ICGC LEIOMYOSARCOMAS

International Cancer Genome Consortium

INSTITUT NATIONAL DU CANCER

Groupe Sarcome Français GSF GETO Groupe d'Etude des Tumeurs Osseuses

Aider la vie! INSTITUT BERGONIÉ Centre Régional de Lutte Contre le Cancer de Bordeaux et du Sud-Ouest

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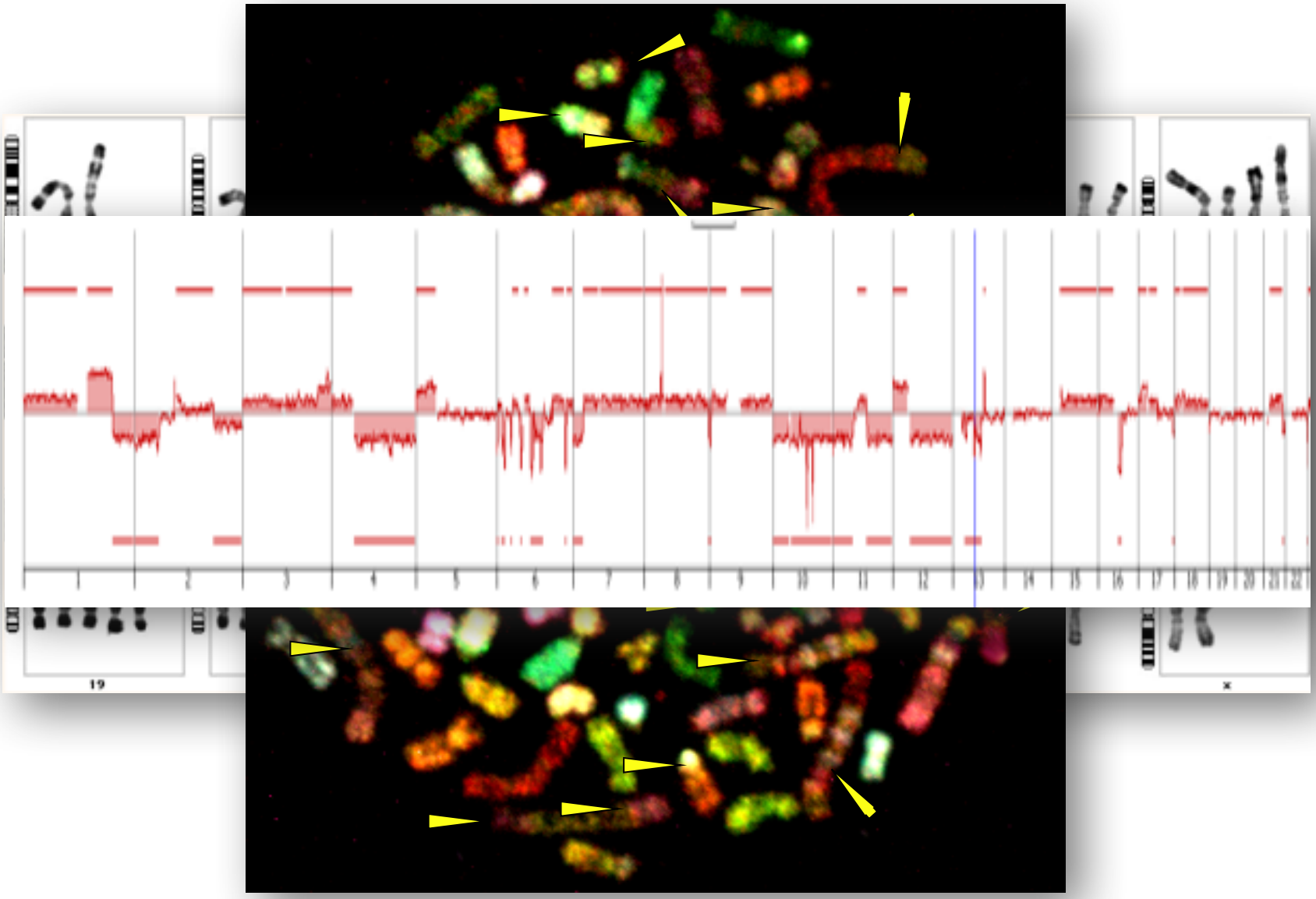
Bordeaux, France

Funded by ITMO cancer (INCa / INSERM)

LMS karyotype and genome



Objectives

- To provide a thorough genomic characterization, based on Next Generation Sequencing (NGS), of a series of LMS (including matched constitutional DNA). This effort will lead to an improved understanding of the pathogenesis of smooth muscle malignant lesions and to the identification of new alterations driving the tumoral process.
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- A first series of 60 characteristic LMS from each location, i.e. 20 from extremity, 20 from retroperitoneal and 20 from uterine, will be exhaustively analysed:
  - 1. Obtain whole genome DNA sequences of primary LMS (coverage >45-fold), and from the matched blood DNAs (coverage >30-fold) for each location,
  - 2. Obtain Whole genome ultra-deep coverage (coverage >200 -fold) of 3 pairs of primary tumours and matched metastasis
  - . This step will search for somatic mutations that may be present in subclones representing less than 15% of the tumor cells.
  - 3. Perform a deep sequencing of the transcriptome (RNASeq) of primary tumour).
  - 5. Analyze data from each cancer and normal genome for the presence of variants compared to the reference human genome and generate a catalogue of somatic mutations and chromosome rearrangements (translocation, copy number variation) for each cancer genome. Identify subclonal somatic mutations in exomes.
  - 6. Assess the false positive rate of somatic mutation/ chromosome alterations in each cancer genome by performing technical replication for 250 candidate alterations per tumour using a different technology.
  - 7. Perform technical replication for the mutations / chromosome alterations which most likely drive of the tumor process. The probability of being a driver will be assessed through their frequency of occurrence in the studied set of tumors and throught the known or inferred function of the genes that they may alter. At least two hundred mutations/alterations should thus be investigated.
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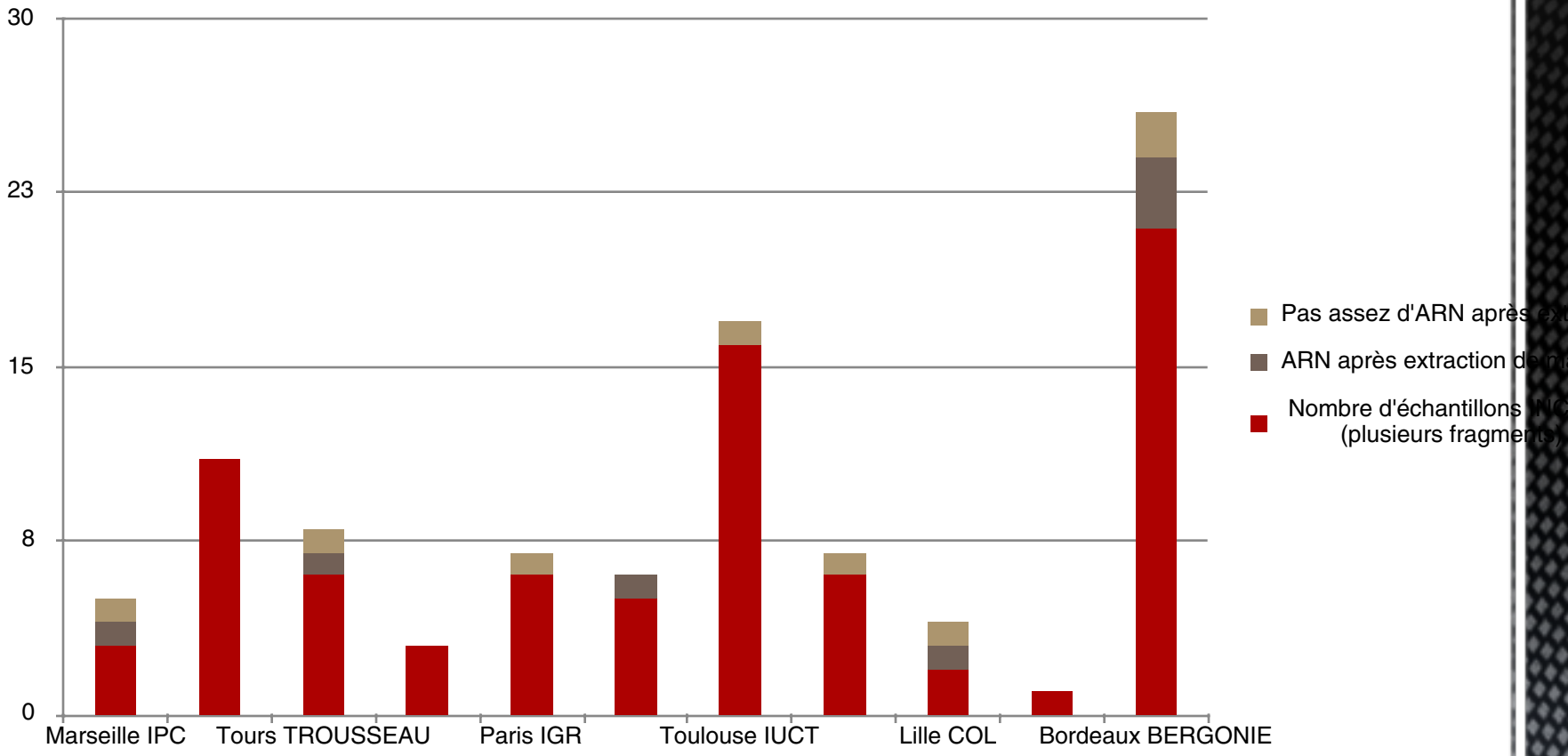
- A second series of >100 LMS will be analysed secondly according to the relevant alterations identified in the first series

Inclusion

- 2 series
  - 60 cases: 2015-2016
  - 200 cases: 2016-2018
- Primary LMS (+ 3 cases with metastasis)
  - Untreated
  - Patient consent
  - Frozen tissue
  - Blood sample
  - Included in NETSARC, RRePS
- 3 locations
  - Intra-abdominal (1/3)
  - Extremities (1/3)
  - GYN (1/3)

