (below FISH resolution).
    - One case with a del11q (ATM) of 211Kb (deleting only partially the FISH probe, false negative).
  - 21/23 were detected only by FISH (n=18 patients) (Figure 2A)
    - All cases were below OGM sensitivity or limitation of OGM pipeline analysis.
- Regarding the percentage of clonality detection in del13q (n=58), trisomy12 (n=19) and del11q alterations (n=29) (Table 1):
  - All these FISH abnormalities present in >24% cells (n=84 abnormalities) were identified by OGM.
  - For the 22 abnormalities detected in ≤24% cells:
    - OGM confirmed 8 abnormalities (8/22, range of FISH clonality percentage: 12-24%, median 18%).
    - OGM did not detect 14 abnormalities (14/22, range: 6-24%, median 12%).
- Regarding the percentage of clonality detection in del17p group (n=20) (Table 1):
  - 7/20 patients (35%) with 17p deletions were not identified by OGM software (range: 13-32%, median 17%).
  - However, 5/7 were detected by reviewing visually the whole genome view. Taking this into account, the percentage of detection of del17p was 90% (18/20).
- If OGM would have been implemented as a stand-alone technique to reproduce Döhner hierarchical model this would result in reclassification of 85% (17/20 patients) of patients with discordant OGM/FISH results.
  - 11/17 patients would change from high-risk to low-risk. Nevertheless, four of these discordant cases showed high complexity by both CBA and OGM, which is a predictor of worse prognosis.
  - One of the two patients with discordant FISH/OGM results, in which additional alterations were detected by OGM, changed from low-risk to high-risk group, and the other moved to a group with more favorable prognosis (Table 2, Figure2B).

Figure 1. Number of del13q, trisomy 12, del11q and del17p abnormalities detected by FISH and OGM.

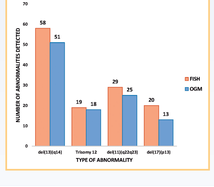


Figure 2. A) Distribution of the alterations detected by FISH and OGM and classification of the discordant alterations by OGM; B) Patients reclassification according to Dönher's hierarchical model using OGM.

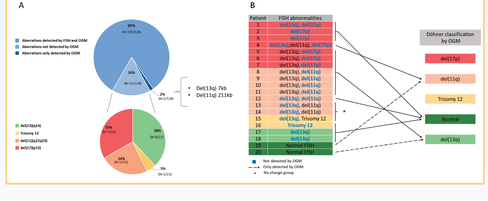
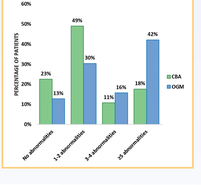


Figure 3. Classification of patients according to the number of anomalies detected by CBA and OGM.



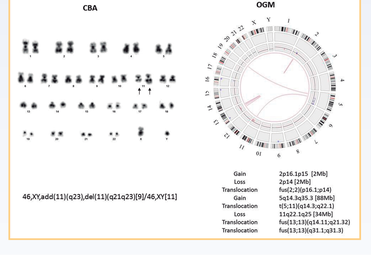
2. Comparison of results between CBA and OGM

- OGM detected a greater proportion of patients with CA than CBA (87% vs. 77%, p= 0.066) (Figure 3).
- The median number of aberrations was also higher with OGM (4 (1-66) vs 2 (1-14), p<0.001).
  - Patients with a normal karyotype (n=23): 11/23 showed 1 to 5 aberrations and 1/23 showed high complexity by OGM.
  - Patients with abnormal karyotype (n=79):
    - OGM detected abnormalities in 74/79 patients.
    - OGM failed to detect CA in 5/79 patients:
      - an isolated 6q deletion present in 14% nuclei.
      - two 11q deletion present in 8% and 12% nuclei.
      - a trisomy 12 in three metaphases.
      - a dic(4;17)(p14;p12) in three metaphases.
  - In 7/102 patients, catastrophic events were detected: 2 showed chromothripsis, 2 showed chromoplexy and 3 cases presented both events.

3. Complexity detection by OGM

- Patients with CK (n=25; 17/25 with ≥5 CA) showed a significantly higher median number of aberrations by OGM than by CBA (11 (5-66) vs 5 (3-14), p<0.001).
- Among patients with abnormal non-CK, 15/54 (28%) showed ≥5 aberrations by OGM (Figure 4), which was the minimum number of CA in the CK group.
- In cases with ≥5 CA by CBA (High-CK), the minimum number of aberrations by OGM was 6.

Figure 4. Example of a CLL case with two abnormalities detected by CBA and eight abnormalities by OGM. While 11q deletion was detected by both methods, OGM results suggested that the additional material in 11q found in the karyotype was an unbalanced t(5;11) and also revealed the presence of other CNV and structural abnormalities missed by CBA.



Conclusions

1. OGM is a robust method to assess genome-wide aberrations in CLL:
   a. Its independence of cell cultures and a higher genomic resolution increases the detection rate of genomic complexity.
   b. To define clear genomic complexity criteria by OGM, additional studies are needed and its clinical impact should be validated.
2. OGM sensitivity is limited and small cell clones could be missed. Thus, FISH for del17p (*TP53*) detection should be maintained in the routine management of CLL patients.

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