XIX CONGRESSO NAZIONALE
SIES 2026

Intraclonal diversification in immunoglobulin light chain genes refines prognostication in early-stage chronic lymphocytic leukemia

Jana Nabki - Università del Piemonte Orientale, Novara, Italy

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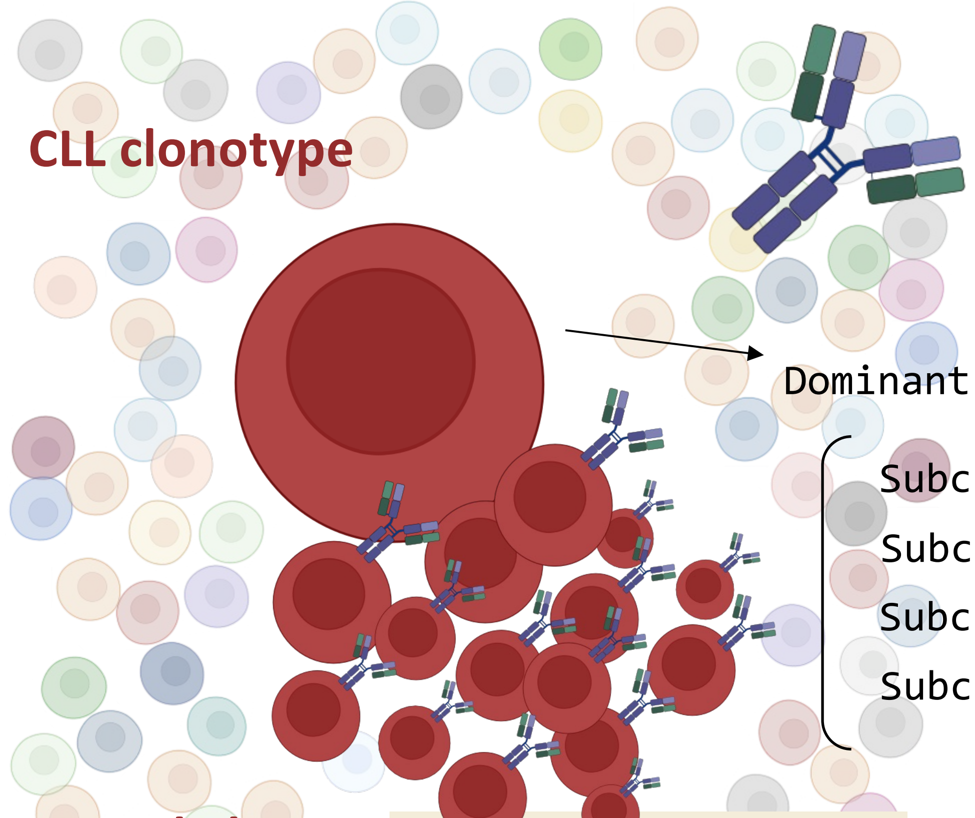
Palazzo degli Affari

Disclosures of Jana Nabki

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
No disclosures							

Intraclonal diversification in CLL



IGHV genes (98% identity)

Light chain genes (99% identity)

Mutational status

```
                -------------V-REGION------------->
                FR1----CDR1----FR2----CDR2----FR3------CDR3
Dominant clone  CCTACTTGGAGCTGAGGAACCTGAGATCTCACGACACCAGGTGTG…   IGLV3-21*04
Subclone 1      -----------------------------T---------------…   IGLV3-21*04
Subclone 2      -----A------------G----------T---------------…   IGLV3-21*04
Subclone 3      ------------G----------------T---------------…   IGLV3-21*04
Subclone 4      -----A------G-----G----------T---------------…   IGLV3-21*04
```

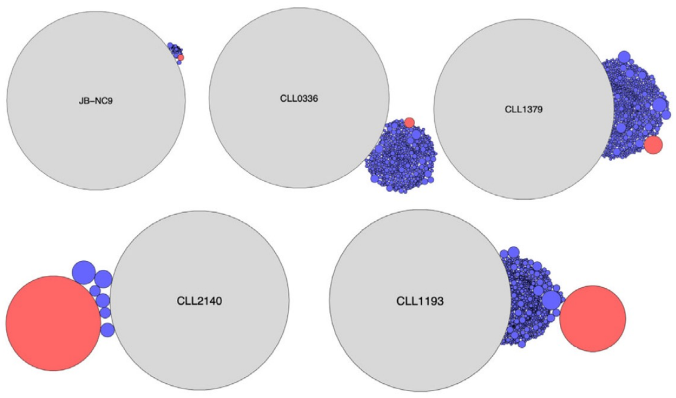
Intraclonal Diversification (ID)

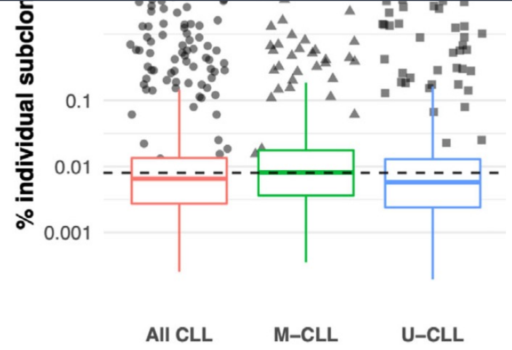
A process of **clonal evolution** that affects immunoglobulins within the CLL clone even after the initial neoplastic transformation

Davi et al. *Leukemia.* 2020; Agathangelidis et al. *Leukemia.* 2022; Sofou et al. *Leukemia.* 2023; Gurrieri et al. *J. Exp. Med.* 2002; Nabki, Al Deeban et al. *Leukemia.* 2024

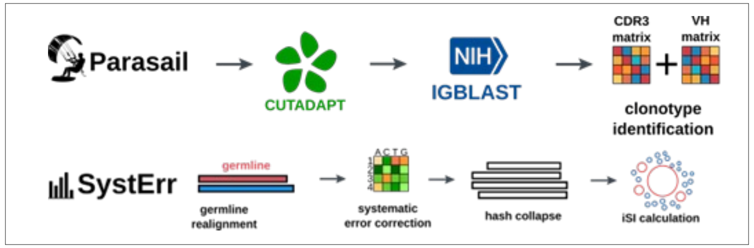
Intraclonal diversification in IGHV genes in CLL

ID is virtually ubiquitous and extends to both **M-CLL** and **U-CLL** patients although at various degrees