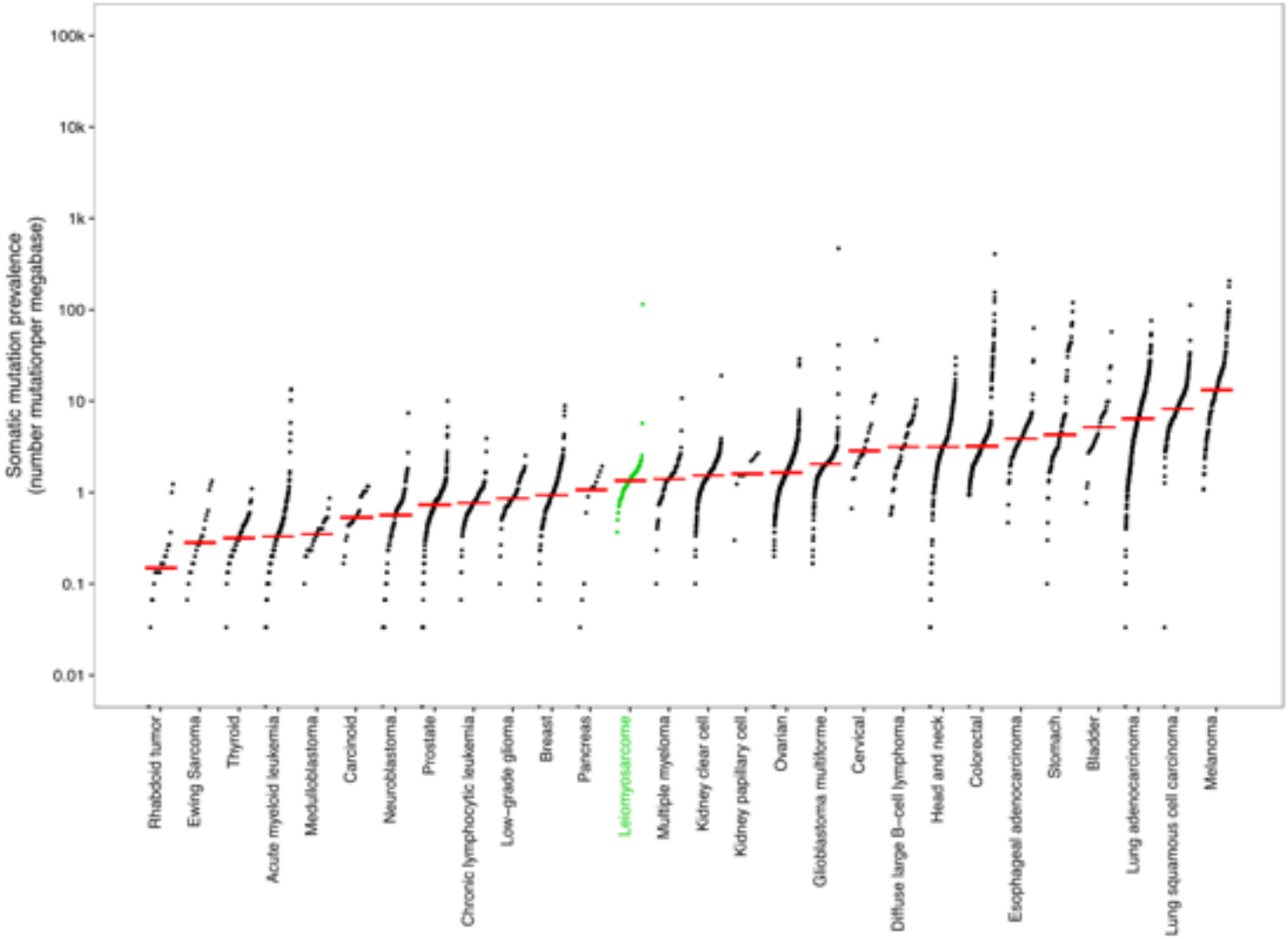
Inclusion


30
23
15
8
0
Marseille IPC
Tours TROUSSEAU
Paris IGR
Toulouse IUCT
Lille COL
Bordeaux BERGONIE
Pas assez d'ARN après ex
ARN après extraction de m
Nombre d'échantillons INC
(plusieurs fragments)

Characteristics	Cohort (n=68)
Median follow-up (months) [95% CI]	30.8 [26.0-36.8]
Median age at diagnosis (years) [95% CI]	64 [61-66]
Genders (%)	
Males	15 (22.06)
Females	53 (77.94)
FNCLCC grade (%)	
I and II	34 (50.00)
III	32 (47.06)
Unknown	2 (2.94)
Location (%)	
Internal trunk	38 (55.88)
Member	22 (33.35)
Uterine	8 (11.77)
Relapse events (%)	
Metastasis	7 (10.29)
Local recurrences	4 (5.88)
Median size (mm)	80
Surgical margins (%)	
R0	44 (64.71)
R1	18 (26.47)
R2	1 (1.47)
Unknown	5 (7.35)

68 cas inclus et séquencés

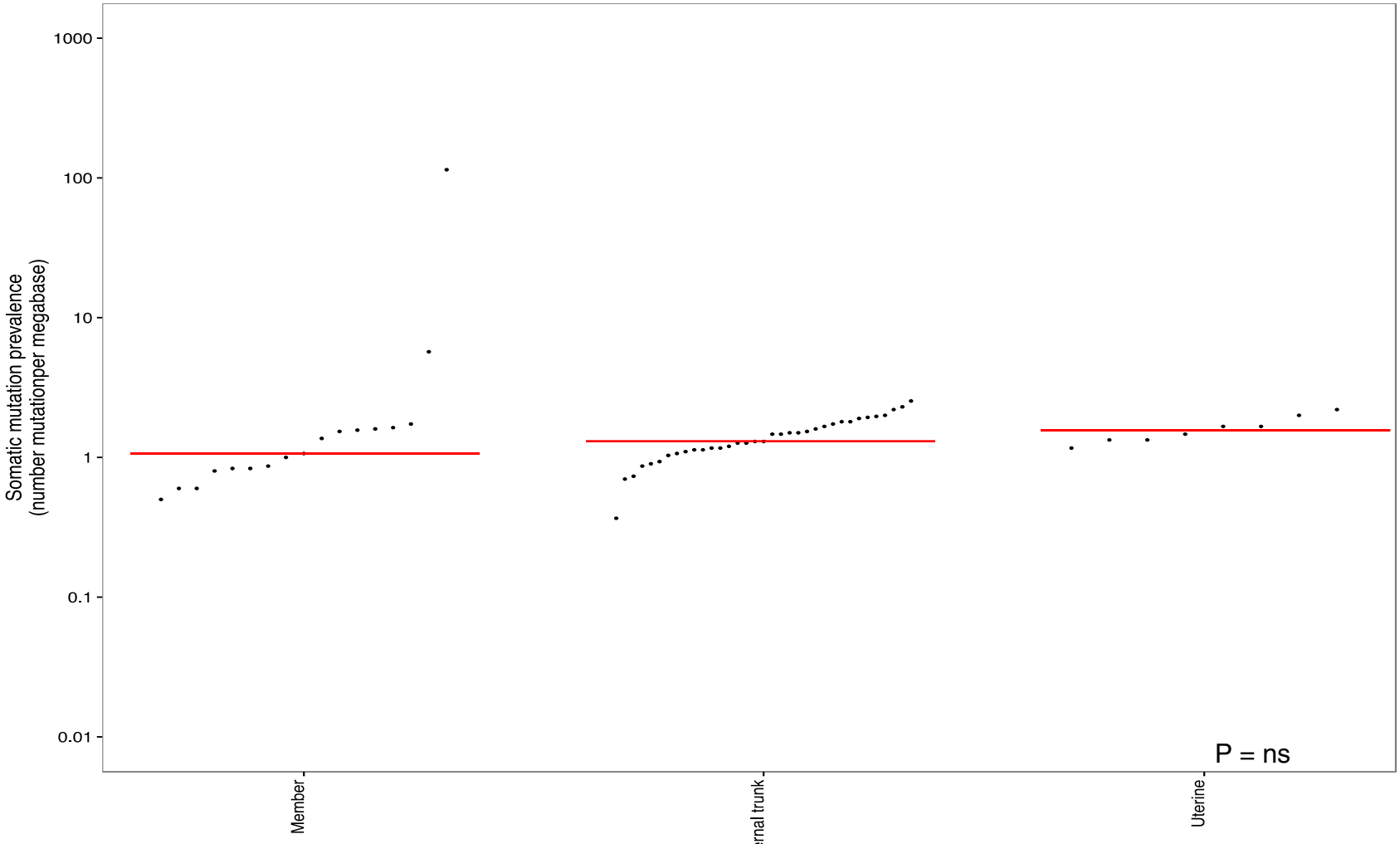
Somatic Coding Variants



1,5 somatic mutations per coding MB

Modifié de Lawrence et al. (Nature, 2013)

