

Prognostic significance of translocations in the presence of mutated IGHV and of cytogenetic complexity at diagnosis of chronic lymphocytic leukemia

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Supplemental Methods

Cytogenetic Analysis

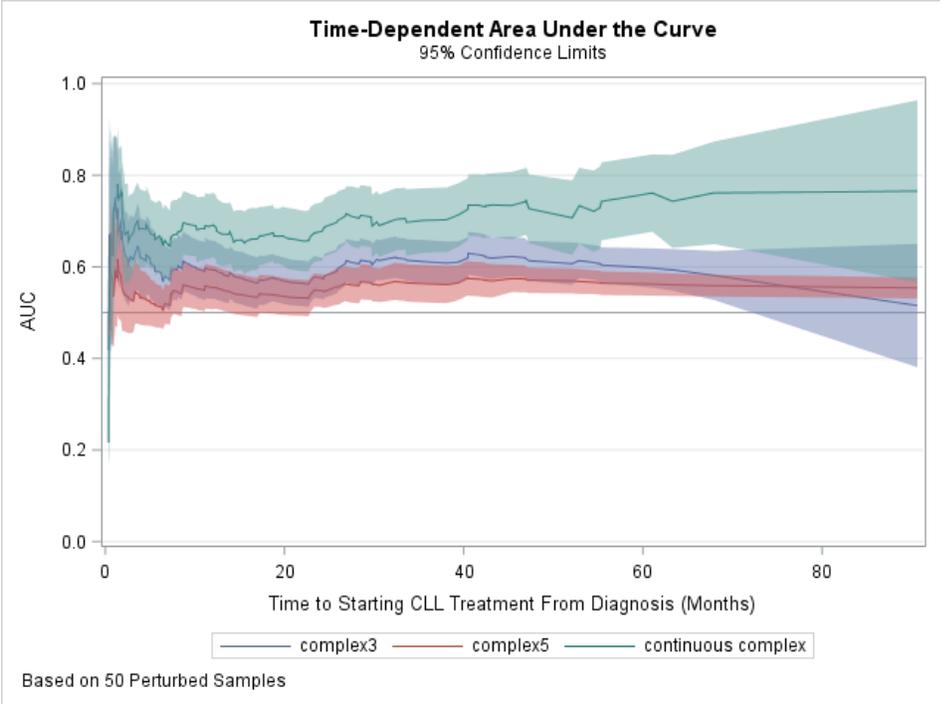
Cells from peripheral blood or bone marrow (2.0×10^6 cells/ml) were incubated in RPMI 1640 medium (Fischer Scientific, Houston, TX) with 2% L-glutamine (Gibco Invitrogen, Carlsbad, CA), supplemented with 20% fetal bovine serum (Hyclone Laboratories, Logan TX), and 2% penicillin and streptomycin (Gibco, Invitrogen, Carlsbad, CA). The mitogens used in these studies were a combination of CpG ODN 685 (20 $\mu\text{g}/\text{ml}$; synthesized by Sigma Aldrich, St. Louis, MO), pokeweed mitogen (PWM, final concentration = 10 $\mu\text{g}/\text{mL}$, Sigma Aldrich) and phorbol 12-myristate 13-acetate (PMA, final concentration = 40ng/ml, Sigma Aldrich), or use of CpG ODN 685 only (20 $\mu\text{g}/\text{ml}$). The mitogens were added to the cultures, and the cells were incubated for 72 hours at 37° C in a 5% CO₂ atmosphere. Harvest was by standard laboratory procedures. All samples were G-banded using trypsin and were stained with Wright stain according to standard laboratory protocols. Each CLL sample was analyzed using both mitogen combinations, unless there were insufficient cells. A total of at least 20 metaphases were analyzed for each sample whenever possible. Analysis of a minimum of 20 cells was required for the sample to be considered to have normal chromosomes. Cases with sole gain or loss of a sex chromosome were considered normal, as those abnormalities are most likely age-related rather than disease related. A clone was defined as two cells with the same chromosomal gain(s) or structural abnormality(ies) or three cells with loss of the same chromosome(s) [ISCN 2013] ¹.

1. International Standing Committee on Human Cytogenetic Nomenclature., Shaffer LG, McGowan-Jordan J, Schmid M. ISCN 2013 : an international system for human cytogenetic nomenclature (2013). Basel: Karger; 2013.

Supplemental Table 1. Area Under the Curve at Different Time Points to First CLL Treatment Using Continuous Complexity Score, Complex3 (≥ 3 Aberrations) and Complex5 (≥ 5 Aberrations)

Time to Treatment (months)	AUC		
	Continuous Complex	Complex3	Complex5
12	0.6537	0.5953	0.5557
24	0.6386	0.5772	0.5461
36	0.6599	0.6174	0.5622
48	0.679	0.6145	0.5609
60	0.7164	0.6000	0.5539
72	0.7304	0.5874	0.5481
84	0.7304	0.5874	0.5481

Supplemental Figure 1. Time-Dependent Area Under the Curve With 95% Confidence Limits for Time to First CLL Treatment Using Continuous Complexity Score, Complex3 (≥ 3 aberrations) and Complex5 (≥ 5 aberrations)



Supplemental Figure 2. Treatment Free Survival Curves by Translocation and IGHV, Stratified by Complex Karyotype (≥ 3 Aberrations).