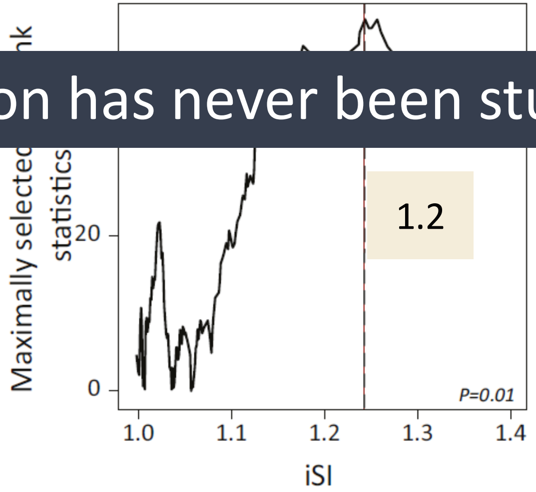


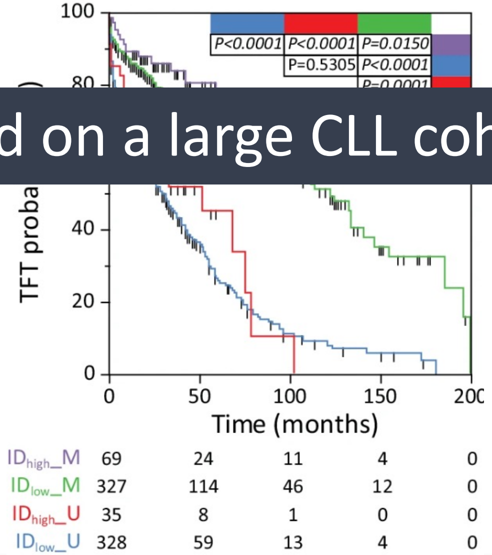


The **inverse Simpson Index (iSI)** was implemented as a diversity measurement



A subset of **M-CLL** with **High levels of ID** in IGHV genes was identified with **longer TTFT** than Low-ID patients





Light chain intraclonal diversification has never been studied on a large CLL cohort

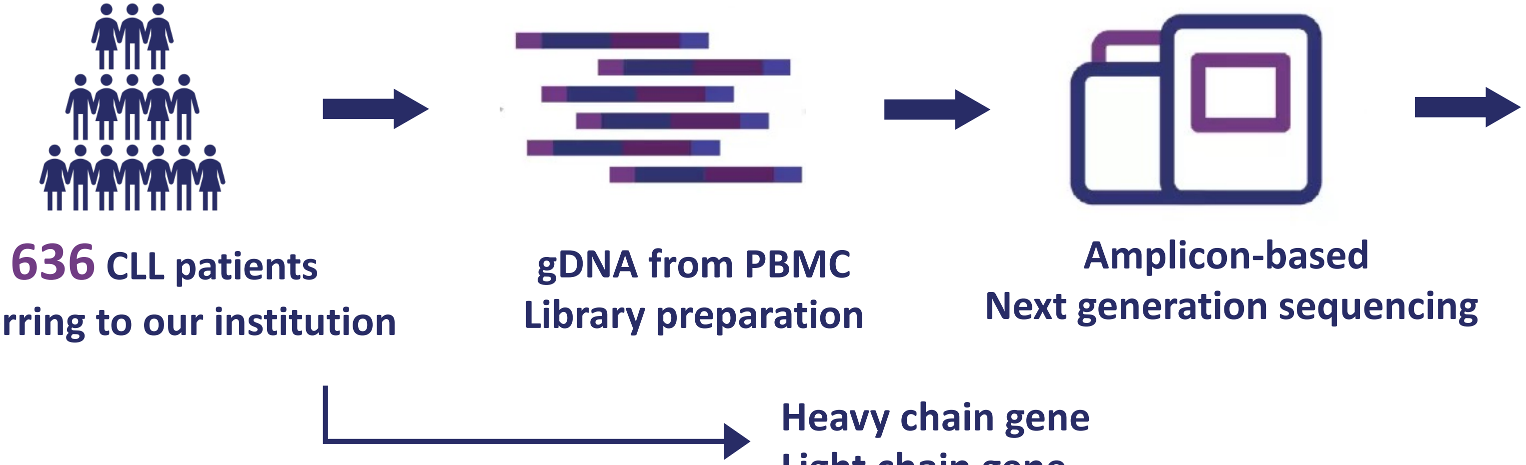
Bagnara et al. *Front Oncol.* 2021; Vit et al. *Leukemia.* 2025

TTFT: Time to first treatment

Aims and Experimental workflow

Analyse intraclonal diversification in **light chain genes** in CLL

Assess the possible synergistic role of **IGHV** and **light chain ID levels** in the prognosis of CLL patients



636 CLL patients referring to our institution

gDNA from PBMC
Library preparation

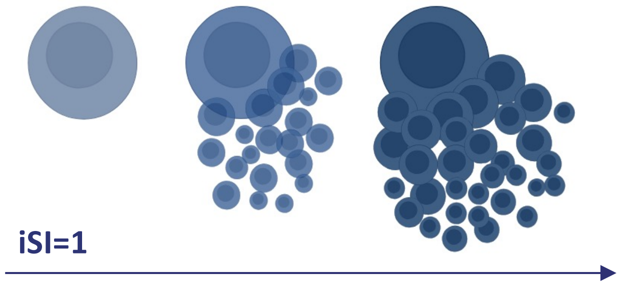
Amplicon-based
Next generation sequencing

Heavy chain gene
Light chain gene

Kappa & Lambda

SystErr
Systematic error supression for RepSeq data
(Patent #102022000027138)

iSI calculation representing ID levels



Vit et al. *Leukemia.* 2025

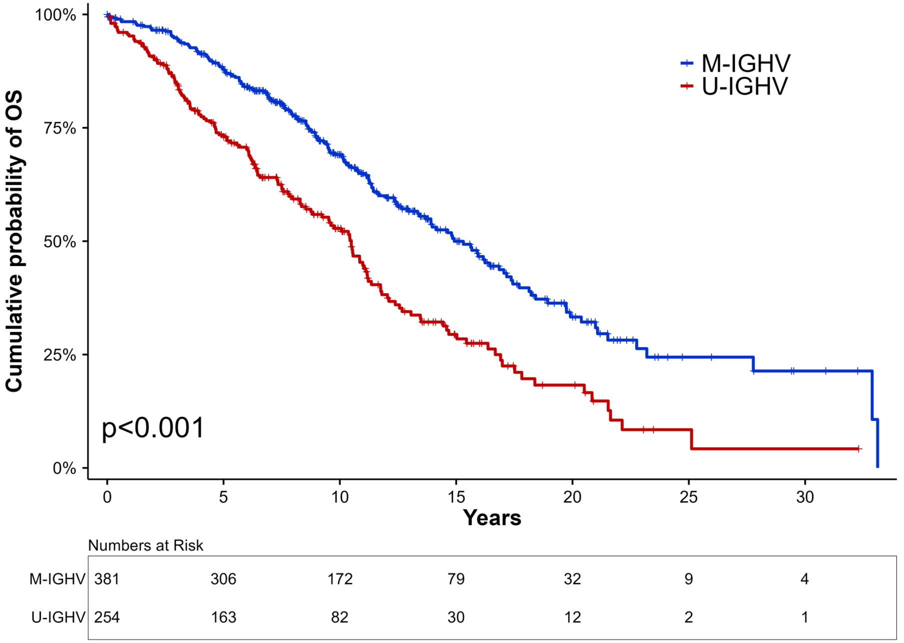
PBMC: Peripheral Blood Mononuclear Cells