Somatic Coding Variants

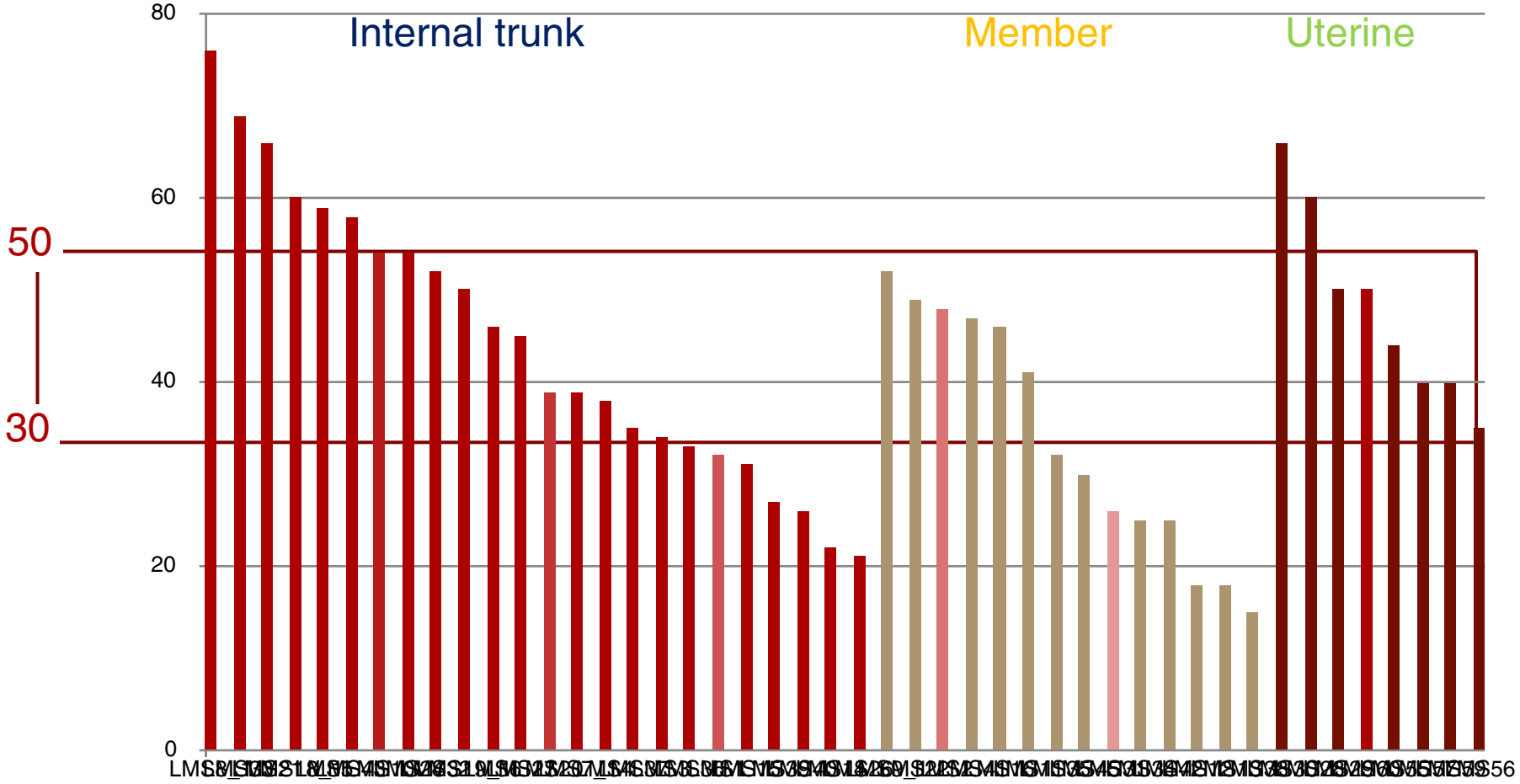


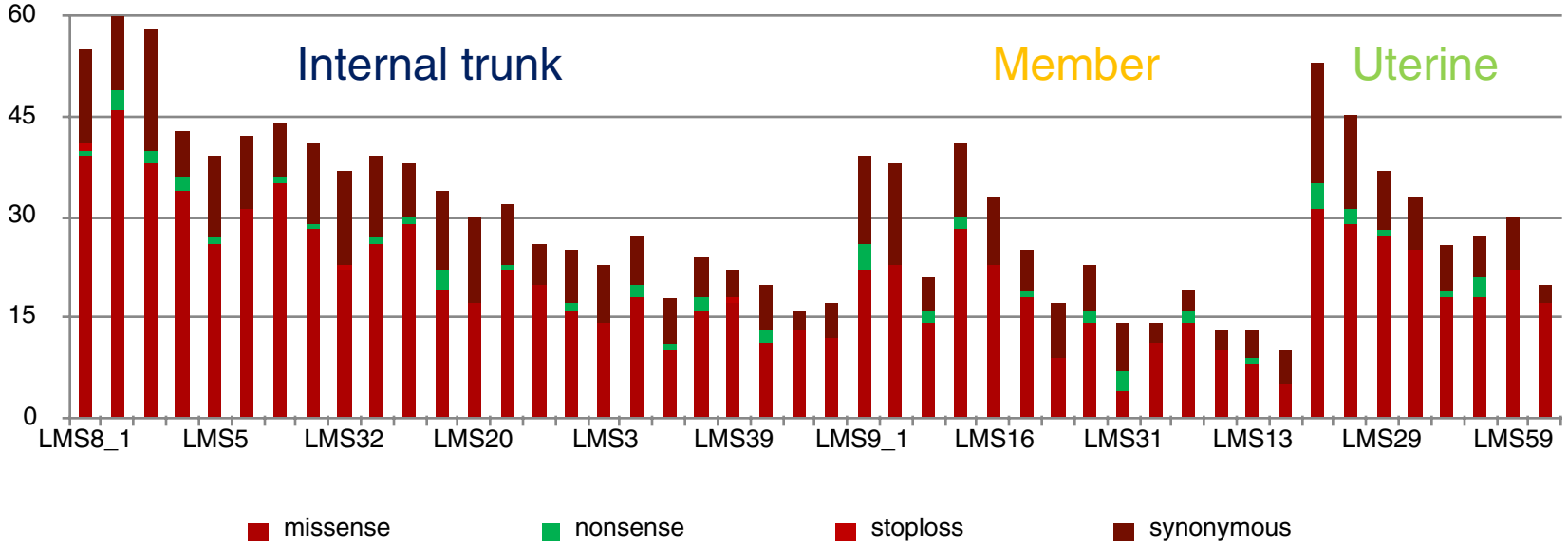
Internal trunk mut. / Mb

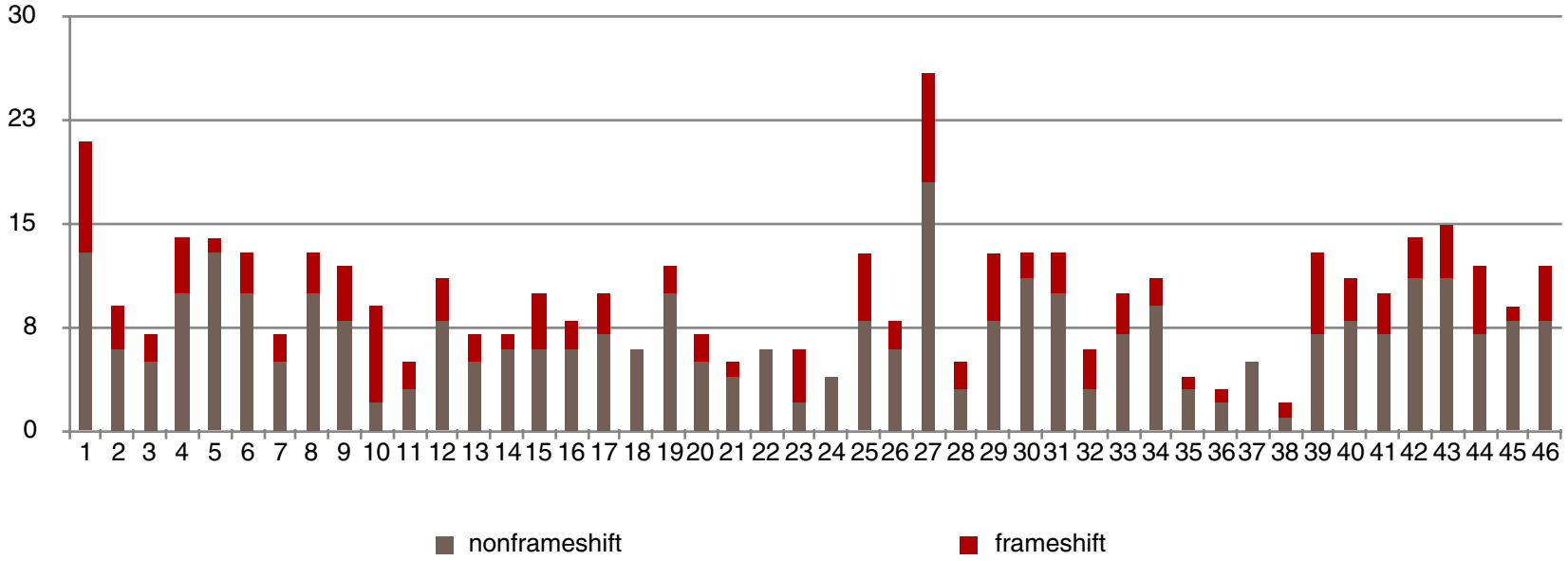
Member mut. / Mb

Uterine mut. / Mb

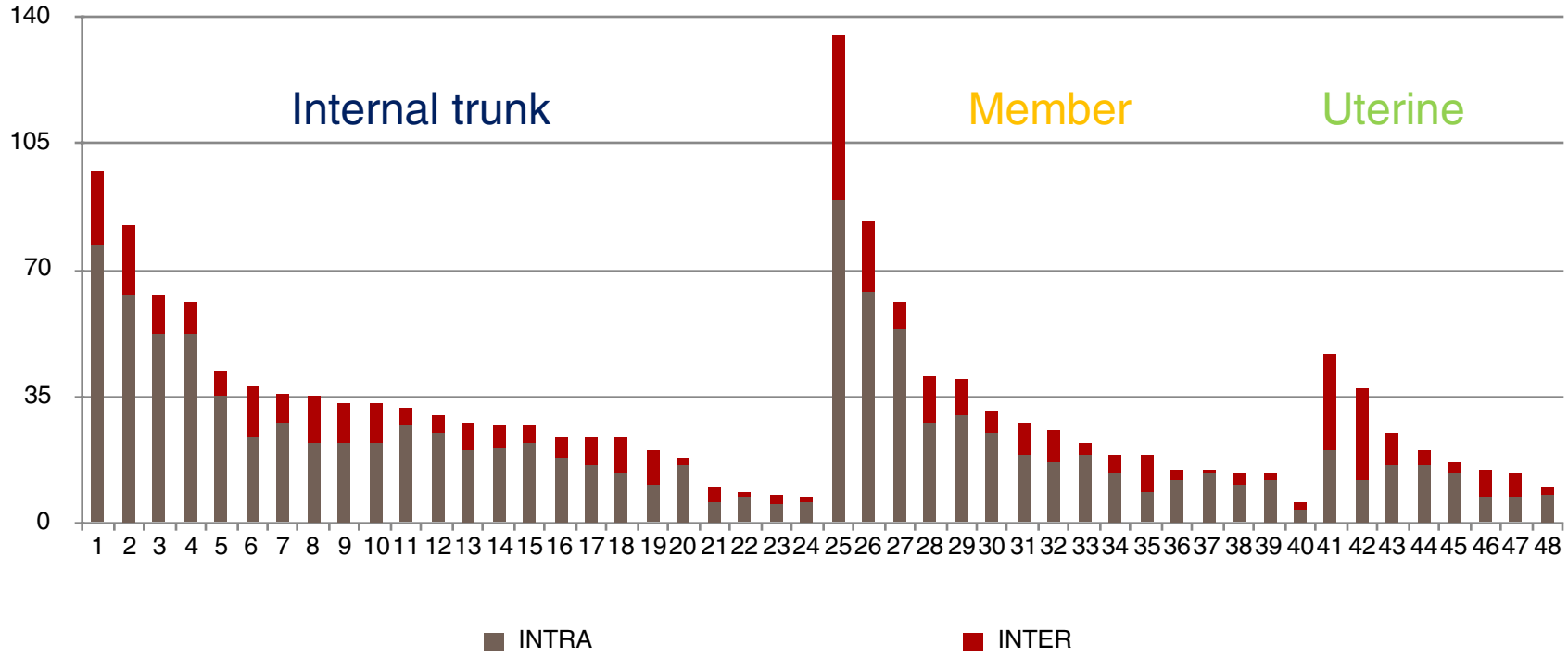
Somatic Exonic Variants



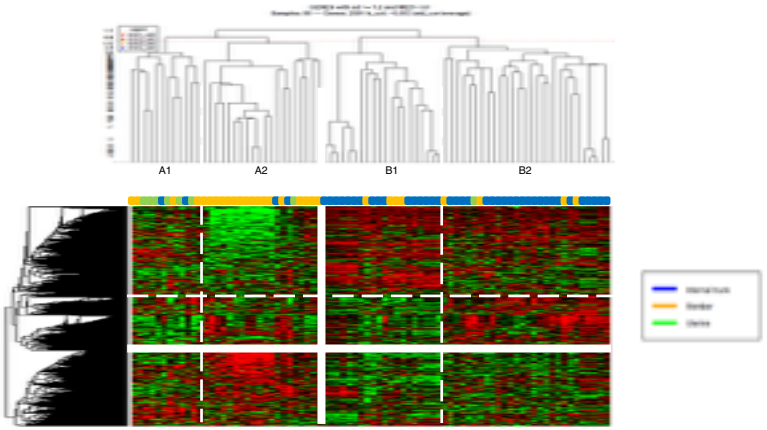

Internal trunk
Member
Uterine
60
45
30
15
0
LMS8_1
LMS5
LMS32
LMS20
LMS3
LMS39
LMS9_1
LMS16
LMS31
LMS13
LMS29
LMS59
missense
nonsense
stoploss
synonymous


30
23
15
8
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46
nonframeshift
frameshift

