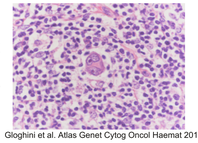
Fragmentome profiling reveals distinct molecular subgroups of Classic Hodgkin lymphoma

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Pirosa MC, Bruscaggin A, Salehi M, Spina V, Pini K, Bocchetta S, Giordano C, Condoluci A, Forestieri G, Piffaretti D, Marques De Almeida J, Annunziata S, Bergesio F, Borsatti E, Bulian P, Chauvie S , Cuzzocrea M, Di Trani M, Gerber B , Kurlapski M, Moccia A, Moia R, Rinaldi A, Rodari M, Romanowicz G, Sacchetti GM, Stasia A , Stathis A, Stüssi G, Zangrilli I , Larocca LM, Pinto A, Santoro A, Cavalli F, Zucca E, Gattei V, Zaucha JM, Carlo-Stella C, Hohaus S, Gaidano G, Ceriani L, Rossi D

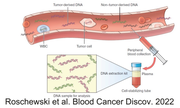
Background & Aims

Classic Hodgkin lymphoma (cHL) is a phenotypically heterogeneous disease with enigmatic biology and pathogenesis. The paucity of malignant cells in the biopsies and the need for their enrichment before sequencing have limited the sample size of genomic studies of cHL, and consequently the granularity of its molecular characterization.



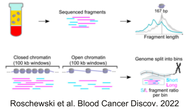
Gloghini et al. Atlas Genet Cytog Oncol Haematol 2016

Several studies confirmed that ctDNA mirrors the genetic profile of cHL malignant cells.



Roschewski et al. Blood Cancer Discov. 2022

Distribution of **cfDNA fragments can inform on patient specific chromatin patterns** and lead to subtype identification



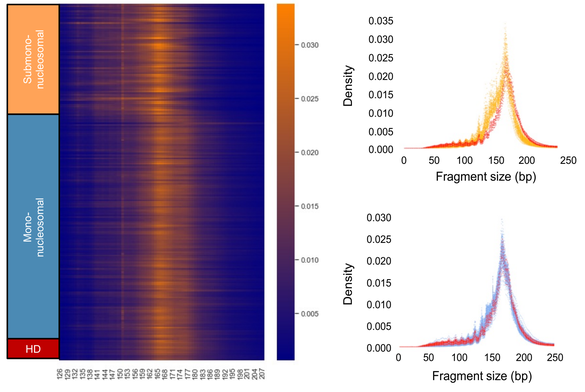
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By leveraging ctDNA analysis, here **we aim to identify molecular groups of cHL** with phenotype- and outcome-associated signatures.

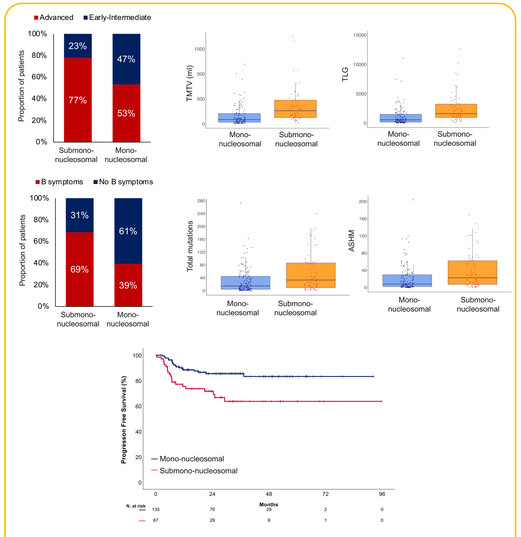
Methods

We sequenced 202 cHL patients targeting ~344 kb of genomic space. Length of ctDNA mapped reads was extracted and unsupervised clustering was performed.
PET/CT scan and 71 plasma cytokines were obtained for clinical correlation.
Single-cell RNA-sequencing was performed for 8 patients.

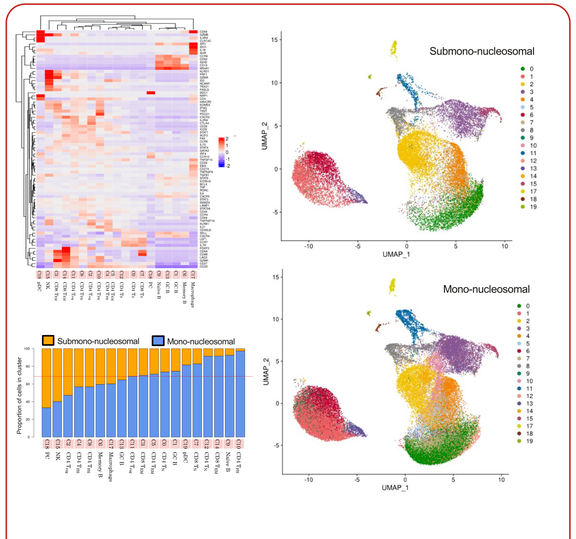
Results



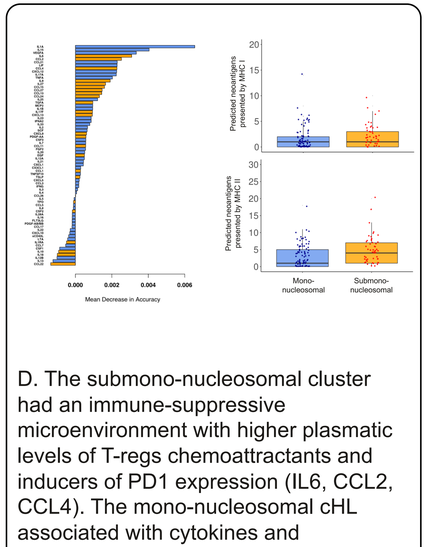
A. To identify molecular subgroups within cHL, we focused on fragmentation profile of cfDNA, a non-genetic way of detecting tumor-derived DNA in the cfDNA samples. We identified two distinct clusters, one with a fragmentation profile close to healthy (N=135), named mono-nucleosomal, and a submono-nucleosomal cluster characterized by a global shift toward shorter fragments, whose length was less than the typical wrap around the nucleosome (N=67).



B. Patients in the submono-nucleosomal cluster showed more frequent advanced stage and B-symptoms at the clinical level, had higher tumor volume (TMTV and TLG) at the radiomic level, had higher number of total and ASHM mutations, and lower PFS in terms of outcome.



C. By single cell RNA-seq, we found that he submono-nucleosomal cluster had an immune-suppressive microenvironment enriched of T-regs Conversely, mono-nucleosomal cHL and a microenvironment enriched of TFH-cells and cytotoxic CD8 TEM-cells.



D. The submono-nucleosomal cluster had an immune-suppressive microenvironment with higher plasmatic levels of T-regs chemoattractants and inducers of PD1 expression (IL6, CCL2, CCL4). The mono-nucleosomal cHL associated with cytokines and chemokines that are known for being attractants and activators of effector T-cells (IL-1a, CCL21, CXCL12, IL-17A). Sub-mono cHL showed a higher predicted number of presented neo-antigens by both MHC-I and MHC-II.

Conclusions

Our observations indicate that cHL belonging to the submono-nucleosomal cluster have a more aggressive disease, a higher mutation load and more neoantigens likely due to denser ASHM, and an immune-suppressive microenvironment. Instead, cHL belonging to the mono-nucleosomal cluster have a less aggressive disease, a lower mutation load and lower neoantigens likely due to liter ASHM, and a tumor-suppressive microenvironment.