Patient characteristics

Characteristic	Value
Median age at diagnosis (range)	70 (37 - 95)
Sex	
Male	380 (59.7%)
Female	256 (40.2%)
Median lymphocyte count	9.7x10^3/µl
Median hemoglobin level	13.7 g/dl
Median platelet count	194x10^3/µl
Light chain	
Kappa	362 (56.9%)
Lambda	196 (31.8%)
Undefined	78 (12.3%)
IGHV	
Mutated	381 (59.9%)
Unmutated	255 (40.1%)
Binet staging system	
Binet A	477 (76.8%)
Binet B	87 (14%)
Binet C	57 (9.2%)
***TP53* disrupted**	71 (11.3%)
del17p	50 (8.5%)
***TP53* mutated**	57 (9.1%)
Trisomy 12	115 (19.5%)
***NOTCH1* mutated**	42 (12.3%)
del13q	284 (48.1%)
del11q	50 (8.5%)
***MYD88* mutated**	12 (3.6%)
***SF3B1* mutated**	23 (6.7%)
***BIRC3* mutated**	5 (1.5%)
Stereotyped cases	
Subset 1	16 (2.5%)
Subset 2	13 (2.1%)
Subset 4	8 (1.3%)
Subset 8	5 (0.8%)

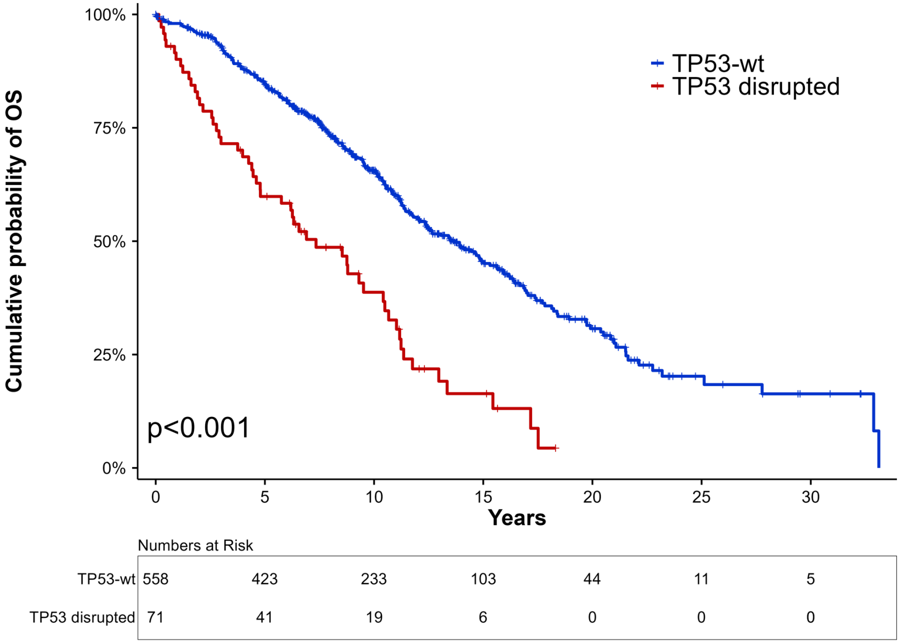
Patient characteristics were representative of a **real-world consecutive cohort of unselected CLL**