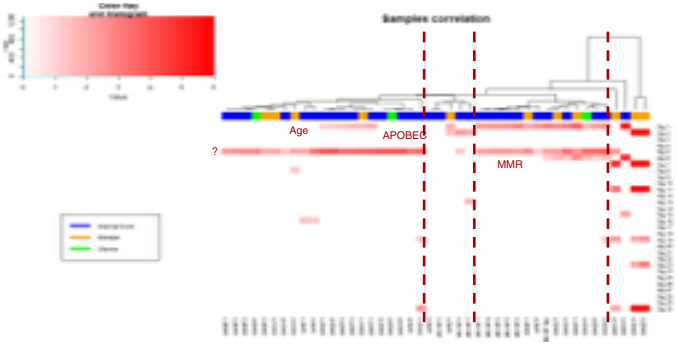
RNAseq: Fusion Trancrits



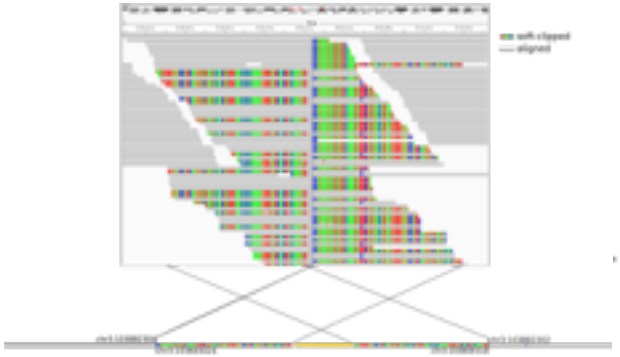
RNAseq: Clustering

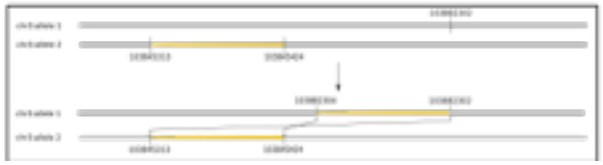


Mutational signatures: Clustering

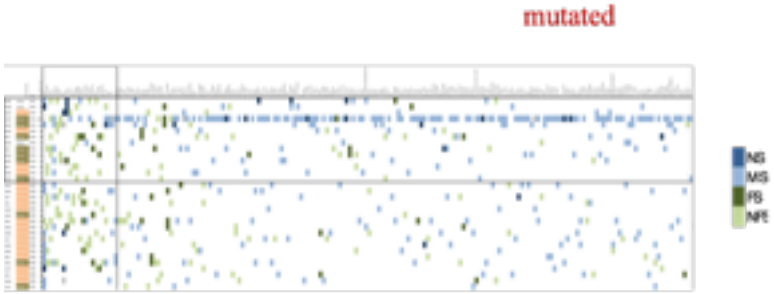


Restructuration du génome: CNV & Translocations



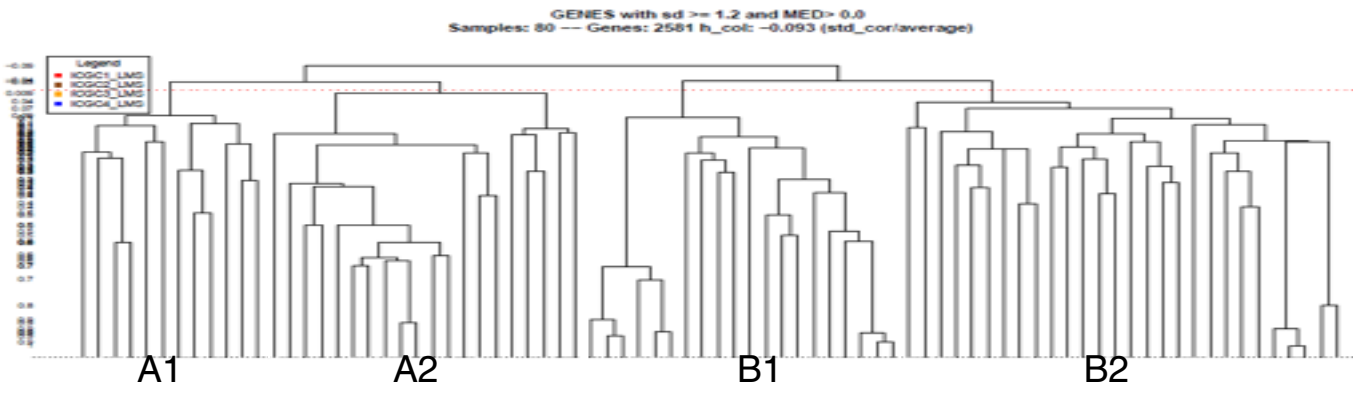


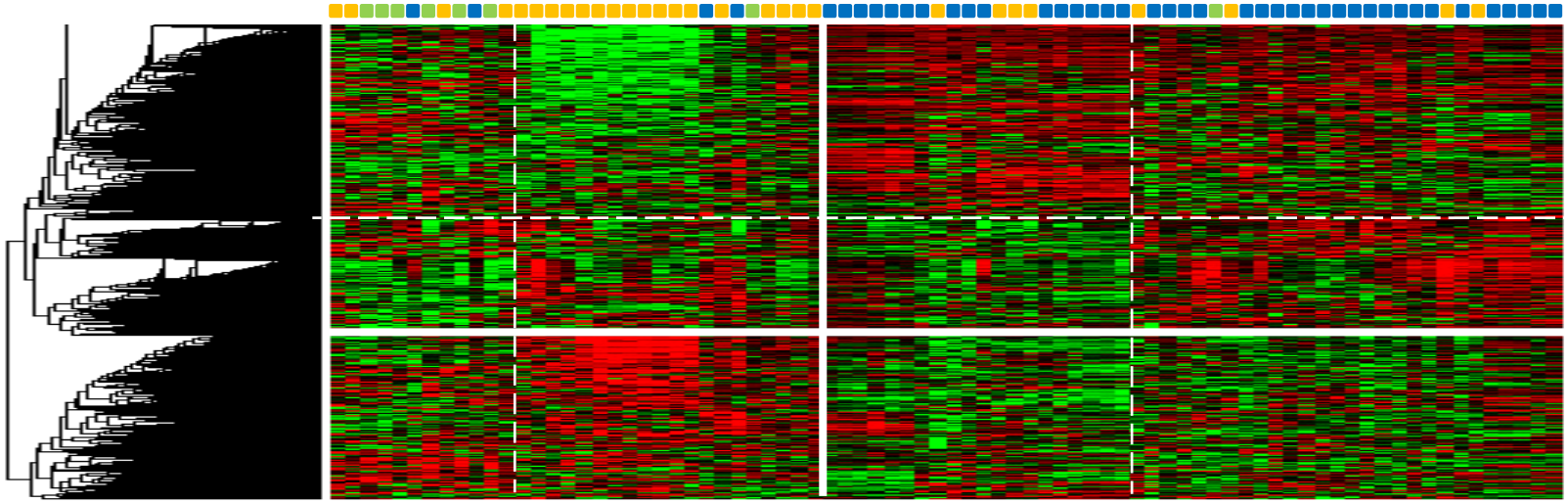
