Biomarker Analysis for Early Signs of Cancer Treatment Response

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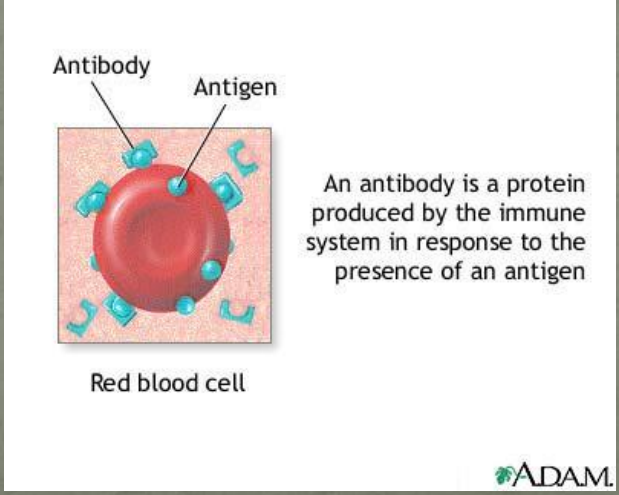
Background

- The term leukemia is used to describe cancer of white blood cells in the blood or bone marrow.
- Chronic Lymphocytic Leukemia (CLL) is the most common leukemia in the western world.
- CLL is a type of leukemia that affects lymphocytic white blood cells.

Background

- A lymphocyte is a type of white blood cell in the vertebrate immune system.
- One type of lymphocyte is the B cell, which is responsible for humoral immunity (antibodies).
- T cells are the other type of lymphocyte present in human body.



Pathogenesis

- CLL starts in the bone marrow and spreads to the blood.
- Lymphocytes are made in the bone marrow.
- CLL results from an acquired injury to the DNA of a single marrow cell that is to become a lymphocyte.

Pathogenesis

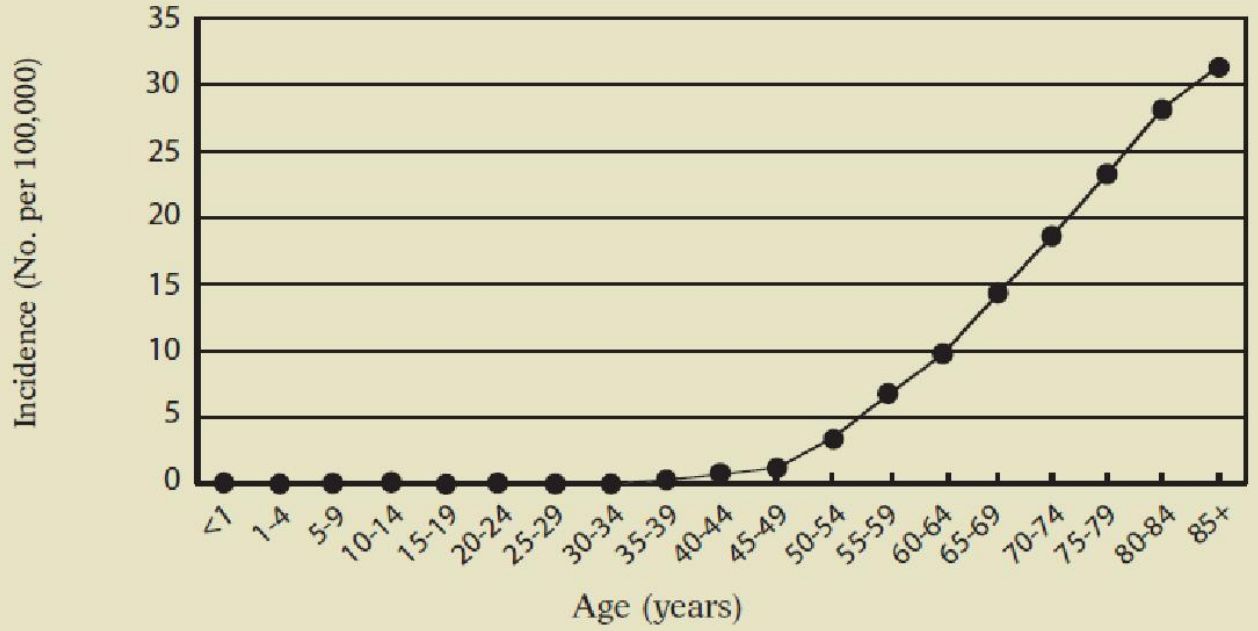
- When the damaged cancerous cell reproduces, the new cells are able to proliferate better than normal lymphocytes.
- CLL however does not actually impede the growth of normal white blood cells.
- CLL is characterized by a gradual increase in the number of white blood cells.