Median follow-up = **13.5** years



Numbers at Risk

M-IGHV	381	306	172	79	32	9	4
U-IGHV	254	163	82	30	12	2	1



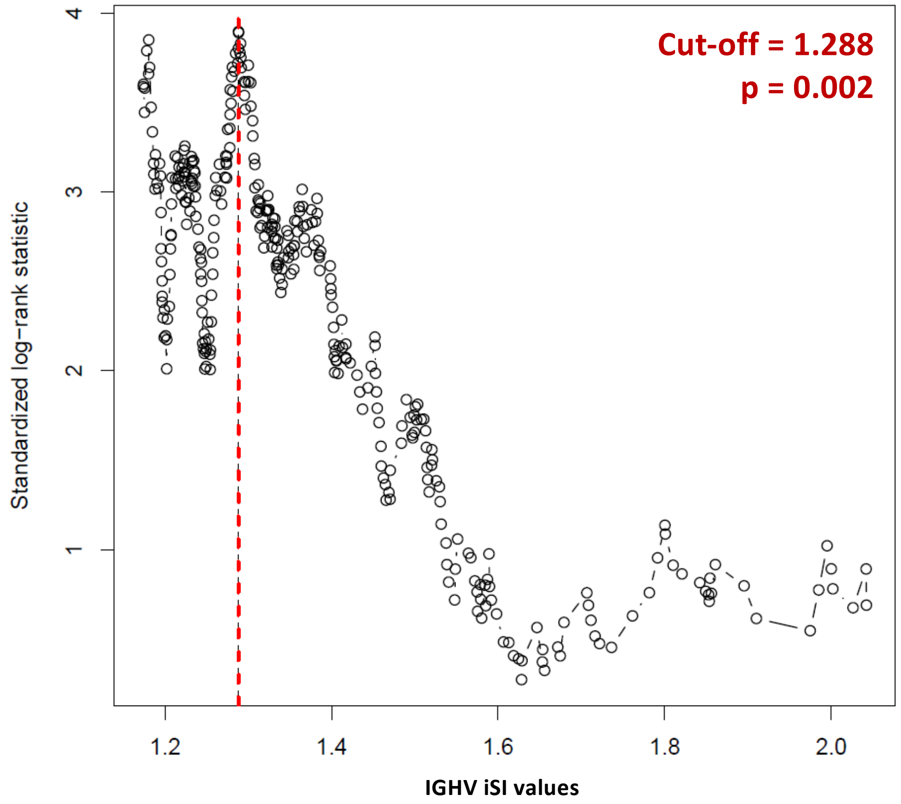
Numbers at Risk

TP53-wt	558	423	233	103	44	11	5
TP53 disrupted	71	41	19	6	0	0	0

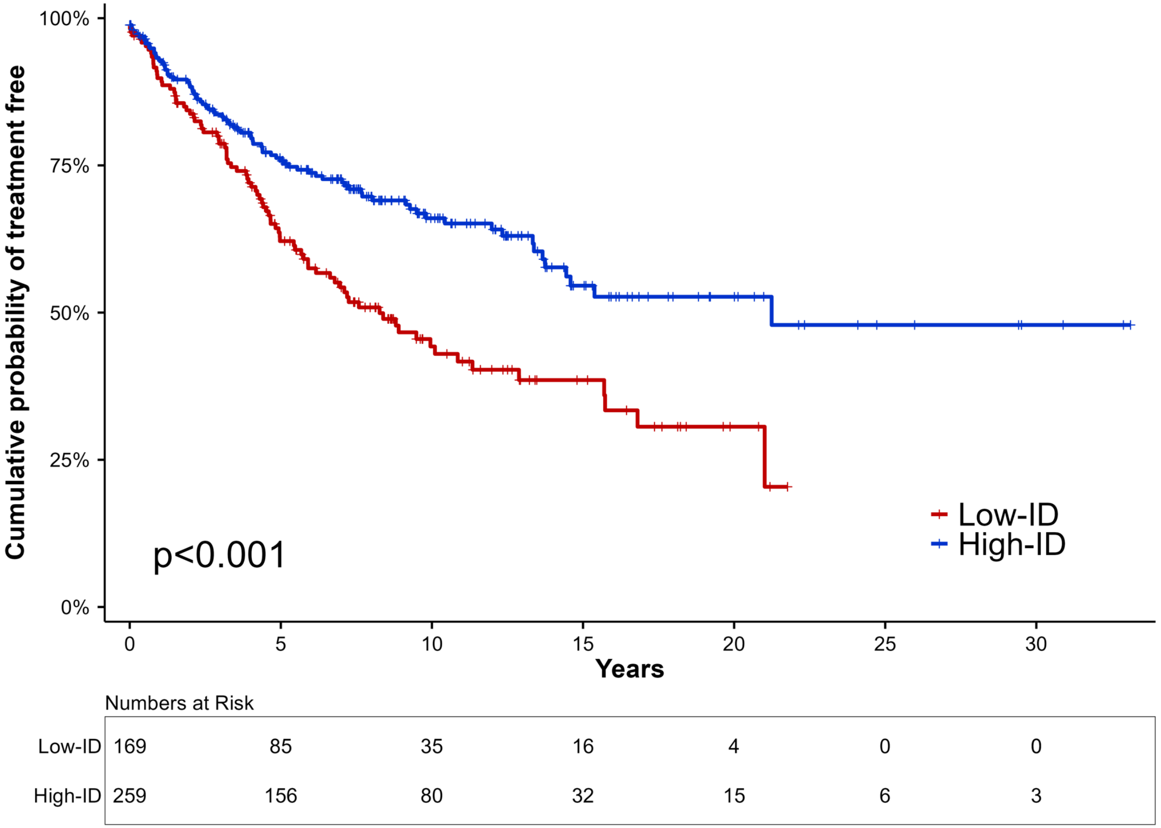
OS: Overall survival

IGHV intraclonal diversification levels correlate with TTFT in early-stage CLL

A recursive partitioning approach (**Maxstat**) identified **IGHV iSI cut-off** to predict TTFT in Binet A CLL



IGHV High-ID patients experienced **longer TTFT** than **IGHV Low-ID** patients in Binet A CLL