Oncogènes et GST: Mutations



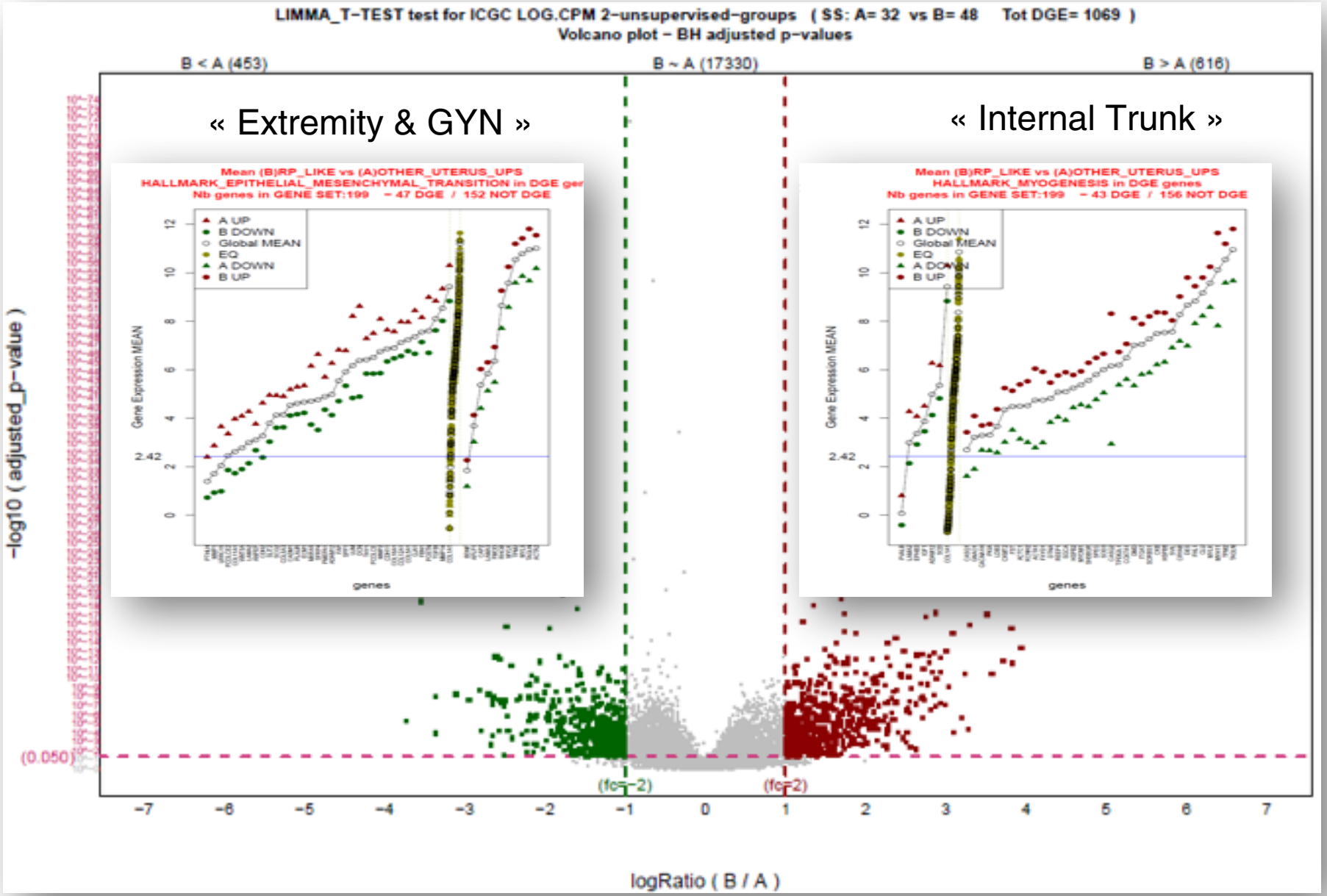
TP53: 50%
« Internal trunk »: 66%
« Member »: 21%

RNAseq: Clustering



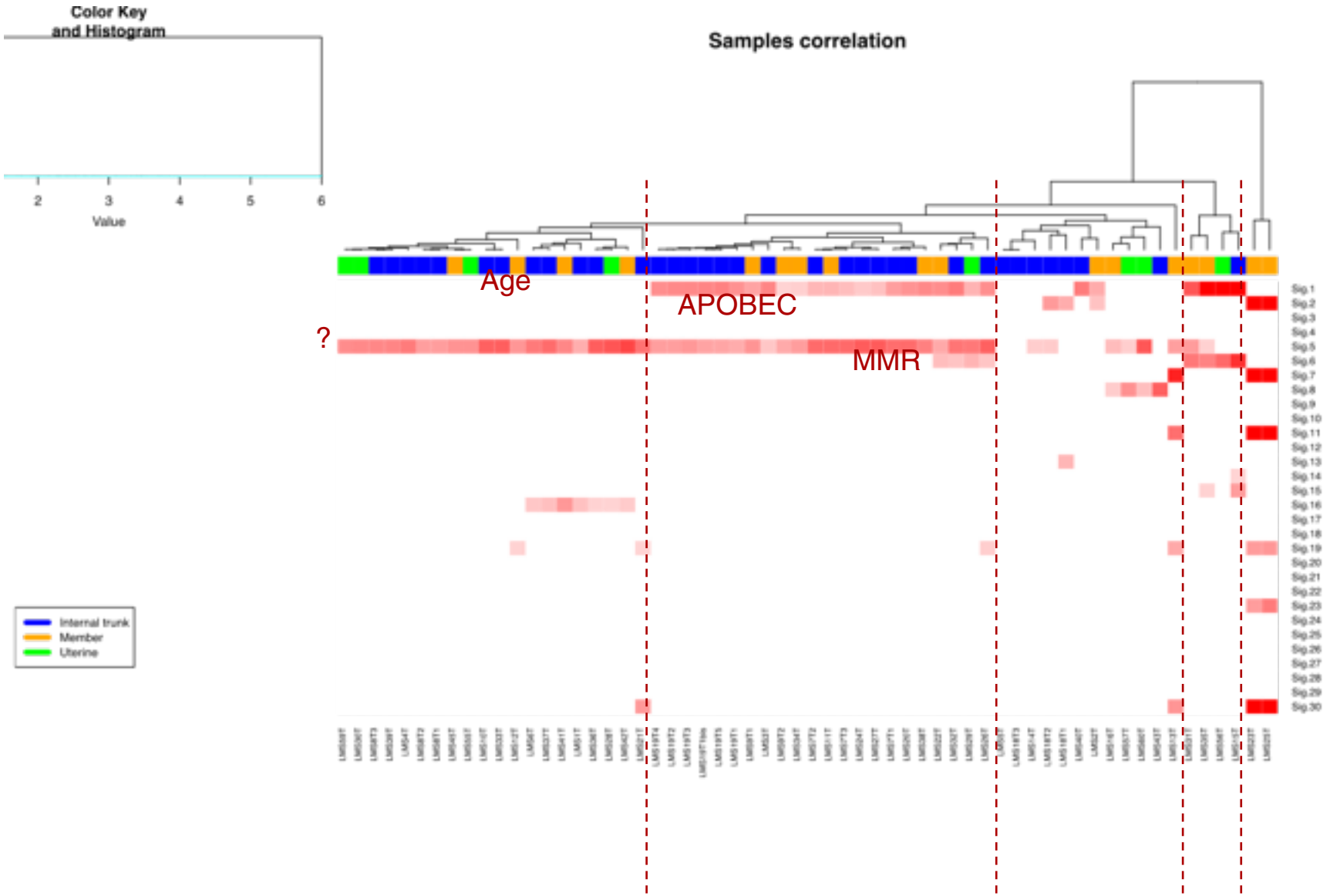


RnaSeq Group A vs B – Limma T-test

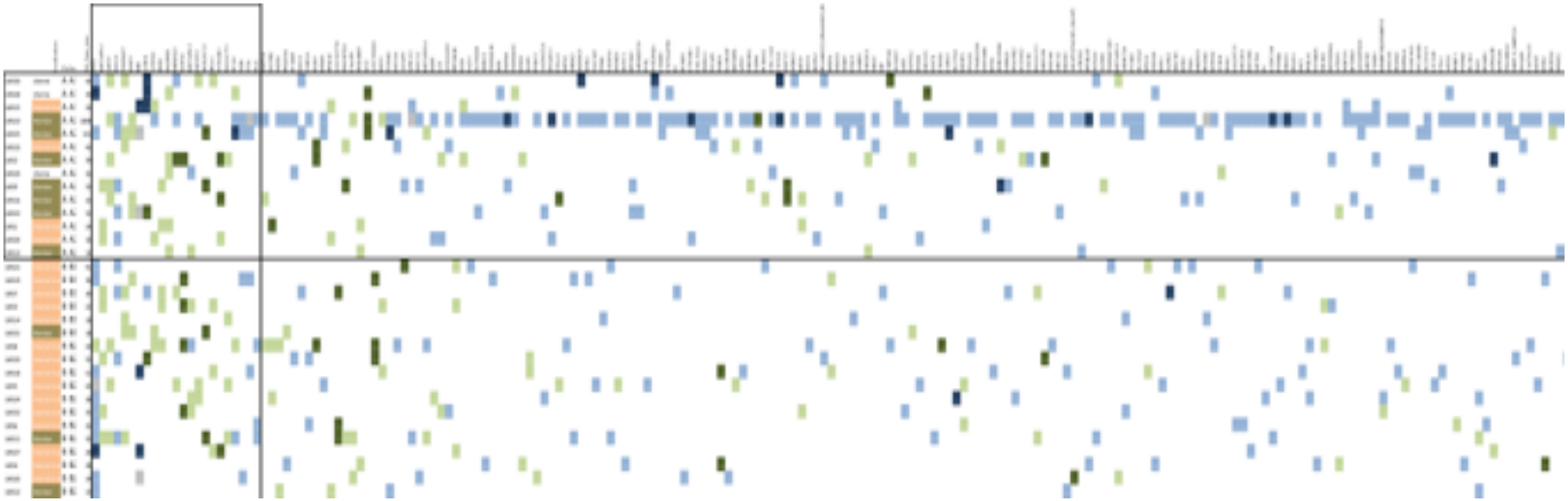



Contribution
Motif
C>A
C>G
C>T
T>A
T>C
T>G
LMS20T
LMS21T
LMS22T
LMS23T
LMS24T
LMS25T