Risks

- CLL has not been associated with any environmental factors.
- Whites have been observed to have a higher risk of developing the disease. Men have a higher incidence of the disease than women.
- Older individuals have a higher risk of developing CLL, with the average age being 70.

Demographics

Chronic Lymphocytic Leukemia— Age-Specific Incidence Rates 1998-2002



Treatments/Side Effects

- Previous and preferred form of treatment was Chlorambucil.
- Now treatment uses Fludarabine, which yielded a higher response rate and longer progression-free survival.
- Side effects: fever, nausea, fatigue, diarrhea, changes in menstrual period, just to name a few.

New Treatment-CpG 7909

- CpG Oligodeoxynucleotides (ODN) can be in the form of Bacterial DNA or synthetic ODN containing the CpG motif.
- CpG ODN induce apoptosis of CLL cells.
- Treatment increases the expression and sensitivity of protein surface markers to antibodies. This helps the body elicit an immune response against the CLL cells.

Clinical Trial

- A clinical trial was carried out jointly at the University of Iowa and Mayo Clinic.
- The objective of the Phase I clinical trial was to establish a safe dose with biological activity.
- The purpose of the clinical trial was to determine the relative increase in surface expression of CD20 and assess the safety of CpG 7909.

Objective

- The goal of our project is to analyze biomarkers, collected in the clinical trial, for early evidence of treatment efficacy.

Study Design

- Phase I Clinical Trial
- 3 Dose levels and 2 routes of administration (IV and SQ)
- 6 patients per group
- Surface protein markers measured at baseline and Days 3, 7, and 28.
- Number of markers observed was 116.

Dataset

- For the actual data, we removed patients that didn't have any observations.
- We also noticed that only a few patients had data for Day 28.

Dataset Numbers

Day3			Day7			Day 28	
IV/Dose	N		IV/Dose	N		IV/Dose	N
1	6		1	5		1	2
2	5		2	4		2	5
3	5		3	5		3	5

Day3			Day7			Day 28	
SQ/Dose	N		SQ/Dose	N		SQ/Dose	N
1	5		1	4		1	0
2	6		2	6		2	5
3	5		3	4		3	5

Protein Markers

- CD22+ CD19-
- CD22+ CD19+
- CD22- CD19-
- CD22- CD19 +
- CD27+CD19-
- CD27+CD19+
- CD27-CD19-
- CD27-CD19+
- CD22+ CD27-
- CD22+ CD27+
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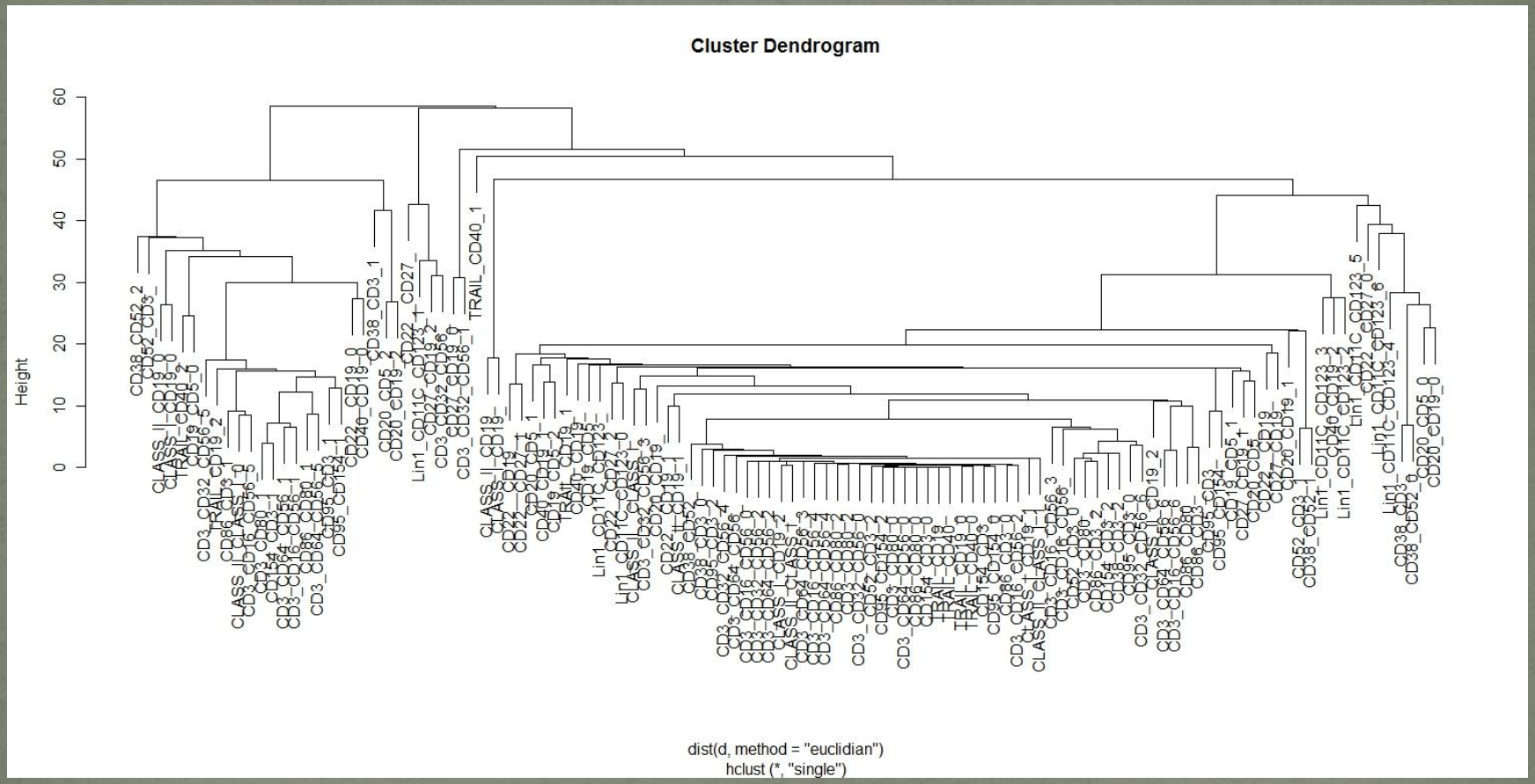
- CD20-CD19-
- CD20-CD19+