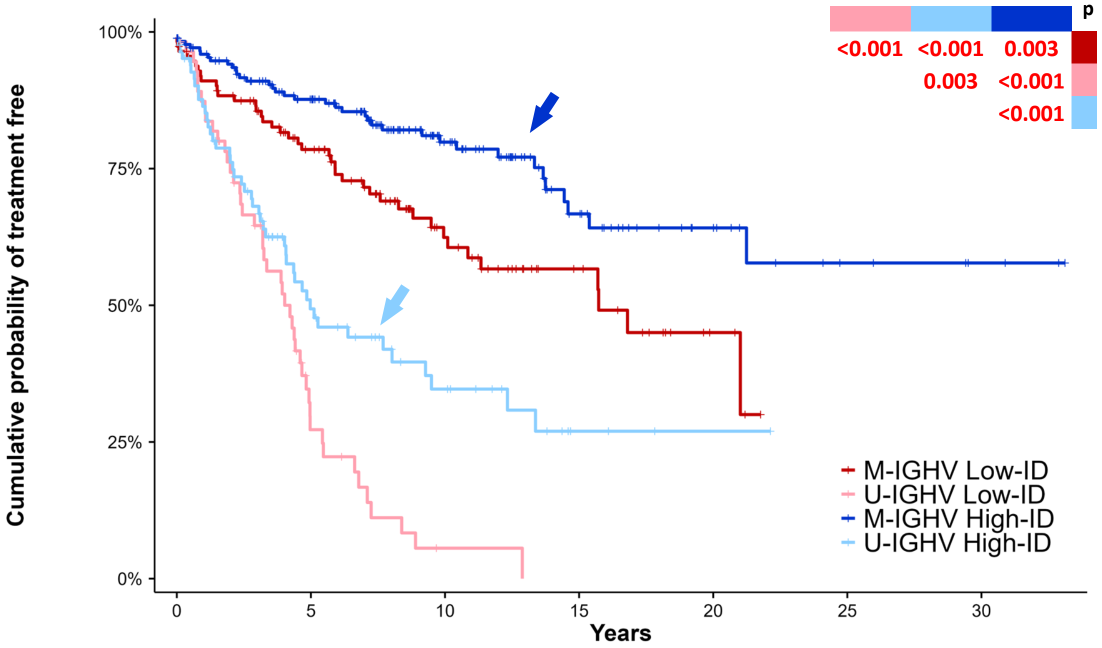


Numbers at Risk

	0	5	10	15	20	25	30
Low-ID	169	85	35	16	4	0	0
High-ID	259	156	80	32	15	6	3

IGHV High-ID associates with longer TTFT also within the IGHV mutational groups

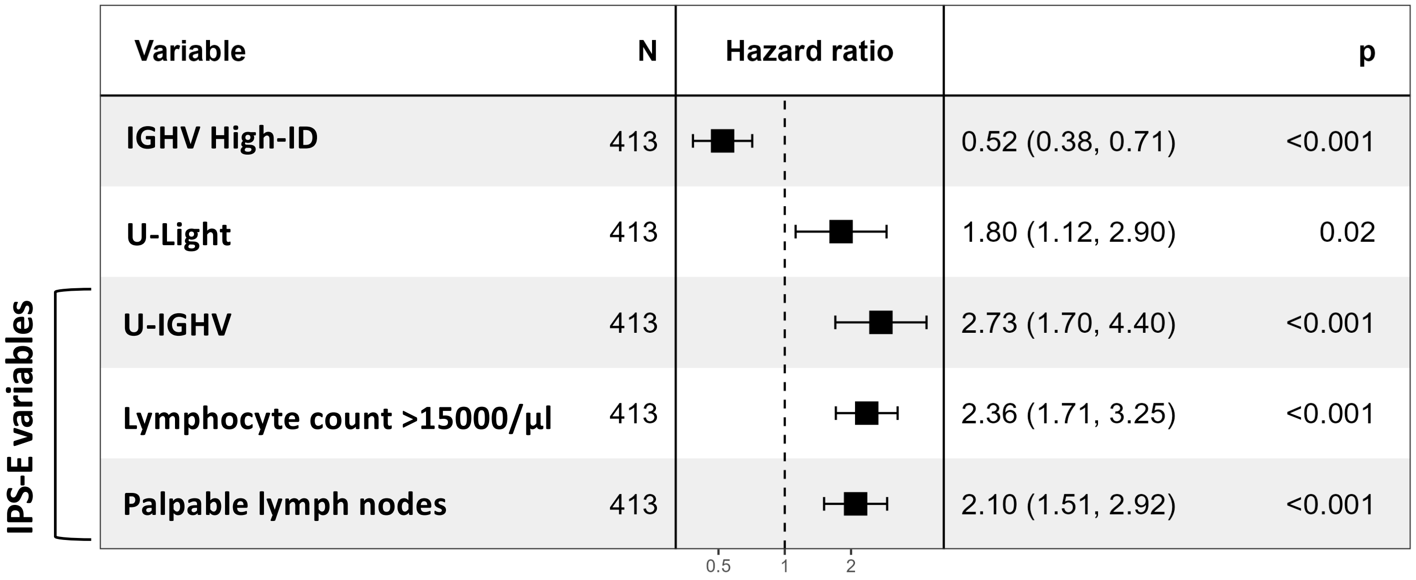
IGHV High-ID levels stratified the outcome of both **M-IGHV** and **U-IGHV** patients in Binet A CLL



Numbers at Risk

	0	5	10	15	20	25	30
M-IGHV Low-ID	113	74	34	16	4	0	0
U-IGHV Low-ID	56	11	1	0	0	0	0
M-IGHV High-ID	176	126	66	29	14	6	3
U-IGHV High-ID	83	30	14	3	1	0	0

IGHV High-ID independently associated with longer TTFT (adjusted for **IPS-E variables** + light chain mutational status)



Variable	N	Hazard ratio		p
IGHV High-ID	413		0.52 (0.38, 0.71)	<0.001
U-Light	413		1.80 (1.12, 2.90)	0.02
U-IGHV	413		2.73 (1.70, 4.40)	<0.001
Lymphocyte count >15000/µl	413		2.36 (1.71, 3.25)	<0.001
Palpable lymph nodes	413		2.10 (1.51, 2.92)	<0.001

IPS-E: International Prognostic Score for Early-stage CLL

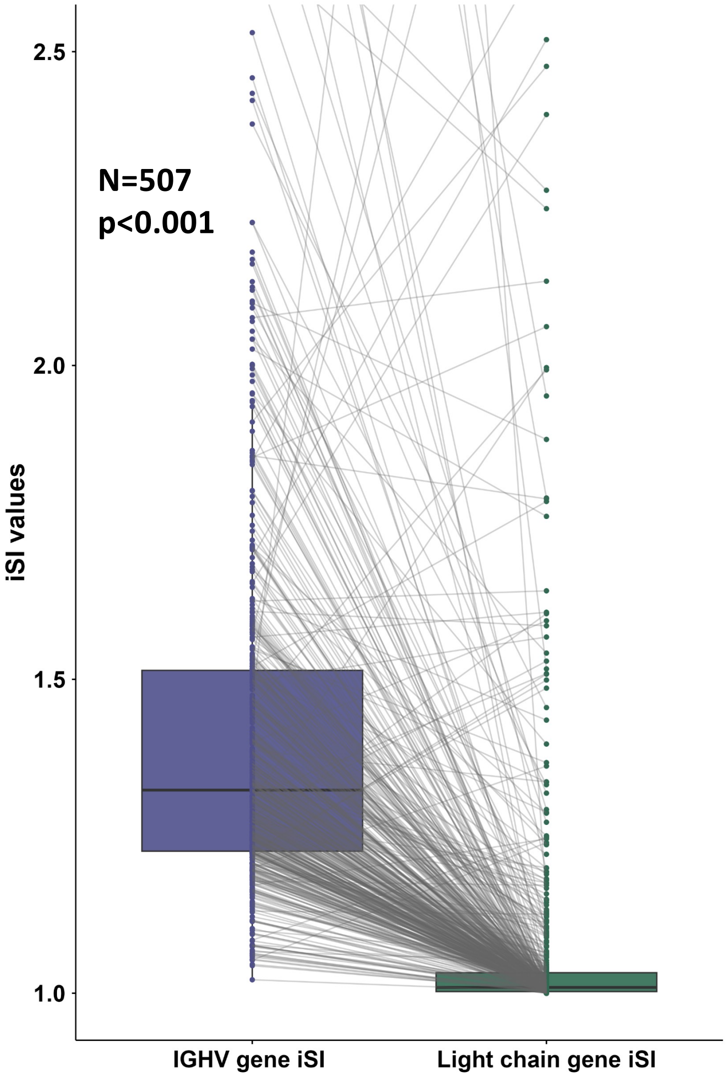
Light chain genes display significantly lower iSI levels than their corresponding IGHV genes