Mutational signatures: Clustering



The 200 most frequently mutated genes



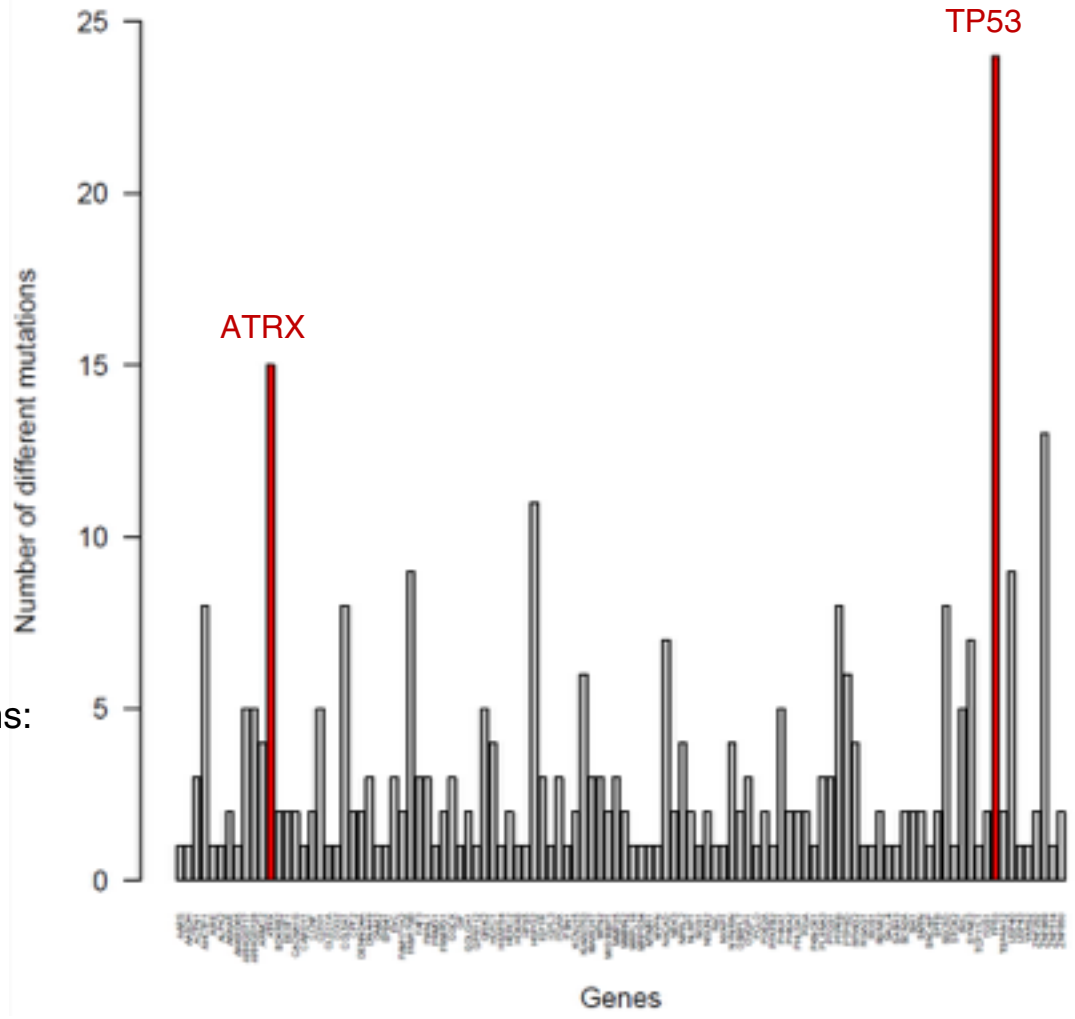
NS
MS
FS
NFS

TP53: 50%

« Internal trunk »: 66%

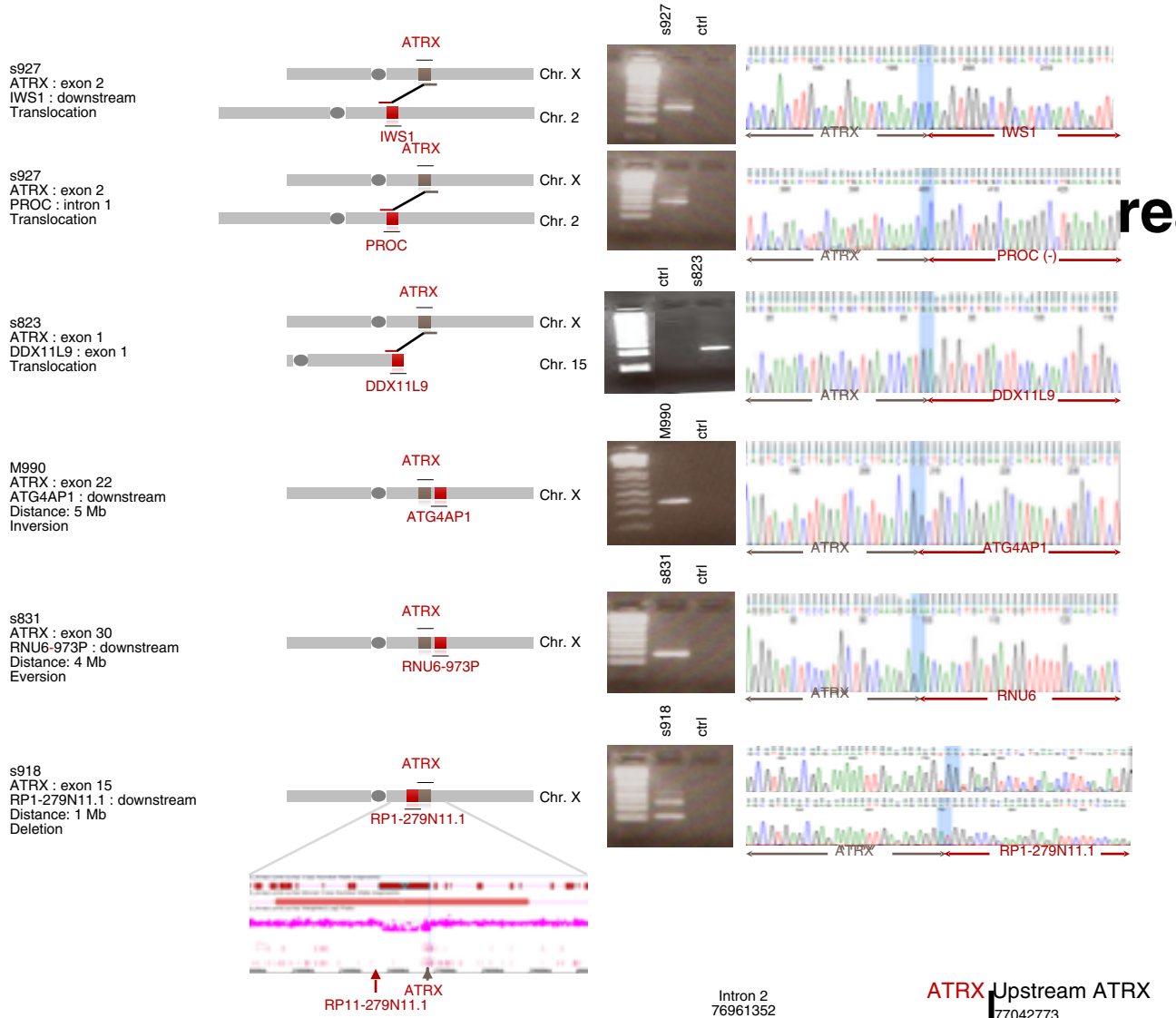
« Member »: 21%

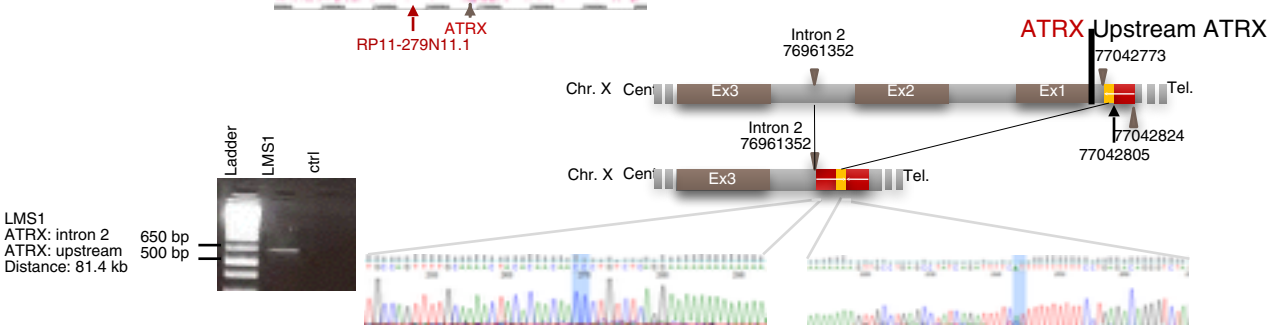
The 200 most frequently **mutated** genes