Methods

- The change in markers was monitored from baseline to day 3, baseline to day 7, and baseline to day 28.
- Hierarchical clustering was used to get some idea of how certain markers would be clustered together later on.
- Similarity was quantified using Euclidian distance to form clusters of markers.

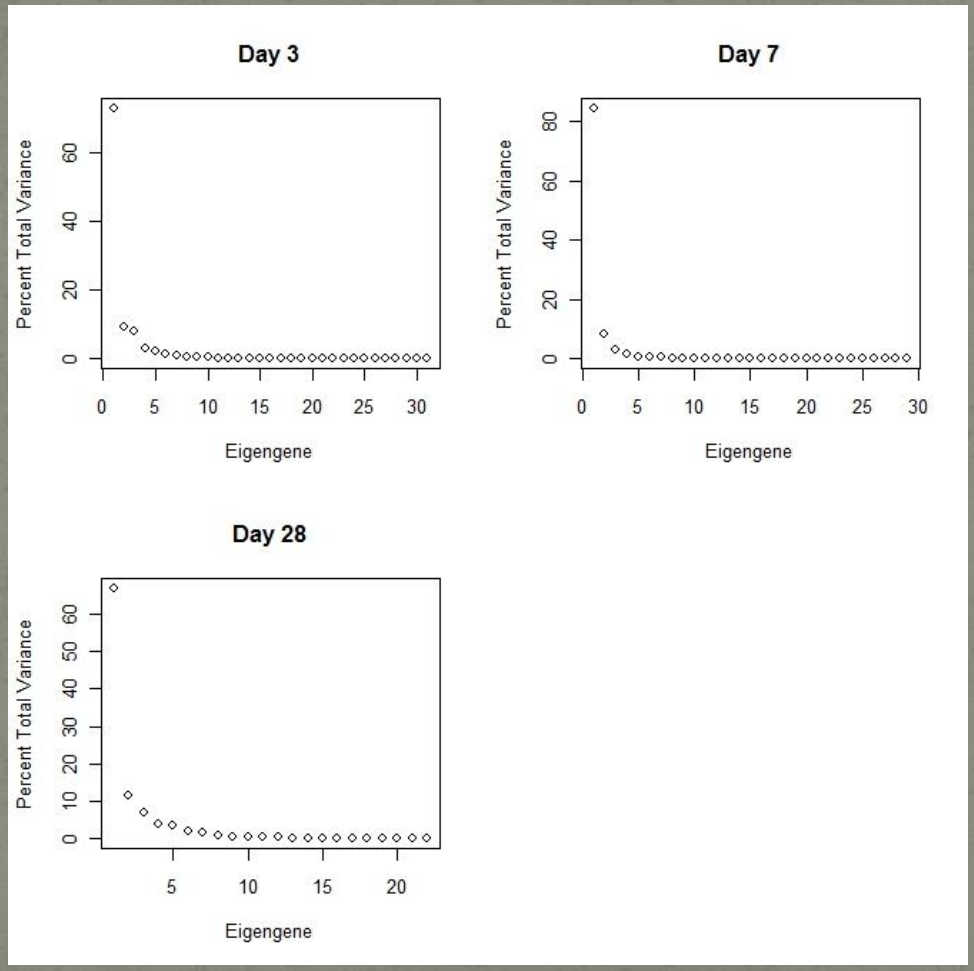
Dendogram


Cluster Dendrogram
Height
0
10
20
30
40
50
60
dist(d, method = "euclidian")
hclust (*, "single")

Principal Components

- Principal components or eigenmarkers are linear combinations of a group of variables (in this case, the markers) that represent the dataset well.
- $Z_i = \alpha_{1i} X_1 + \alpha_{2i} X_2 + \ldots + \alpha_{116i} X_{116}$
- Often the variability in the original markers can be represented by a smaller number of eigenmarkers.

Eigenmarker Plot



Inference

- Linear regression was used to model the eigenmarkers as a function of dose and route of administration.
- Multiple types of linear regression were attempted: regression with categorical variables, regression with numerical and categorical variables, and regression using interaction terms.
- Based on the AIC model fit criterion, the models with categorical variables were chosen.

Linear Regression Formula

- $y = \beta_0 + \beta_1 Dose2 + \beta_2 Dose3 + \beta_3\ SQ + \varepsilon$
- y is the baseline change in a given marker.
- $\varepsilon \sim N(0,1)$
- $Dose_i$ = 1 if subject received dose i
  = 0 if otherwise
- SQ = 1 if subcutaneous
  = 0 if otherwise
- We ran the linear regression model to explain the first three eigenmarkers for all three days.

Day 3 Mean Change/ 95% CI

Modality	Dose (mg/kg)	Eigenmarker 1	Eigenmarker 2	Eigenmarker 3
IV	0.15	20.4 (-7.1, 47.9)	1.3 (-9.8, 12.6)	2.2 (-8.0, 12.5)
	0.45	12.6 (-14.6, 39.8)	-2.7 (-13.8, 8.3)	-3.8 (-14.1, 6.3)
	0.75	29.0 (1.4, 56.5)	-2.9 (-14.1, 8.3)	1.5 (-8.7, 11.8)
SQ	0.15	-19.1 (-46.7, 8.3)	4.2 (-7.0, 15.4)	2.5 (-7.7, 12.8)
	0.45	-26.9 (-52.9, -0.9)	0.1 (-10.4, 10.6)	-3.5 (-13.3, 6.1)
	0.75	-10.5 (-38.1, 16.9)	-0.1 (-11.2, 11.1)	1.8 (-8.4, 12.1)

Day 7 Mean Change/ 95% CI

Modality	Dose (mg/kg)	Eigenmarker 1	Eigenmarker 2	Eigenmarker 3
IV	0.15	-53.3 (-92.8, -13.8)	3.3 (-10.9, 17.6)	3.5 (-4.8, 12.0)
	0.45	-5.4 (-49.3, 38.3)	1.3 (-14.5, 17.2)	0.6 (-8.6, 10.0)
	0.75	-13.0 (-55.0, 29.0)	7.3 (-7.9, 22.6)	-2.6 (-11.6, 6.3)
SQ	0.15	-2.9 (-46.7, 40.8)	-4.6 (-20.6, 11.2)	2.2 (-7.1, 11.6)
	0.45	44.8 (5.4, 84.3)	-6.7 (-21.0, 7.5)	-0.6 (-9.1, 7.7)
	0.75	37.3 (-7.0, 81.7)	-0.7 (-16.8, 15.3)	-4.0 (-13.4, 5.4)

Day 28 Mean Change/ 95% CI

Modality	Dose (mg/kg)	Eigenmarker 1	Eigenmarker 2	Eigenmarker 3
IV	0.15	-4.9 (-58.1, 48.3)	23.7 (-19.1, 15.2)	-2.2 (-19.2, 14.7)
	0.45	6.4 (-22.7, 35.5)	9.6 (0.2, 19.1)	-3.6 (-12.9, 5.6)
	0.75	1.4 (-27.7, 30.6)	-9.8 (-19.3, -0.4)	-0.5 (-9.80, 8.7)
SQ	0.15	-11.8 (-74.8, 51.1)	-1.3 (-21.7, 19.0)	2.4 (-17.6, 2.5)
	0.45	-0.4 (-29.6, 28.7)	10.2 (0.8 , 19.7)	1.0 (-8.2, 10.3)
	0.75	-5.4 (-34.6, 23.7)	-9.3 (-18.7, 0.1)	4.1 (-5.1, 13.4)

Conclusion

- The fact that CD20 markers were clustered together supports the study of this marker for evidence of efficacy.
- There is some evidence of significance in the eigenmarkers.
- Grouping for the principal components tended to be similar to hierarchical clustering.
- The results should be taken with care because multiple comparisons were done without adjustment.

Acknowledgments

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