Median iSI IGHV

1.54

Median iSI Light chain

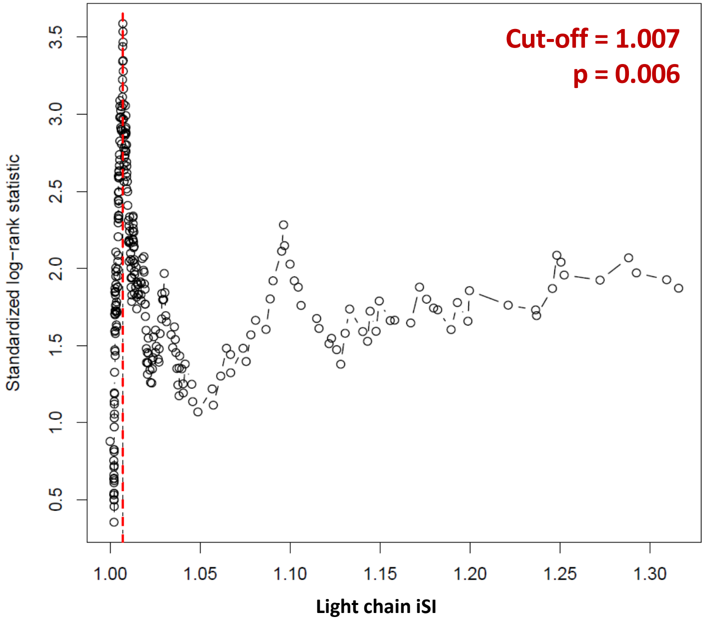
1.008

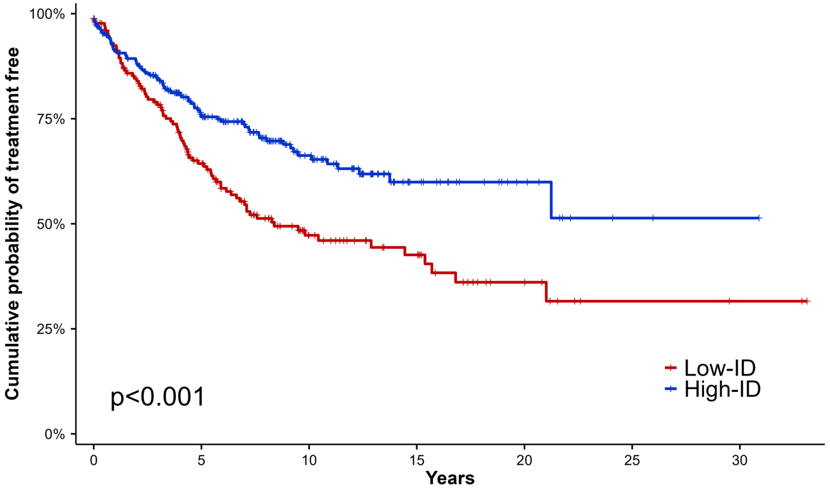


Similar to IGHV genes, light chain genes are subjected to intraclonal diversification in CLL

The extent of ID was markedly less in light chain genes

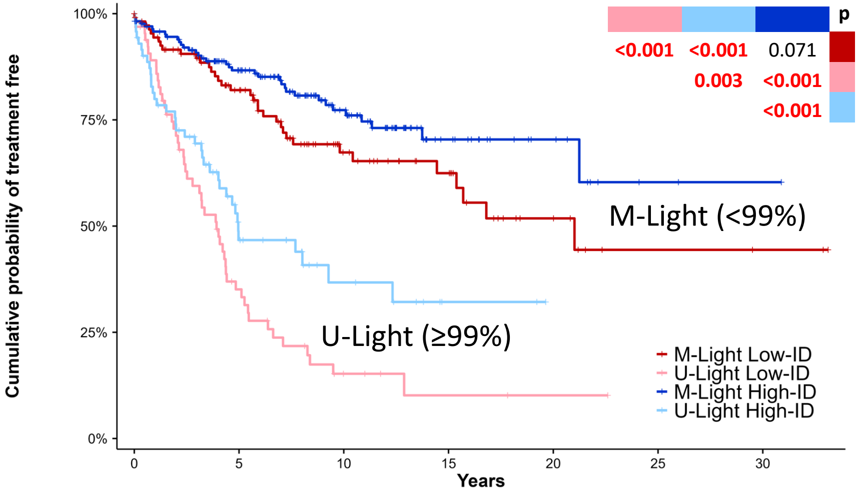
Light chain genes ID levels predict TTFT in early-stage CLL





Numbers at Risk

	0	5	10	15	20	25	30
Low-ID	175	91	39	24	10	3	2
High-ID	240	141	72	22	9	2	1



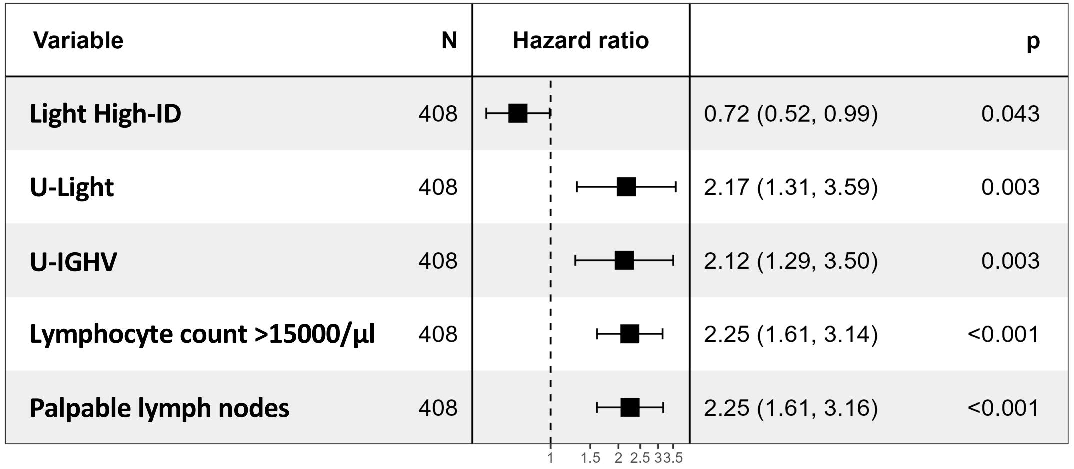
Numbers at Risk

	0	5	10	15	20	25	30
M-Light Low-ID	110	72	34	22	9	3	2
U-Light Low-ID	65	19	5	2	1	0	0
M-Light High-ID	169	119	63	20	9	2	1
U-Light High-ID	71	22	9	2	0	0	0

High ID levels in **light chain genes** associated with **longer TTFT**

Within the U-Light group, **Light High-ID** patients significantly associated with **longer TTFT** compared to Light Low-ID patients

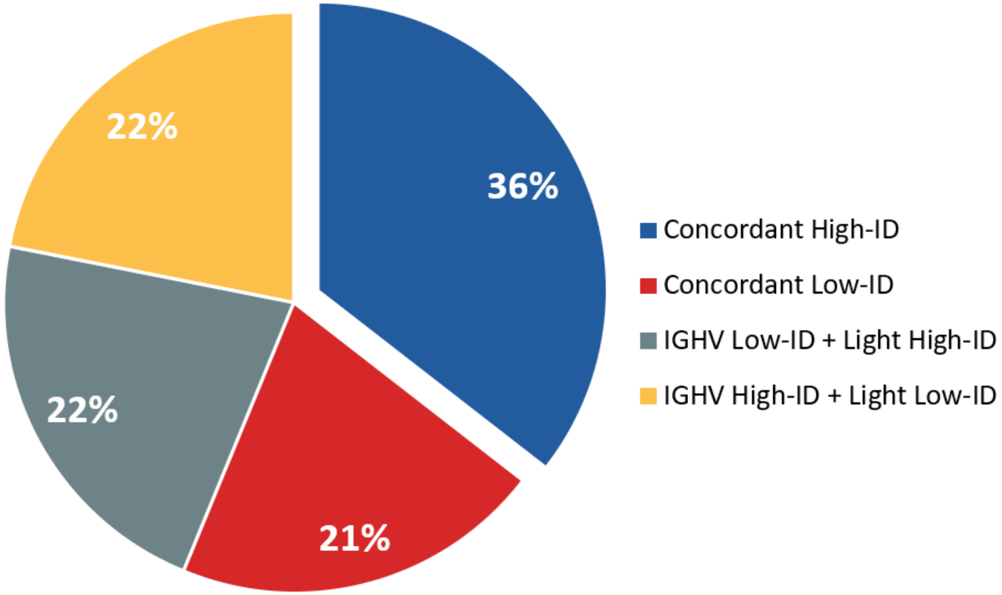
Light High-ID patients independently predicted prolonged TTFT (adjusted for **IPS-E variables + Light chain mutational status)**



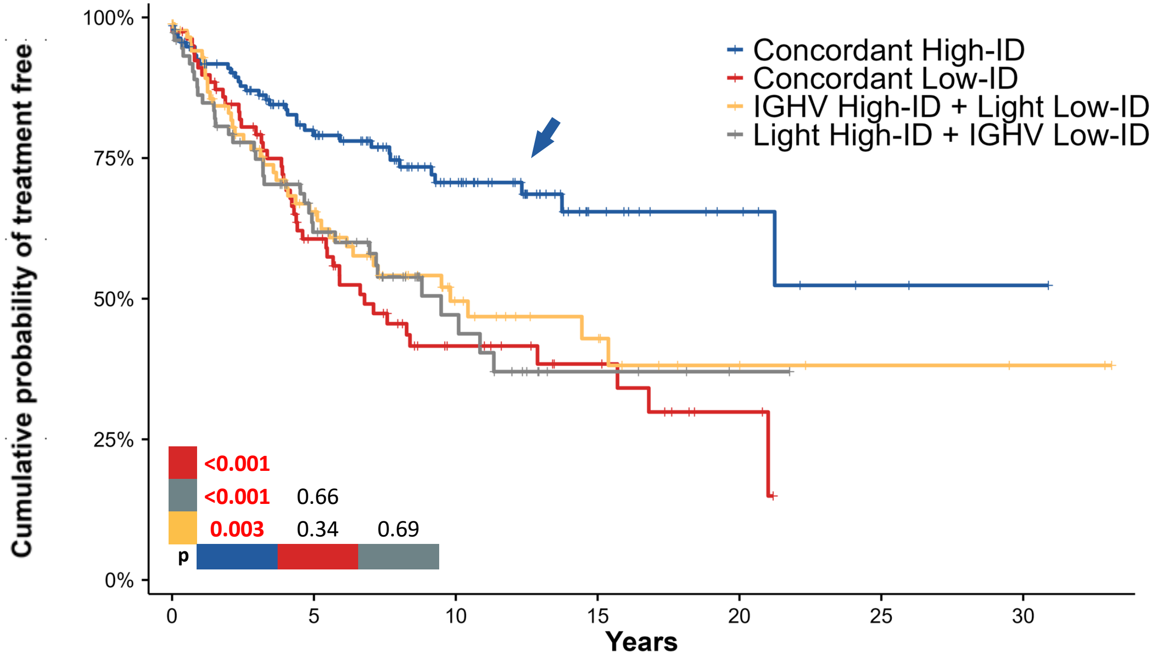
Variable	N	Hazard ratio		p
Light High-ID	408		0.72 (0.52, 0.99)	0.043
U-Light	408		2.17 (1.31, 3.59)	0.003
U-IGHV	408		2.12 (1.29, 3.50)	0.003
Lymphocyte count >15000/µl	408		2.25 (1.61, 3.14)	<0.001
Palpable lymph nodes	408		2.25 (1.61, 3.16)	<0.001

Concordant High-ID patients experience longer TTFT in early stage CLL

Concordant ID levels were found in both chains in 56% of CLL patients **(36% Concordant High-ID** and **21% Concordant Low-ID**)



Concordant high levels of diversification in both chains associated with longer TTFT relative to concordant low-ID patients and discordant cases

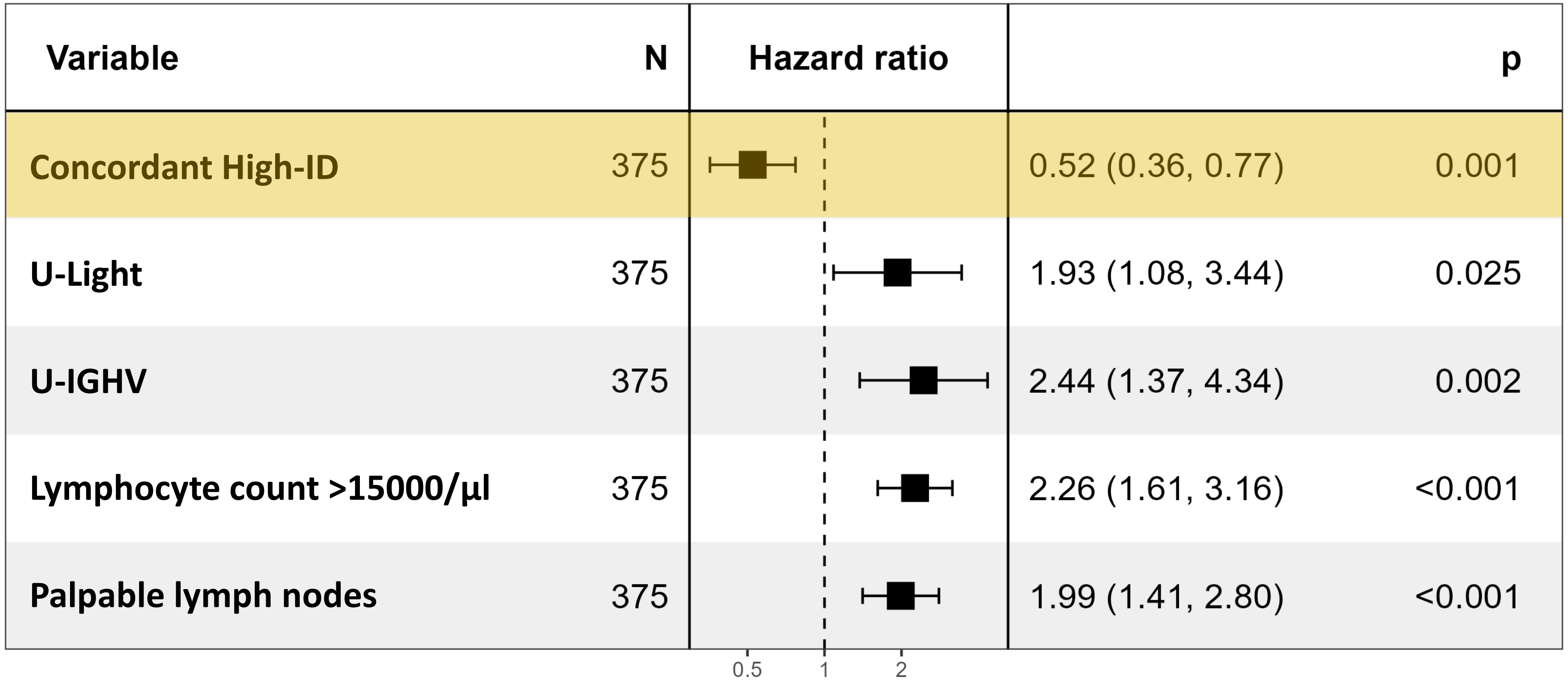


Numbers at Risk

	0	5	10	15	20	25	30
Concordant High-ID	136	85	47	14	7	2	1
Concordant Low-ID	79	39	17	10	3	0	0
IGHV High-ID + Light Low-ID	87	45	19	11	5	3	2
Light High-ID + IGHV Low-ID	74	36	14	4	1	0	0

Concordant High-ID patients experience longer TTFT in early stage CLL

Concordant High-ID maintained a significant association with **longer TTFT** independently of the IPS-E variables with the addition of the light chain genes mutational status



Variable	N	Hazard ratio		p
Concordant High-ID	375		0.52 (0.36, 0.77)	0.001
U-Light	375		1.93 (1.08, 3.44)	0.025
U-IGHV	375		2.44 (1.37, 4.34)	0.002
Lymphocyte count >15000/µl	375		2.26 (1.61, 3.16)	<0.001
Palpable lymph nodes	375		1.99 (1.41, 2.80)	<0.001

Conclusions

This study represents the **largest real-life cohort** of unselected CLL analysed for intraclonal diversification in light chain genes

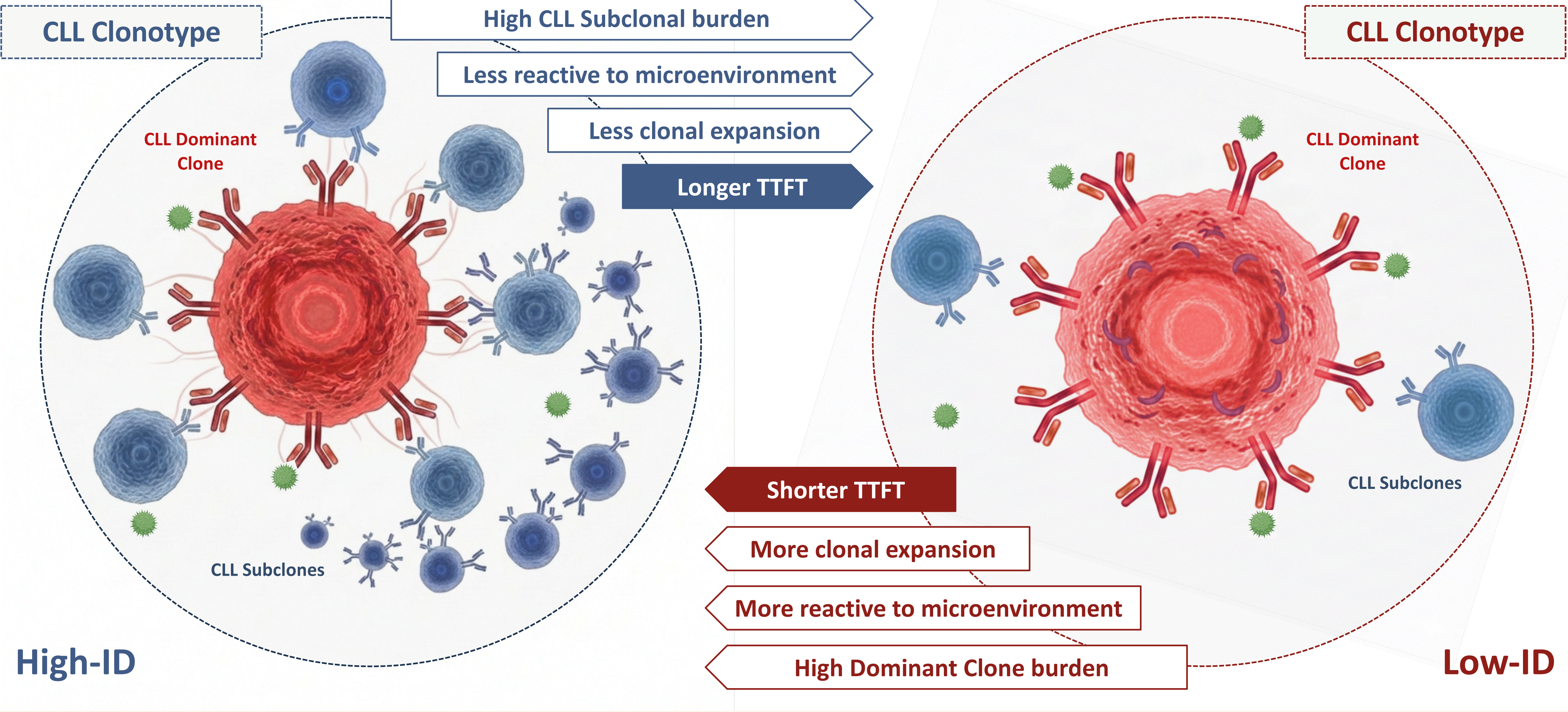
This study provides the first evidence that **ID in both chains** may hold additional **prognostic value** to **early-stage CLL** patients

ID emerged as an **independent predictor of TTFT** that refined prognostic stratification beyond IGHV and light chain mutational status

Future steps

Evaluate the clinical impact of **ID** in the context of **biological agents**, namely BCL2i +/- anti-CD20, in which IGHV mutational status still maintains a prognostic value

BCL2i: B-cell Lymphoma-2 inhibitor



CLL Clonotype

CLL Dominant Clone

CLL Subclones

High-ID

High CLL Subclonal burden

Less reactive to microenvironment

Less clonal expansion

Longer TTFT

CLL Clonotype

CLL Dominant Clone

CLL Subclones

Shorter TTFT

More clonal expansion

More reactive to microenvironment

High Dominant Clone burden

Low-ID

Packham et al. *Haematologica* 2014; Hacken, *Biochim Biophys Acta*. 2016; Oppezzo et al *Blood Cancer Journal*. 2013

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Bashar Al Deeban
Mohammad Almasri
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CRO
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Clinical and Experimental Onco-Hematology Unit

Filippo Vit
Riccardo Bomben
Francesca Rossi
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AGING PROJECT

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Light High-ID patients associate with CLL baseline characteristics