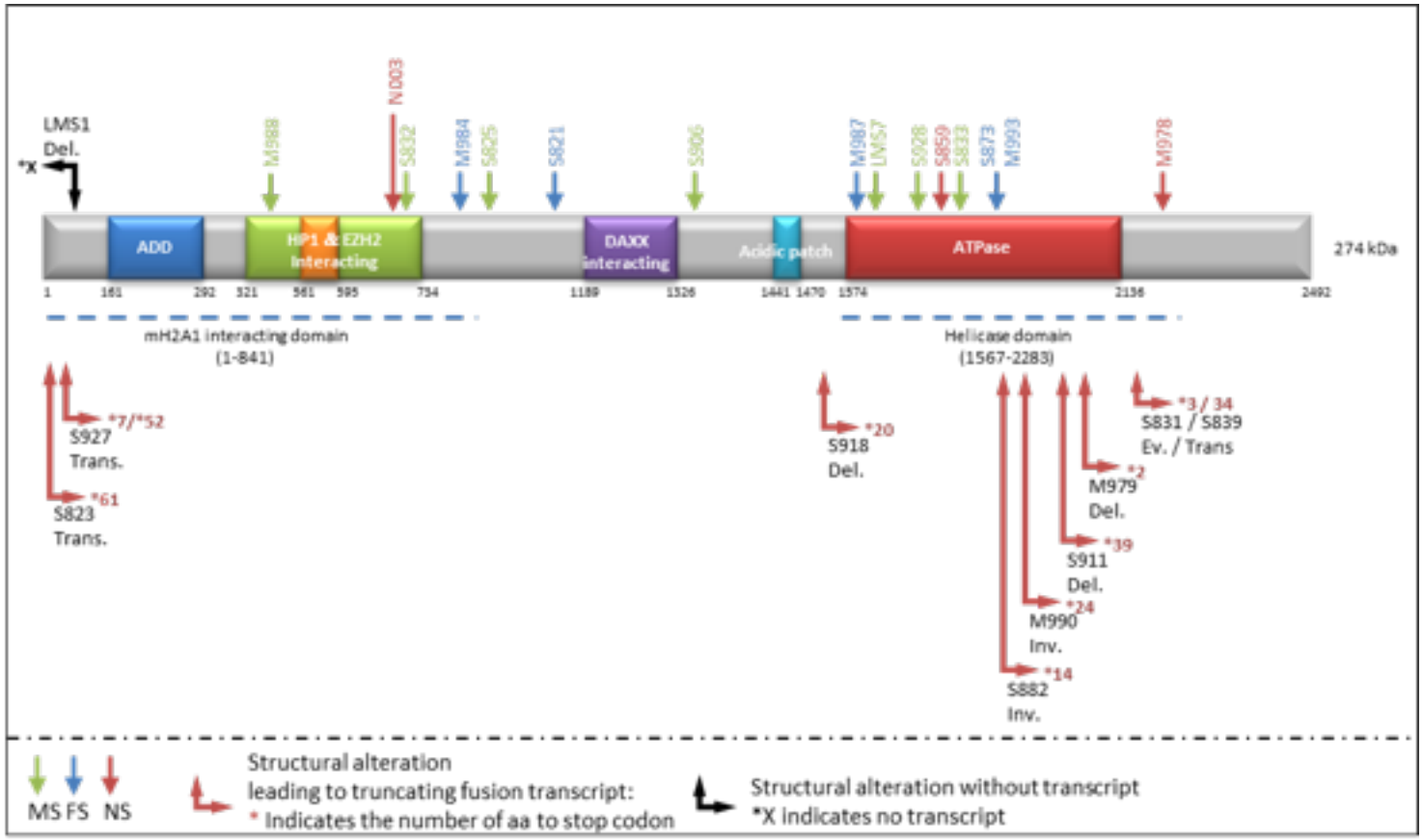
Exonic point mutations:
Nonsense
Misense
Stop Loss

ATRX
Large rearrangements

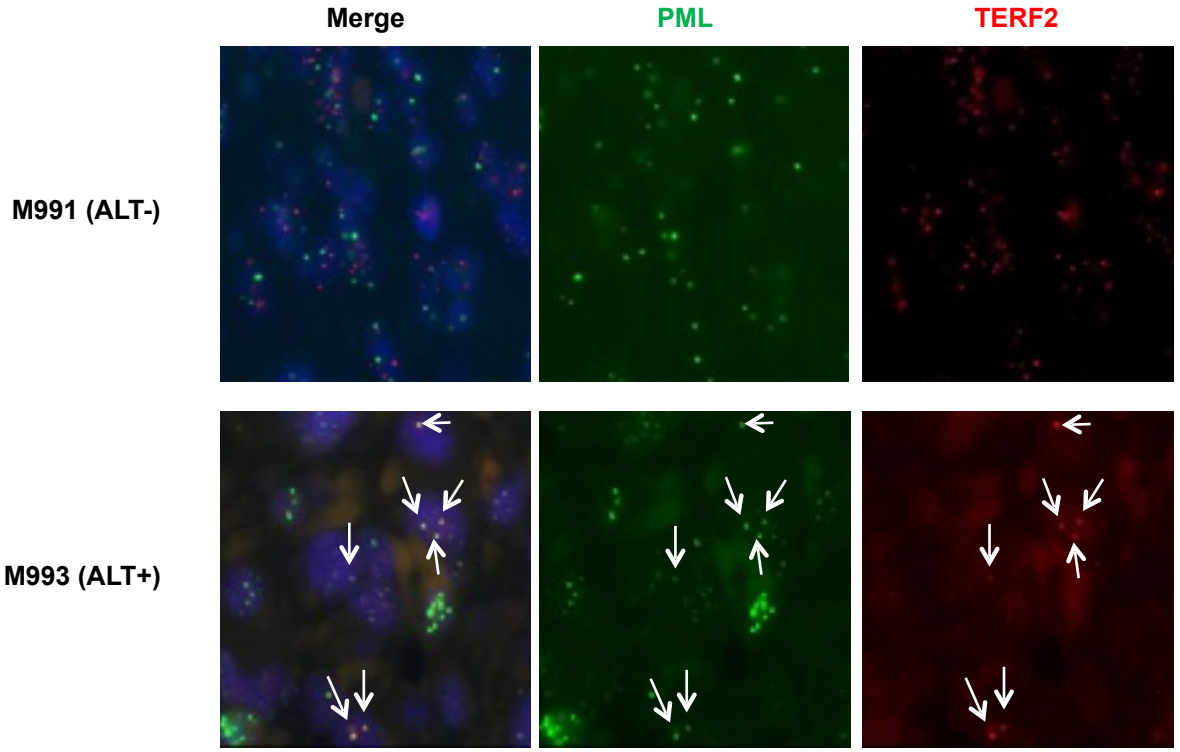


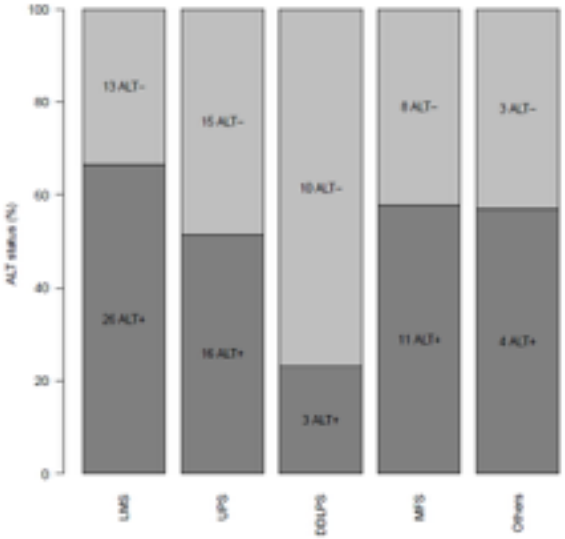


ATRX mutations



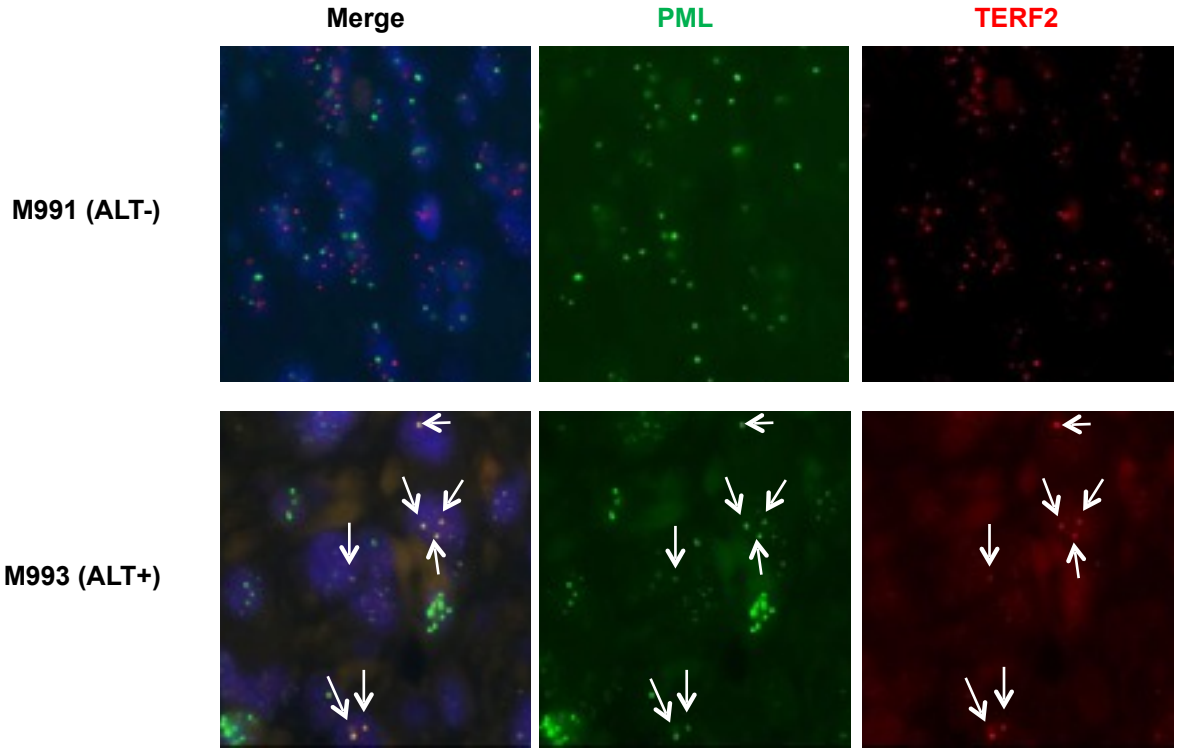
ATRX & ALT phenotype

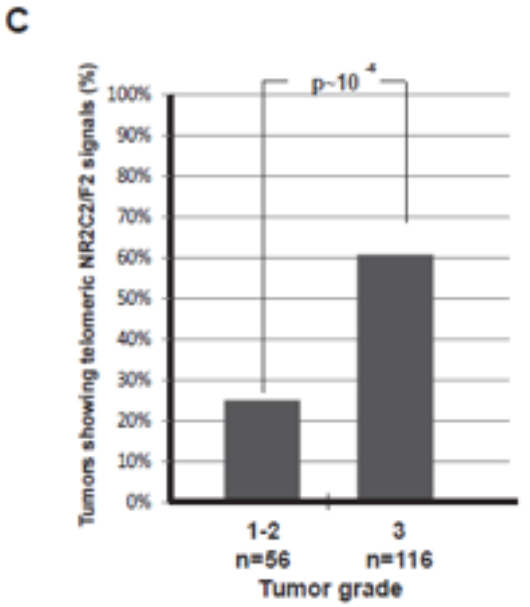




ATRX mutated cases: 97% ALT+

ATRX & ALT phenotype

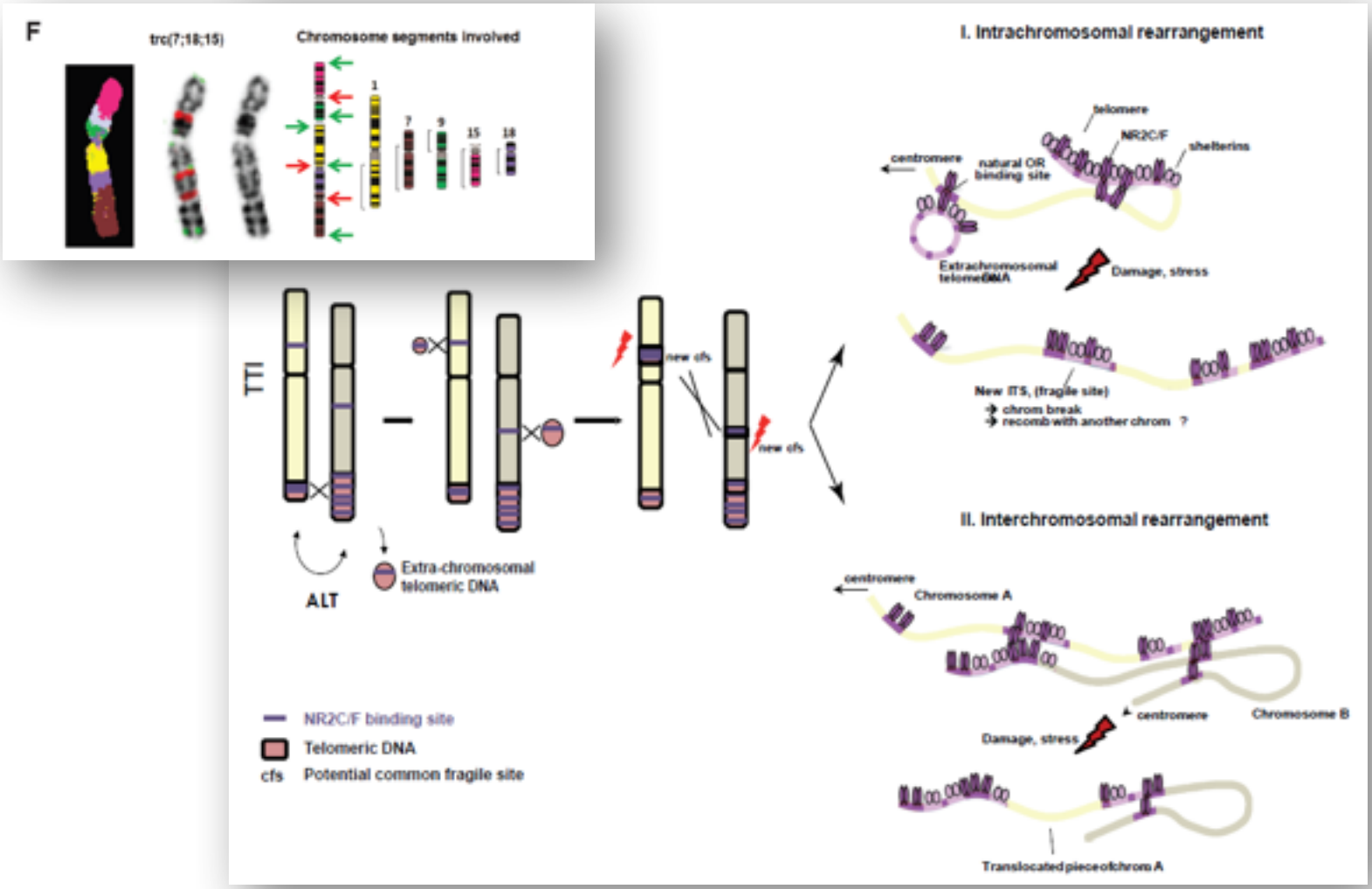




Marzec et al; Cell 2015

ATRX mutated cases: 97% ALT+

ALT & Chromosomal instability
Targeted Telomere Insertion



Marzec et al; Cell 2015

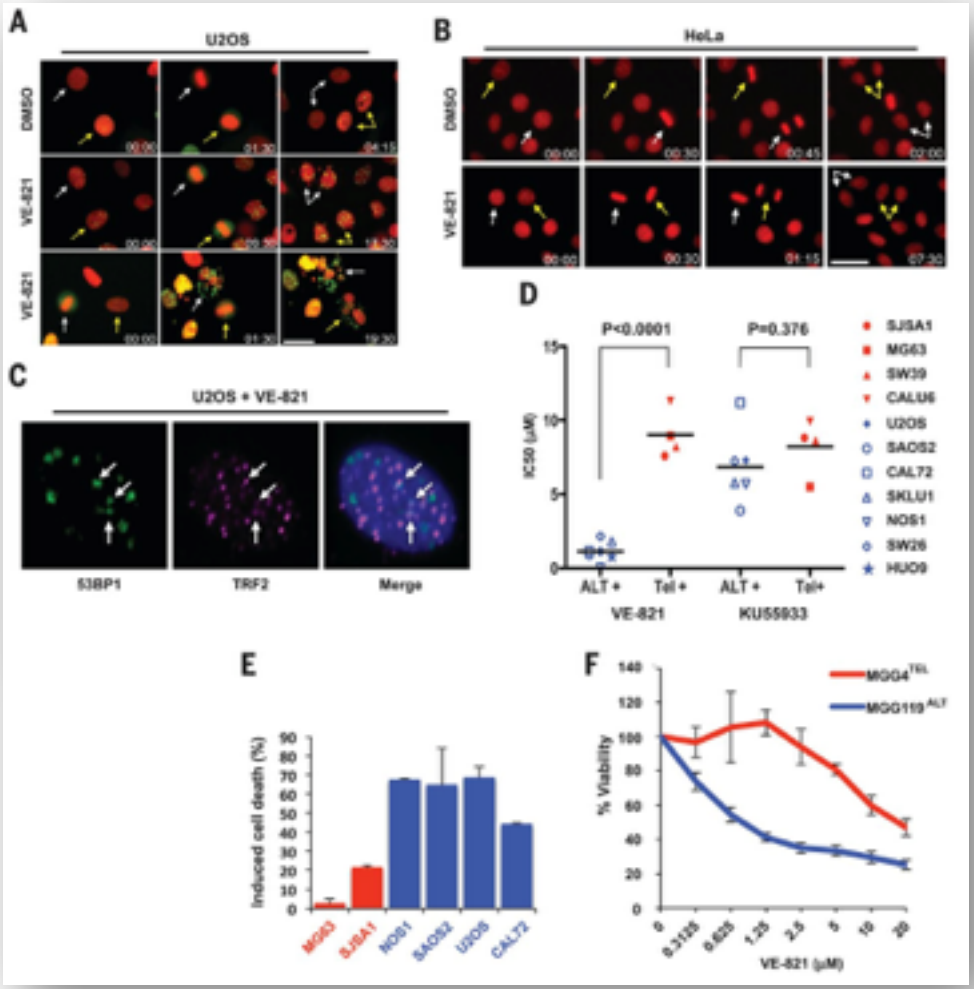
ATRX, ALT & Therapie ?

TELOMERES IN CANCER

Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors

Rachel Litman Flynn,[1,2]* Kelli E. Cox,[1]† Maya Jeitany,[1]† Hiroaki Wakimoto,[3] Alyssa B. Bryll,[3] Nidhi J. Ganesan,[3] Francesco Bertuzzi,[3,4] Jose M. Pilarski,[3] Mario L. Suvà,[3,5] Cyril H. Benes,[1] Daniel A. Haber,[1,6] Francois D. Bonnetin,[3] Lee Zou[1,6]*

Cancer cells rely on telomerase or the alternative lengthening of telomeres (ALT) pathway to overcome replicative mortality. ALT is mediated by recombination and is prevalent in a subset of human cancers, yet whether it can be exploited therapeutically remains unknown. Loss of the chromatin-remodeling protein ATRX associates with ALT in cancers. Here, we show that ATRX loss compromises cell cycle regulation of the telomeric noncoding RNA TERRA and leads to persistent association of replication protein A (RPA) with telomeres after DNA replication, creating a recombinogenic nucleoprotein structure. Inhibition of the protein kinase ATR, a critical regulator of recombination recruited by RPA, disrupts ALT and triggers chromosome fragmentation and apoptosis in ALT cells. The cell death induced by ATR inhibitors is highly selective for cancer cells that rely on ALT, suggesting that such inhibitors may be useful for treatment of ALT-positive cancers.



Acknowledgments

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Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale