

ICGC LEIOMYOSARCOMAS



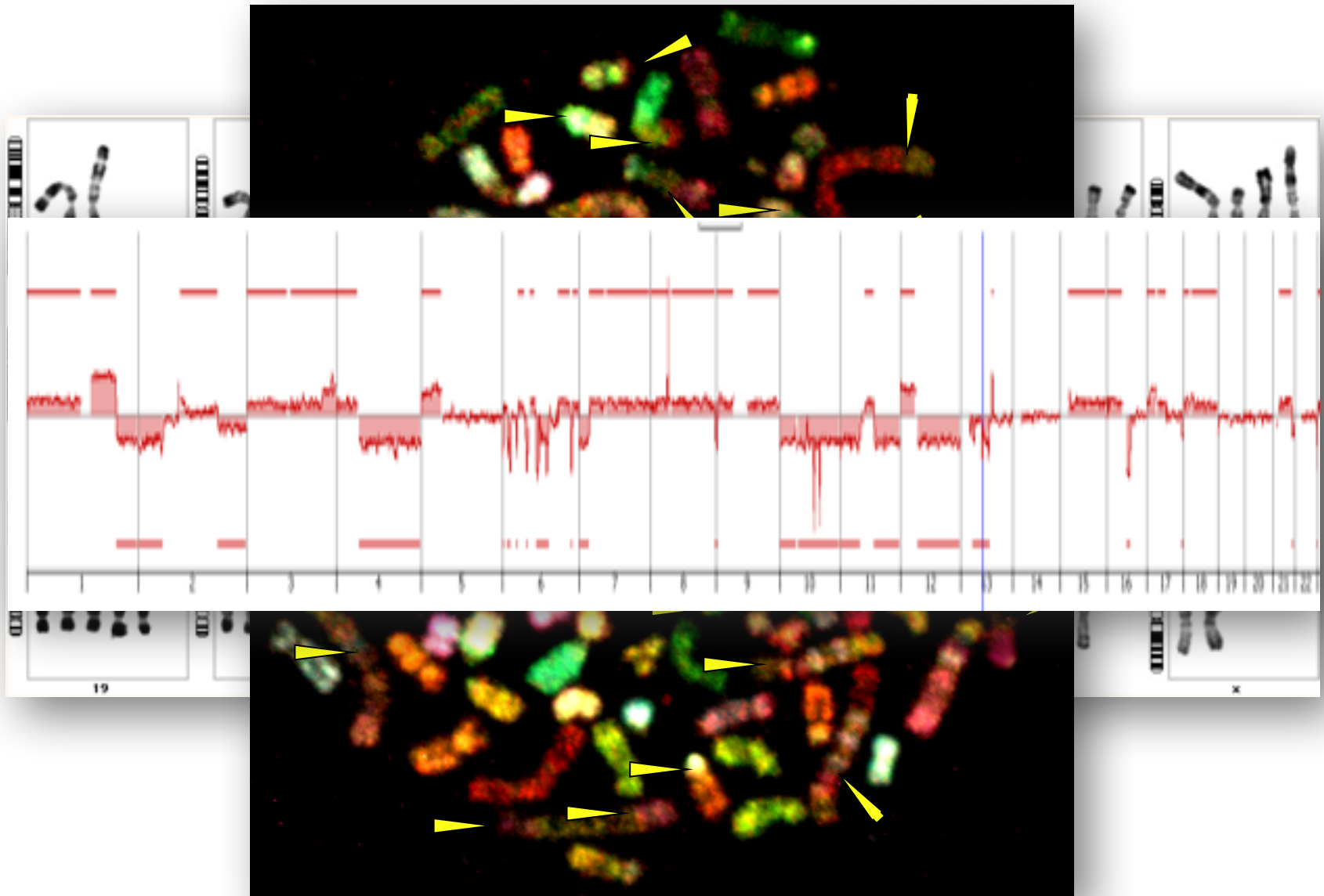
International Cancer Genome Consortium



Fred Chibon
INSERMU1218 – Institut Bergonié
Bordeaux, France

Funded by ITMO cancer (INCa / INSERM)

LMS karyotype and genome



- 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.
-
- 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 through the known or inferred function of the genes that they may alter. At least two hundred mutations/alterations should thus be investigated.
-
- A second series of >100 LMS will be analysed secondly according to the relevant alterations identified in the first series

Objectives

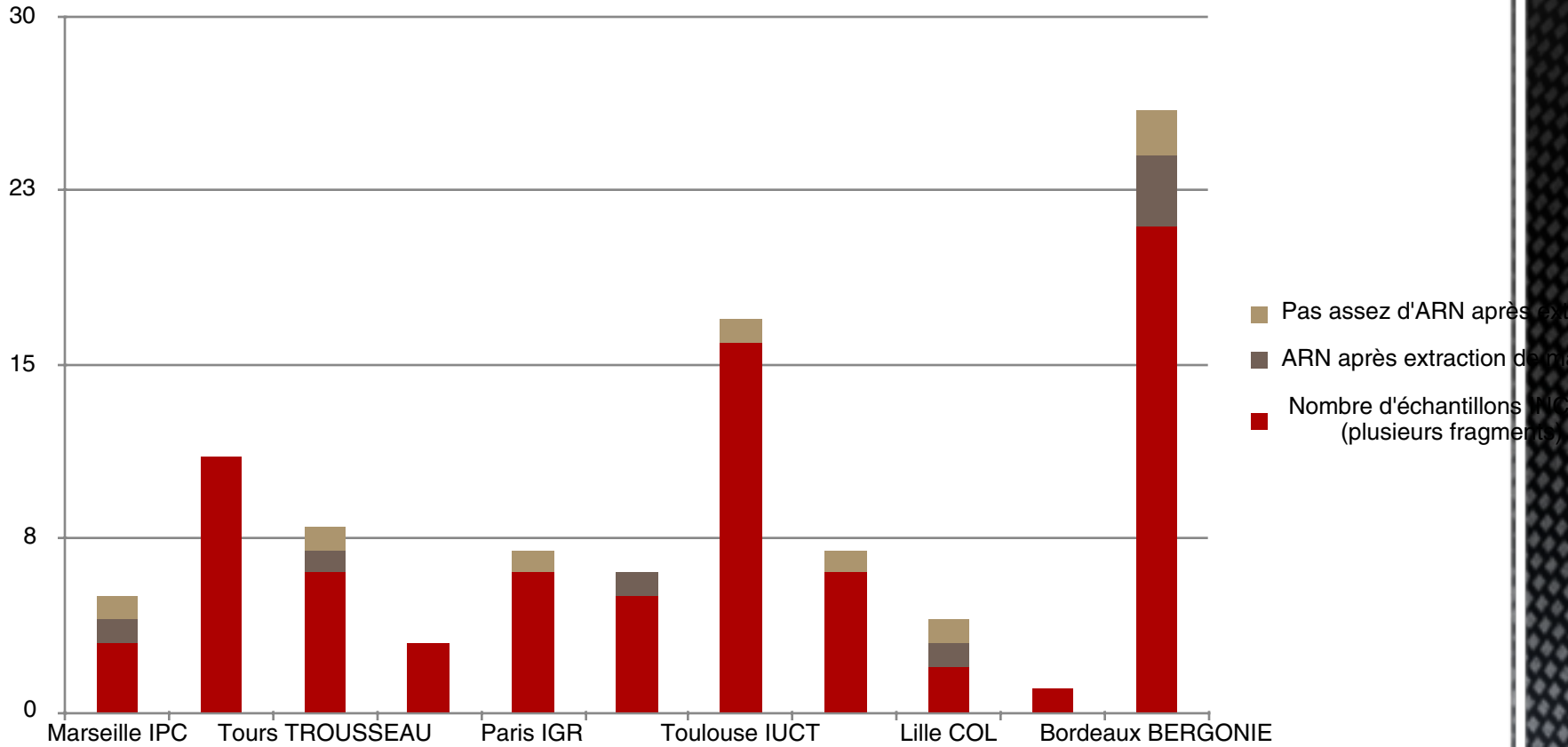
Inclusion



- 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)



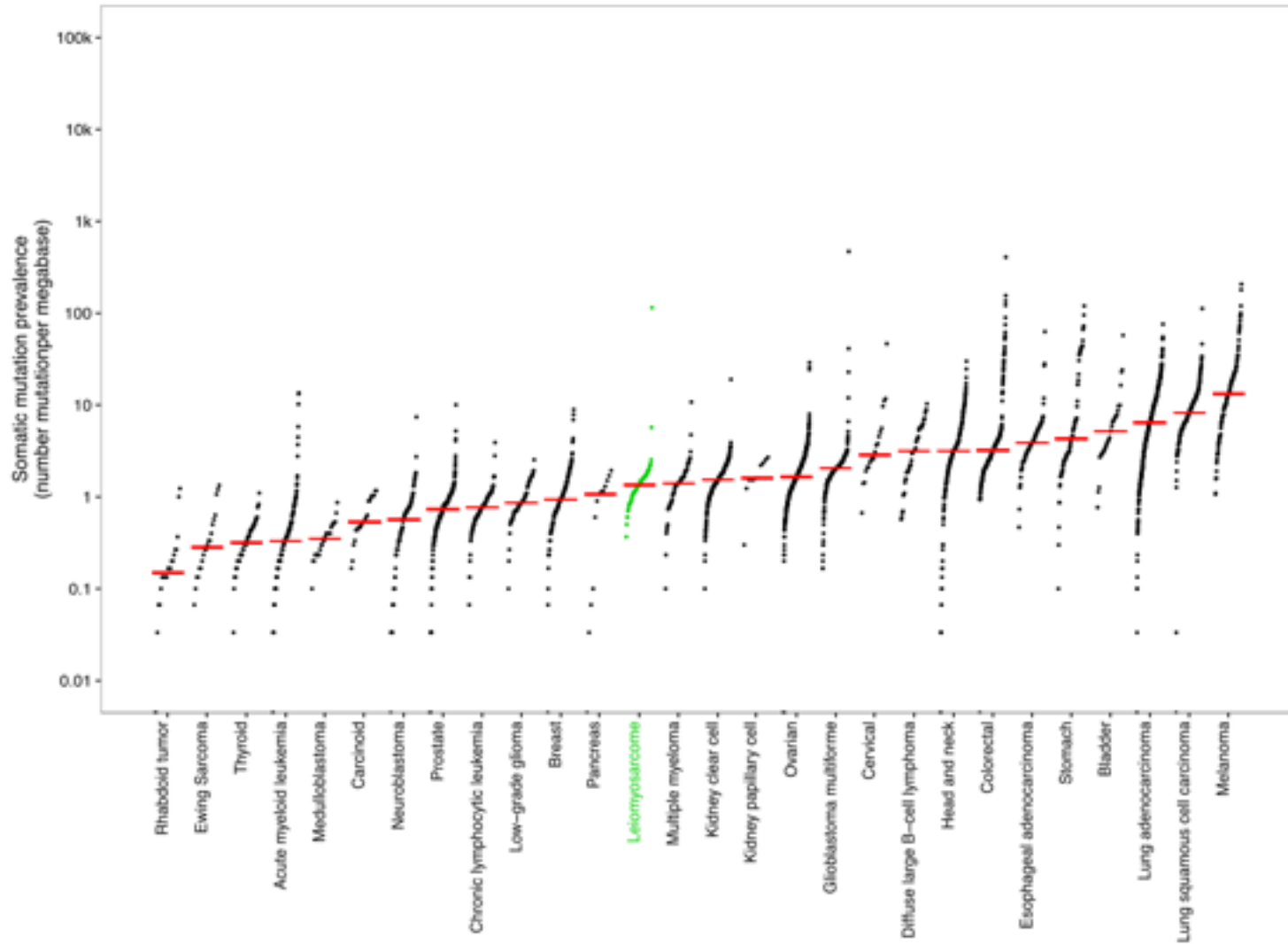
Inclusion



| 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) |

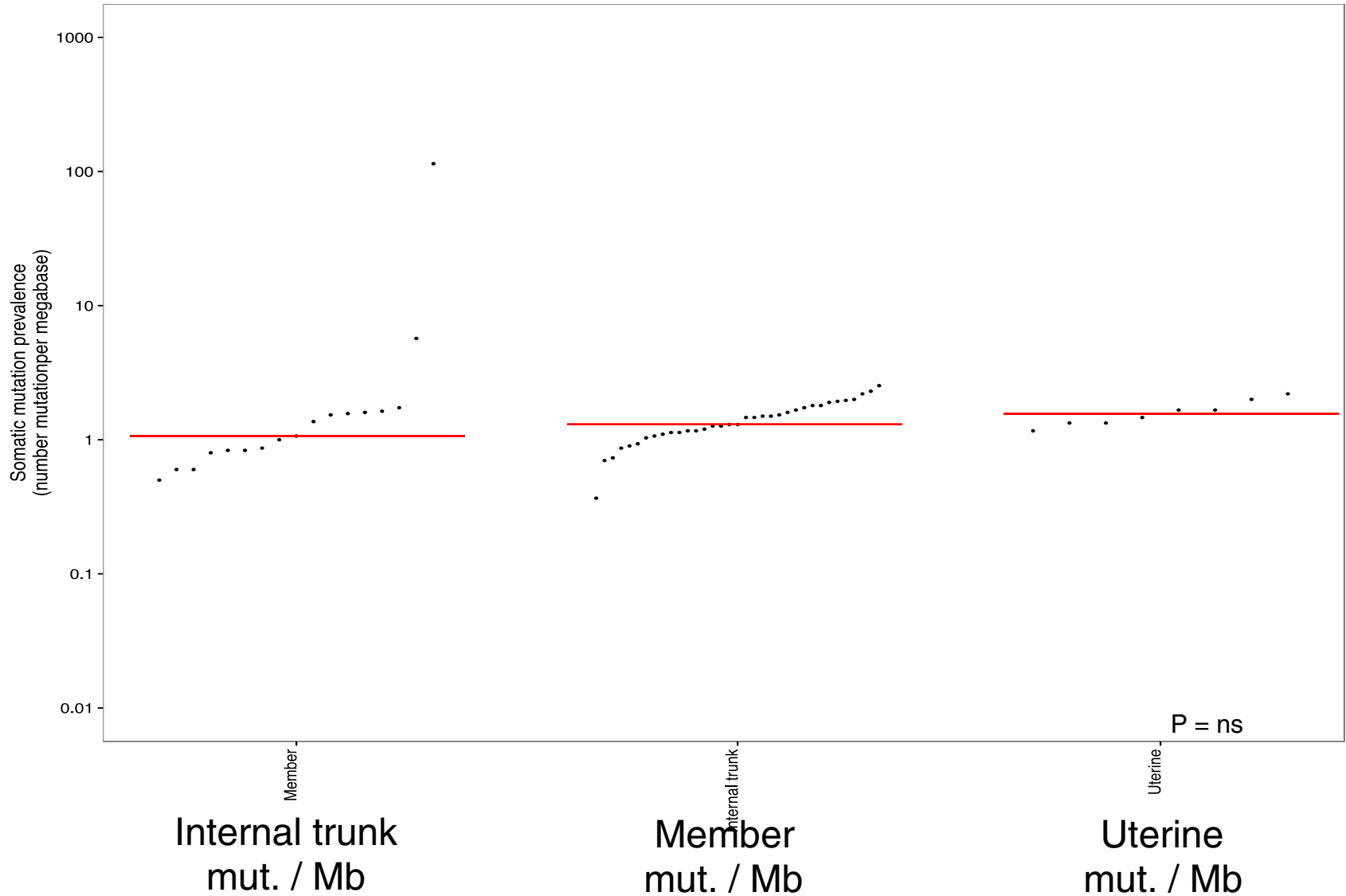
**68 cas inclus et
séquencés**

Somatic Coding Variants

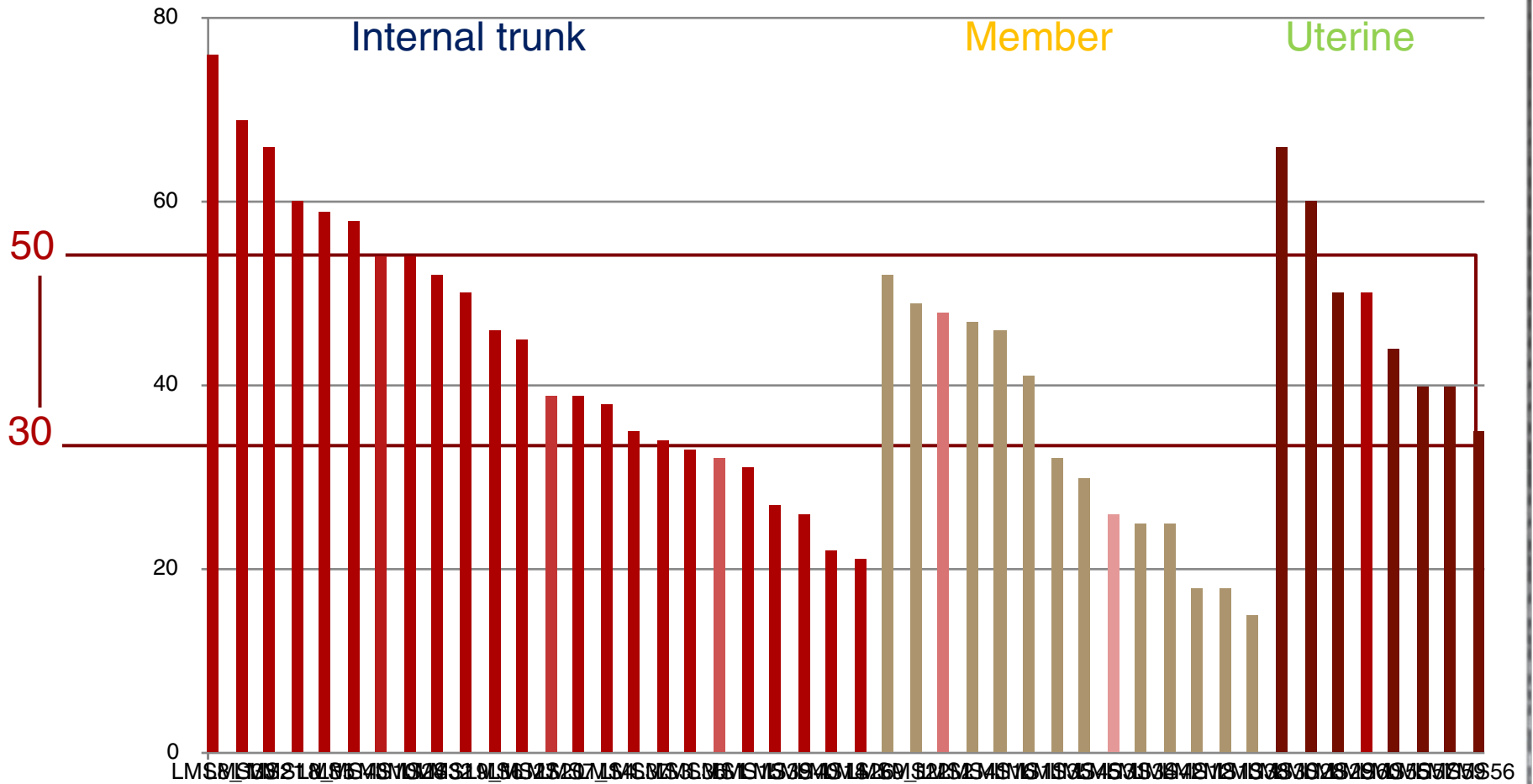


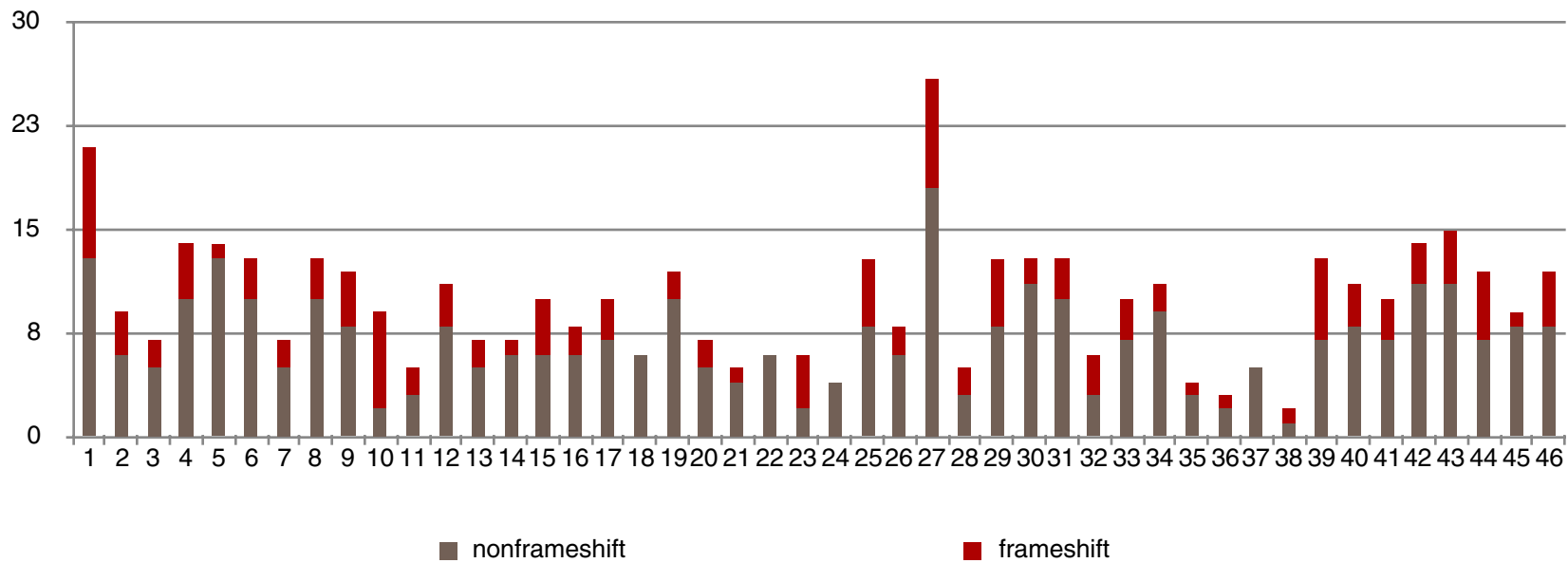
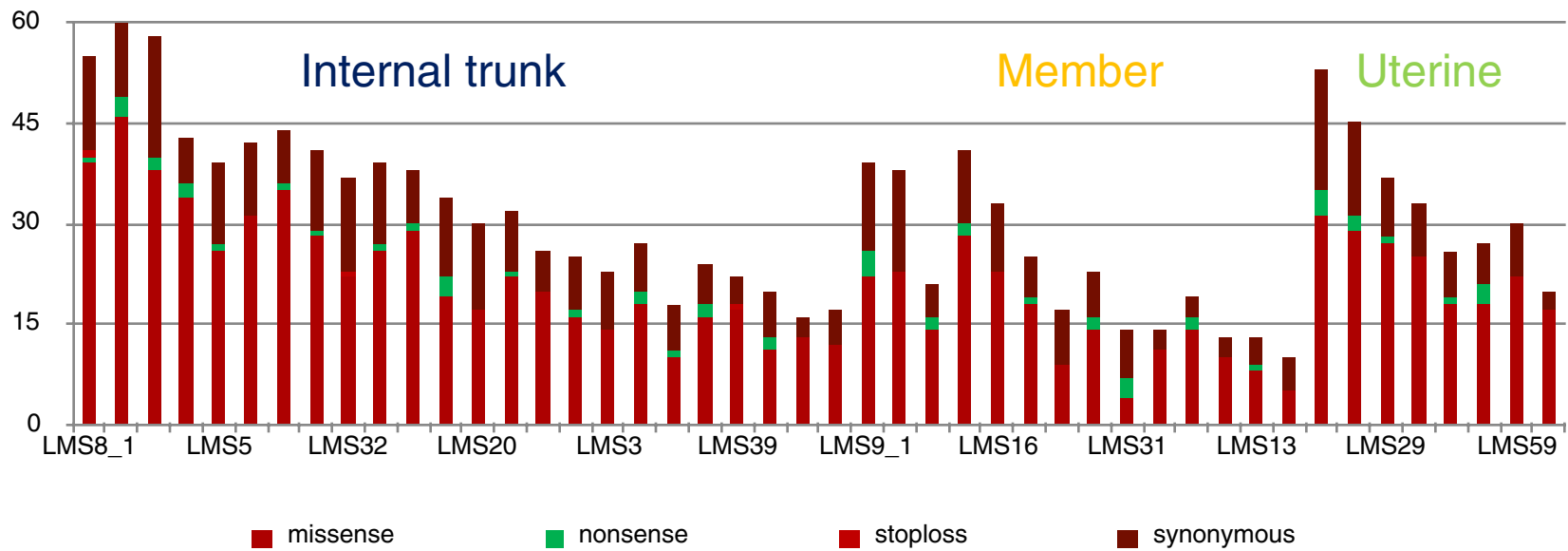
1,5 somatic mutations per coding MB

Somatic Coding Variants

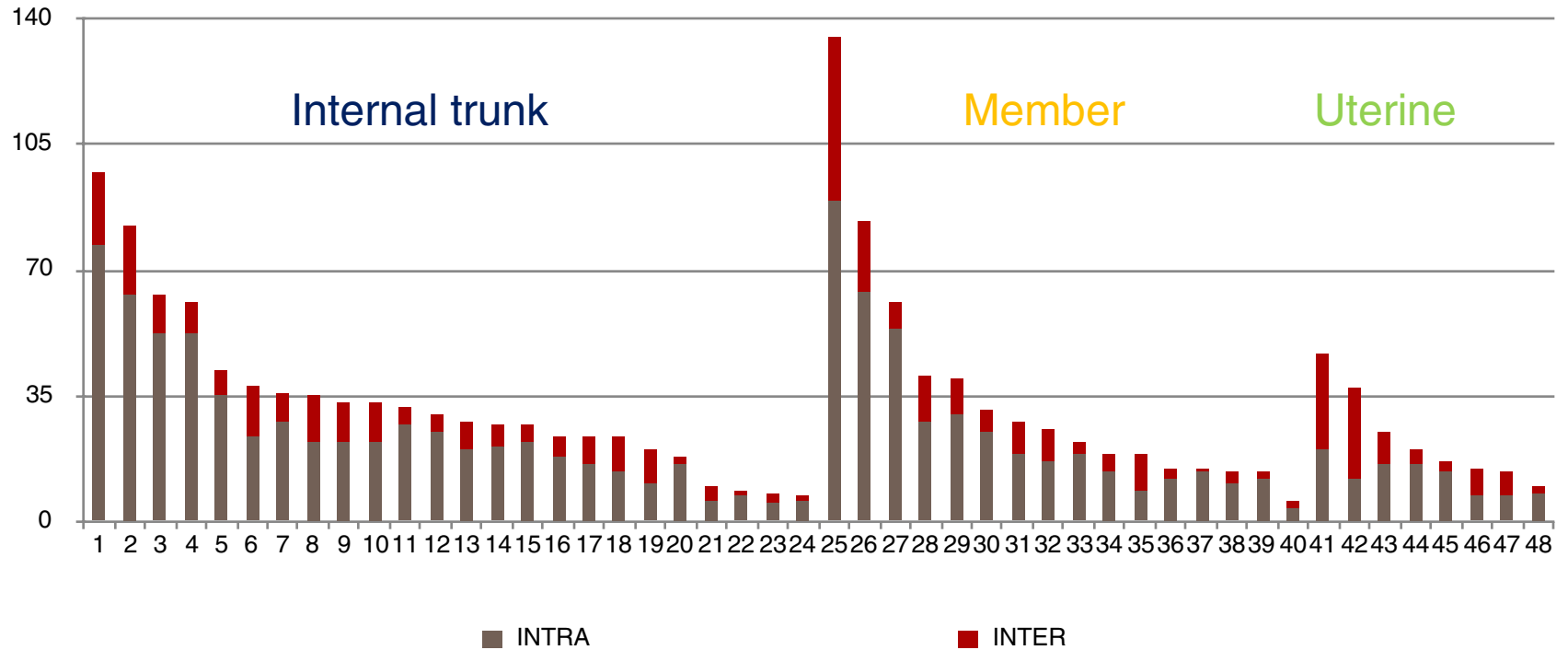


Somatic Exonic Variants

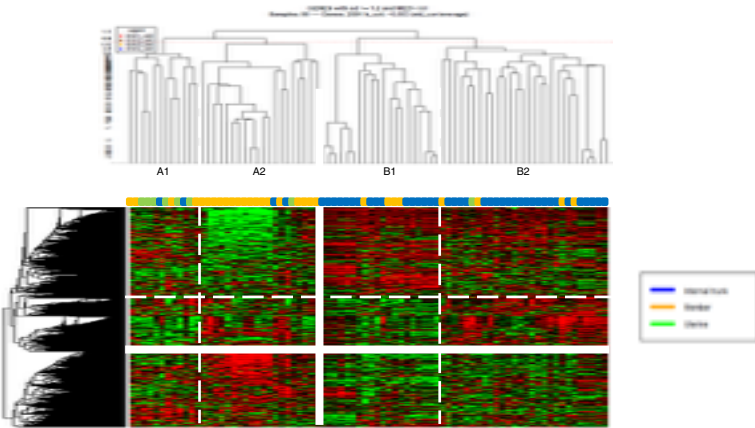




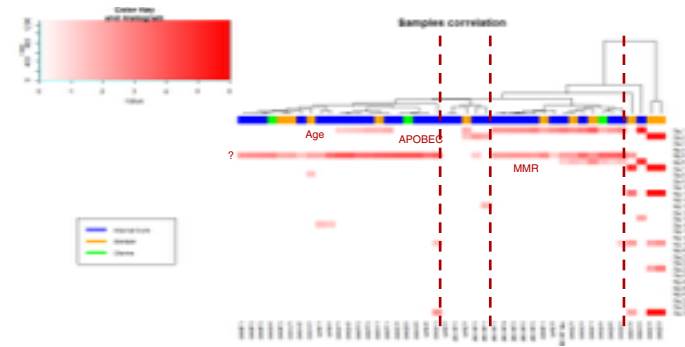
RNAseq: Fusion Transcripts



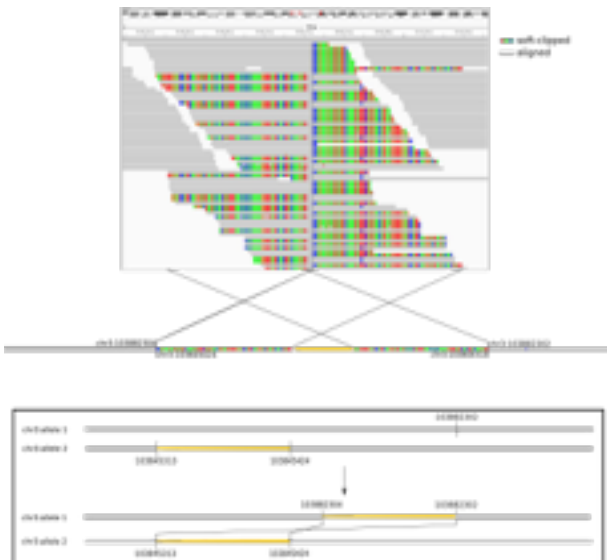
RNAseq: Clustering



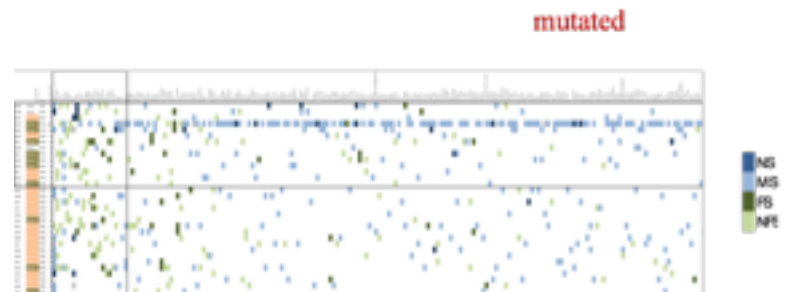
Mutational signatures: Clustering



Restructuration du génome: CNV & Translocations

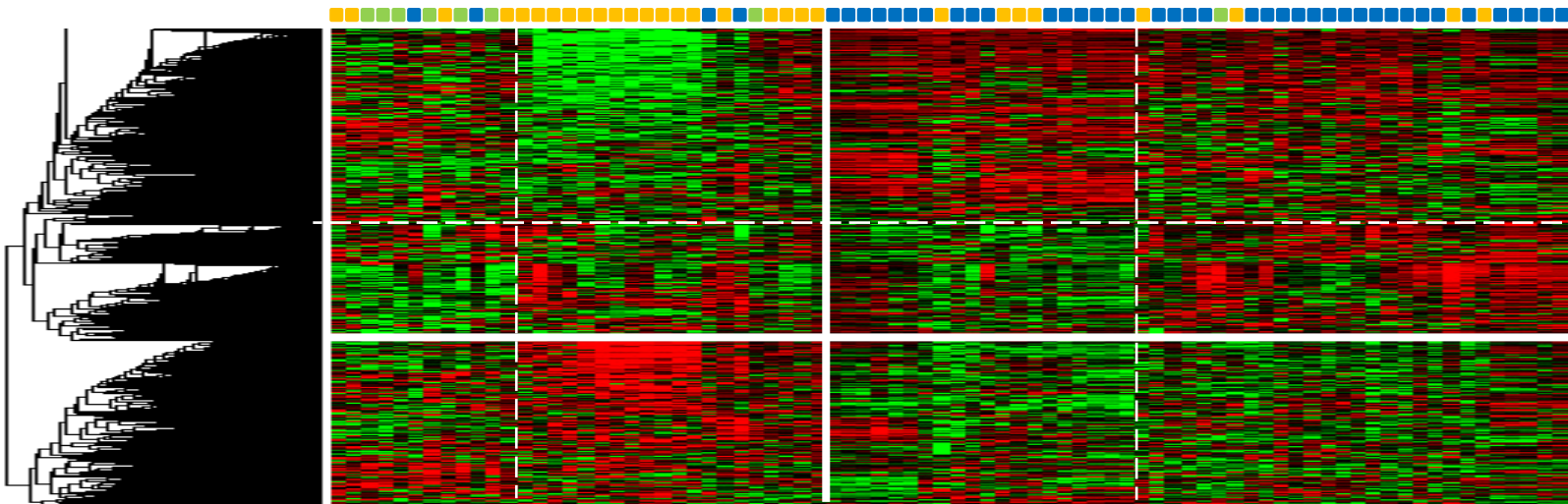
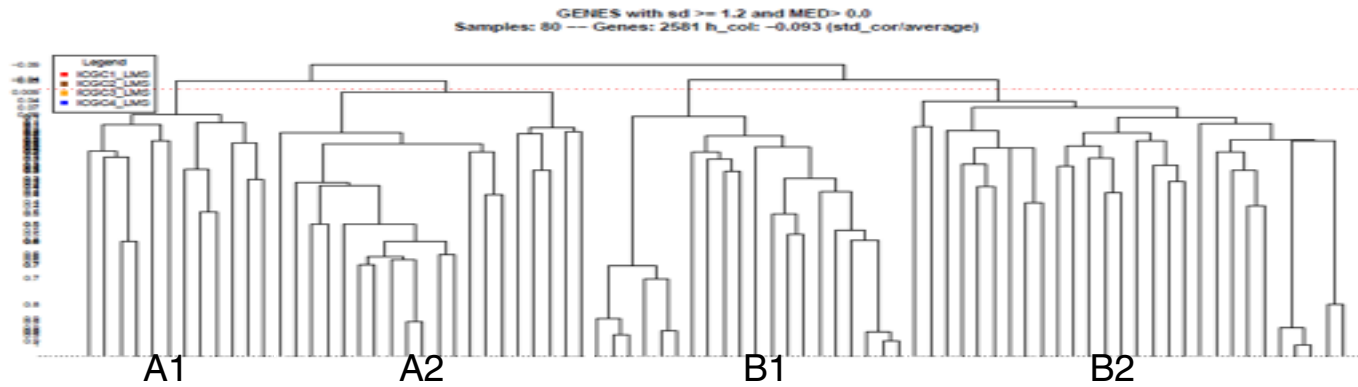


Oncogènes et GST: Mutations



TP53: 50%
 α Internal trunk: 66%
 α Member: 21%

RNAseq: Clustering



RnaSeq Group A vs B – Limma T-test

LIMMA_T-TEST test for ICGC LOG.CPM 2-unsupervised-groups (SS: A= 32 vs B= 48 Tot DGE= 1069)
Volcano plot – BH adjusted p-values

B < A (453)

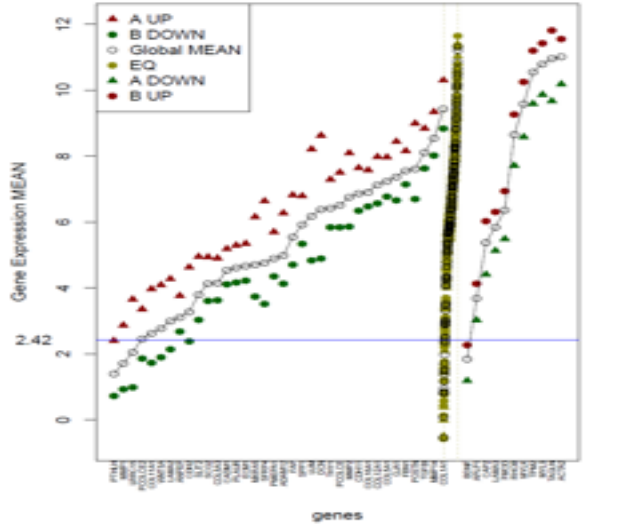
B ~ A (17330)

B > A (816)

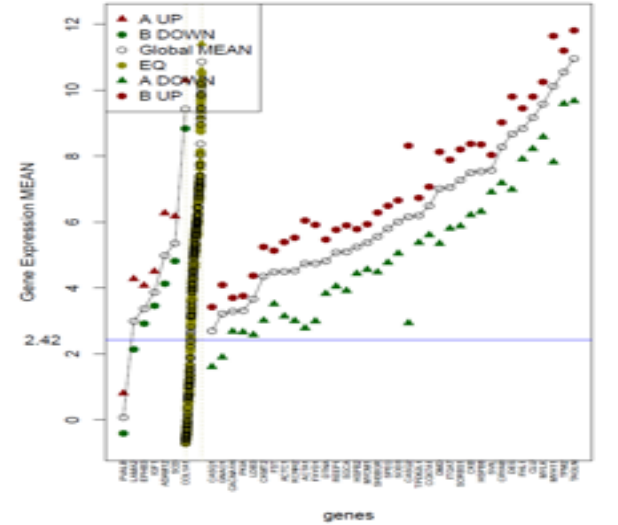
« Extremity & GYN »

« Internal Trunk »

Mean (B)RP_LIKE vs (A)OTHER_UTERUS_UPS
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION in DGE genes
Nb genes in GENE SET:199 - 47 DGE / 152 NOT DGE



Mean (B)RP_LIKE vs (A)OTHER_UTERUS_UPS
HALLMARK_MYOGENESIS in DGE genes
Nb genes in GENE SET:199 - 43 DGE / 156 NOT DGE



-log10 (adjusted_p-value)

(0.050)

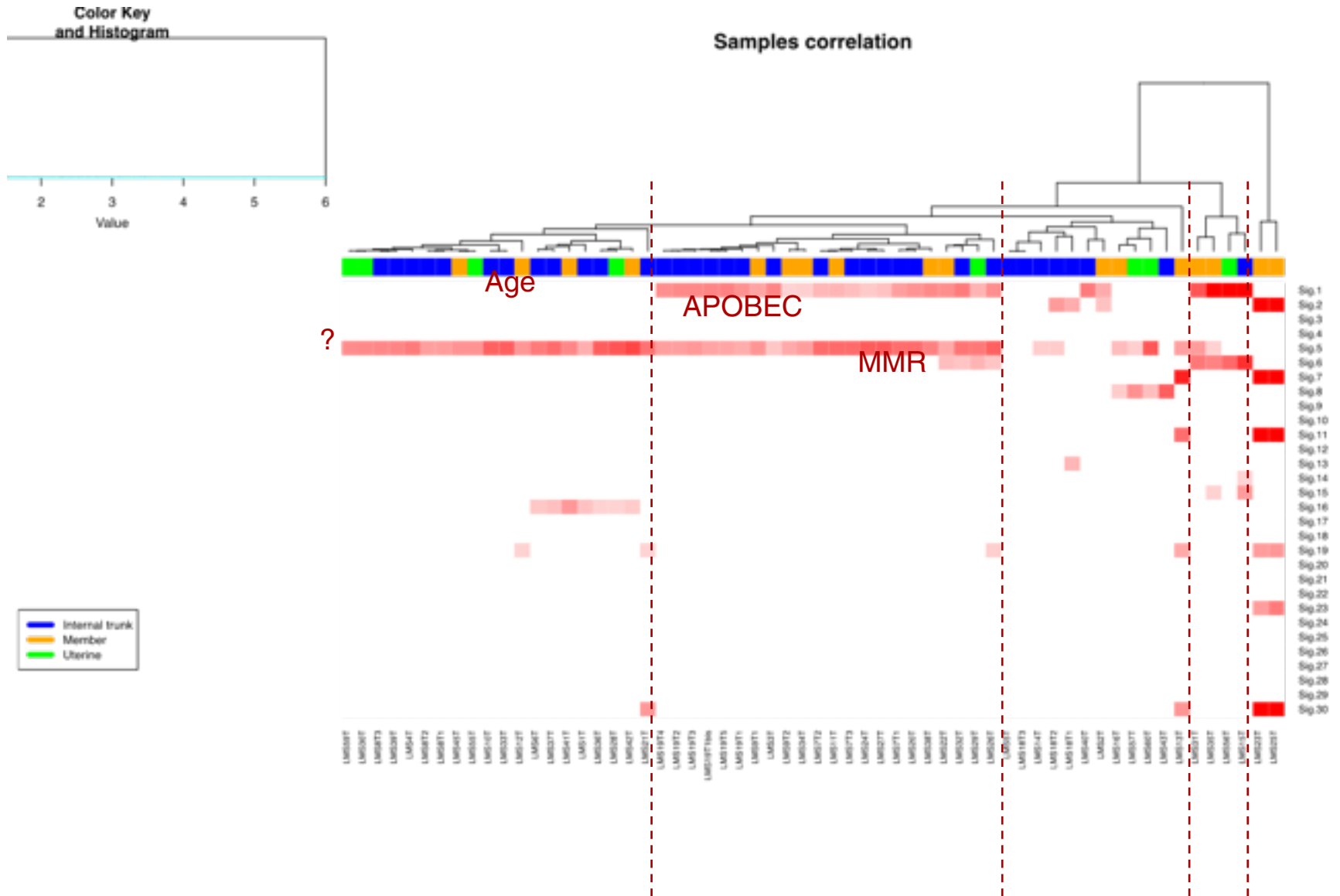
(fc=2)

(fc=2)

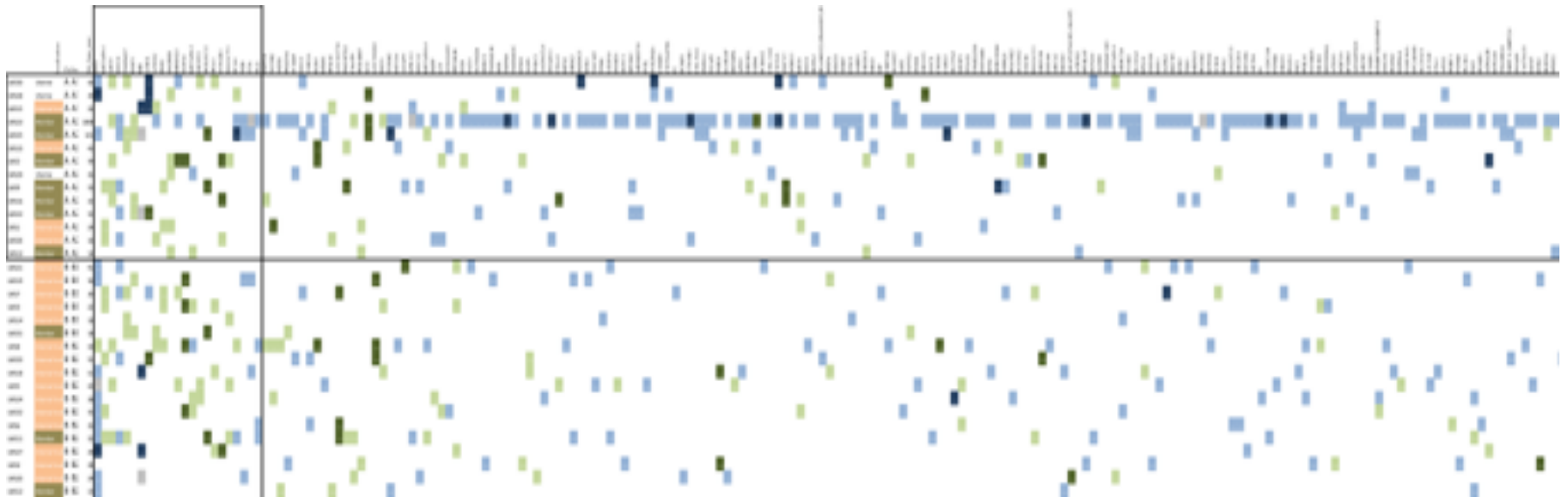
logRatio (B / A)



Mutational signatures: Clustering



The 200 most frequently mutated genes



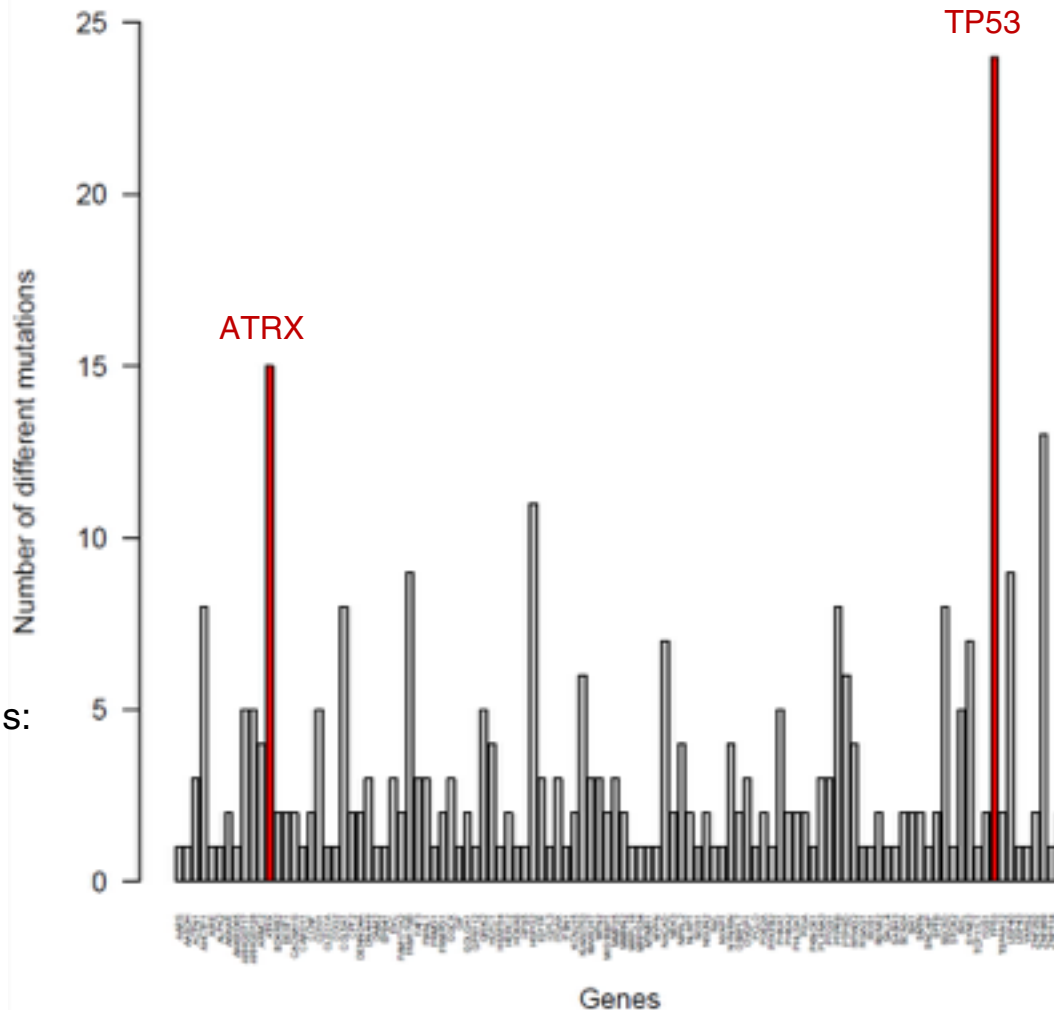
NS
MS
FS
NFE

TP53: 50%

« Internal trunk »: 66%

« Member »: 21%

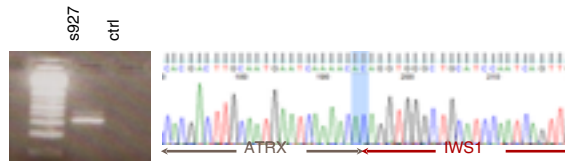
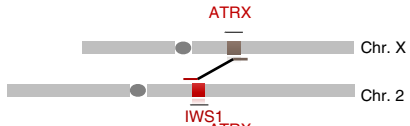
The 200 most frequently mutated genes



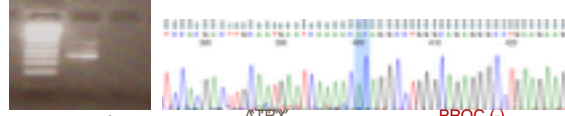
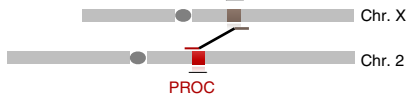
Exonic point mutations:
Nonsense
Misense
Stop Loss

ATRX Large rearrangements

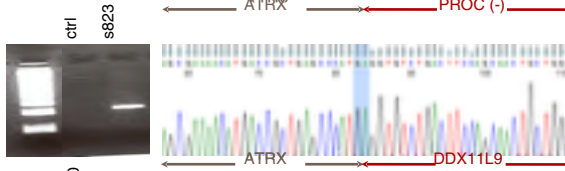
s927
 ATRX : exon 2
 IWS1 : downstream
 Translocation



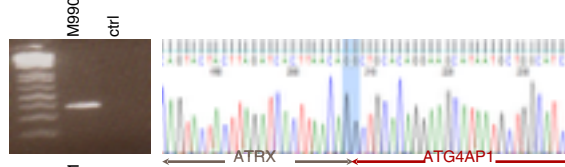
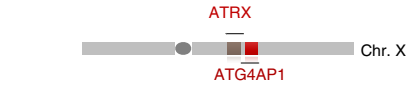
s927
 ATRX : exon 2
 PROC : intron 1
 Translocation



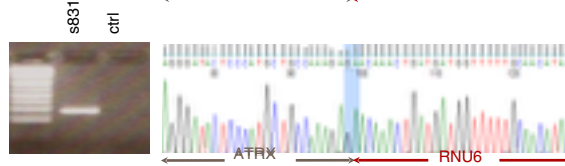
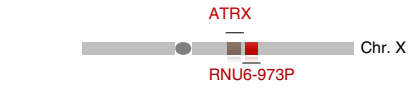
s823
 ATRX : exon 1
 DDX11L9 : exon 1
 Translocation



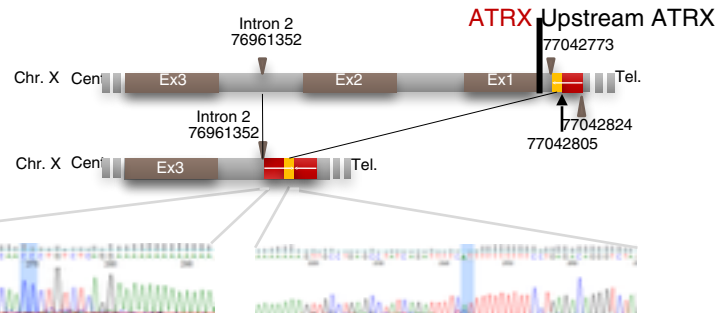
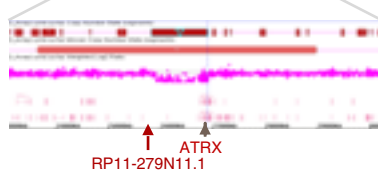
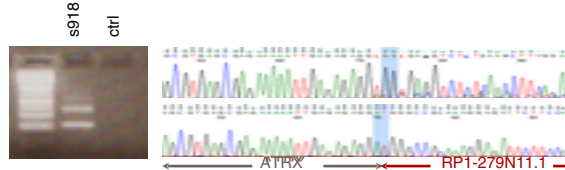
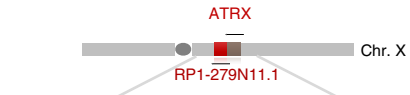
M990
 ATRX : exon 22
 ATG4AP1 : downstream
 Distance: 5 Mb
 Inversion



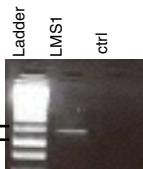
s831
 ATRX : exon 30
 RNU6-973P : downstream
 Distance: 4 Mb
 Eversion



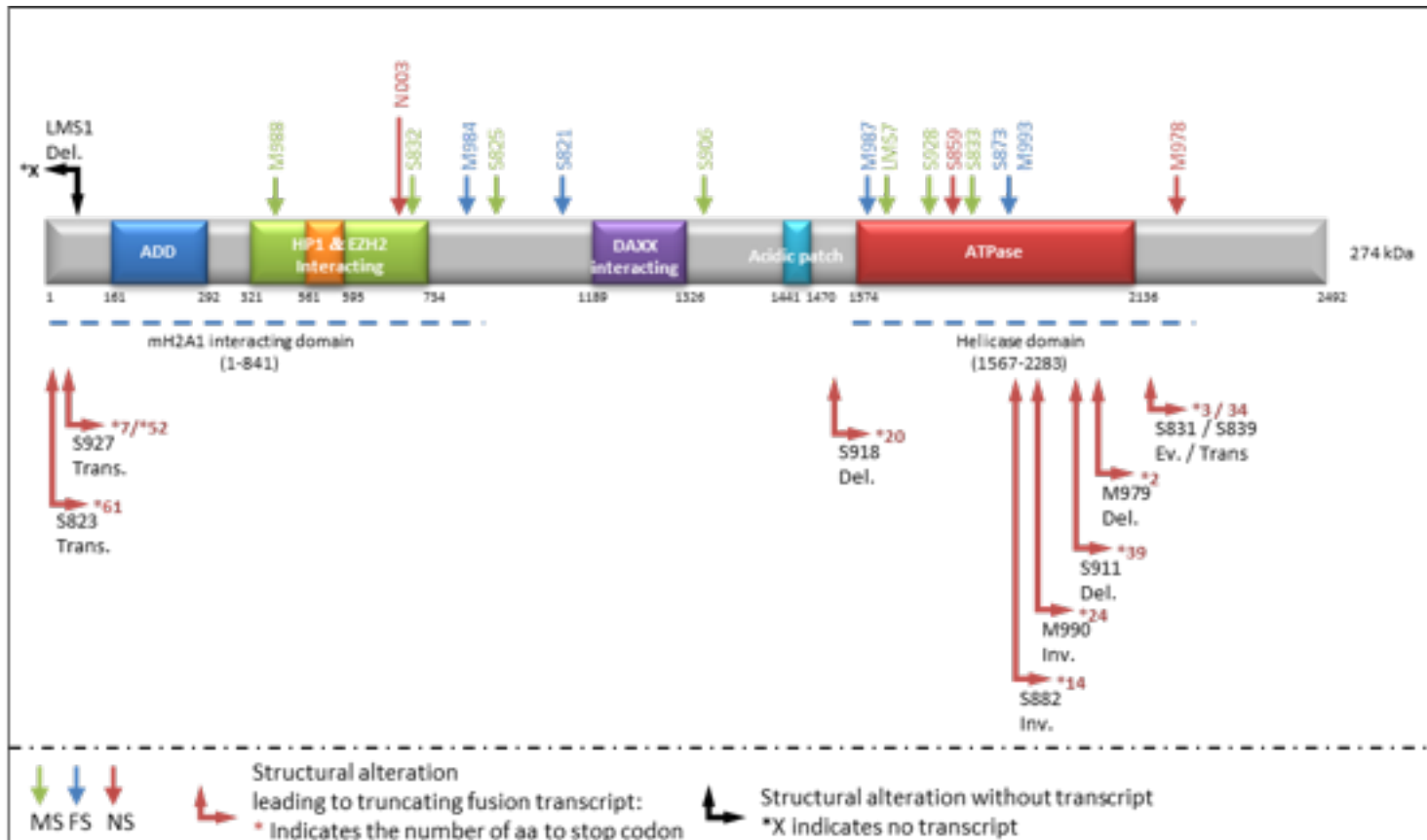
s918
 ATRX : exon 15
 RP1-279N11.1 : downstream
 Distance: 1 Mb
 Deletion



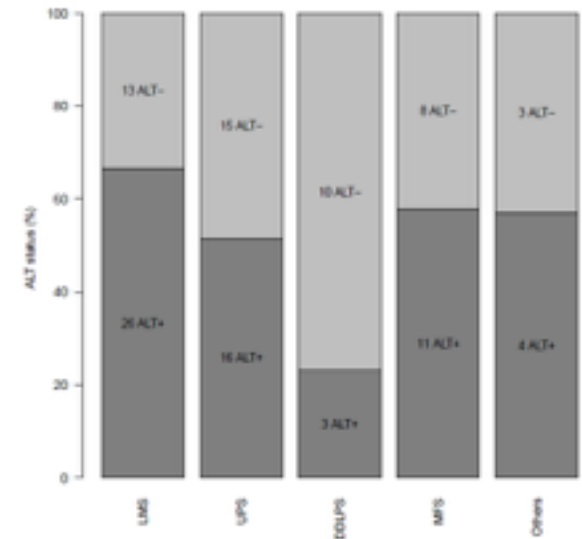
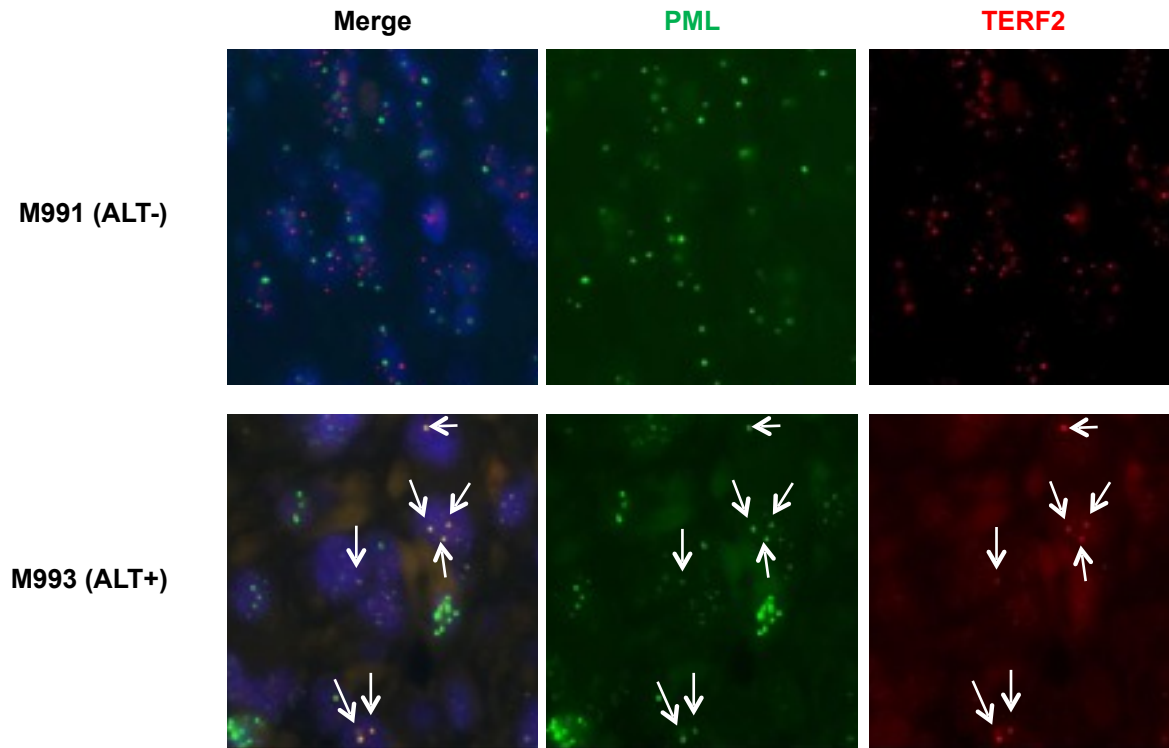
LMS1
 ATRX: intron 2 650 bp
 ATRX: upstream 500 bp
 Distance: 81.4 kb



ATRX mutations

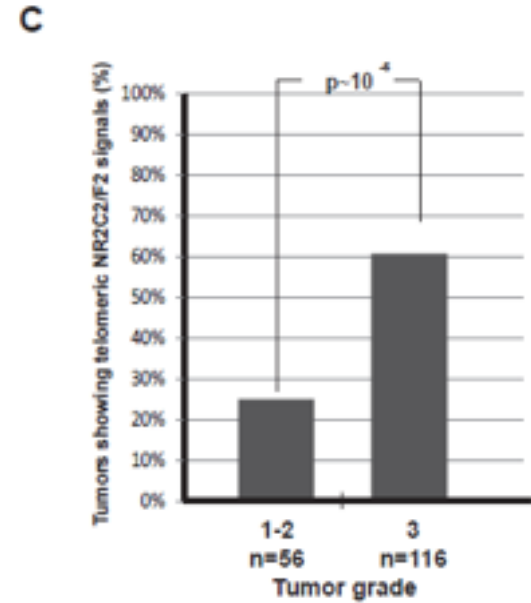
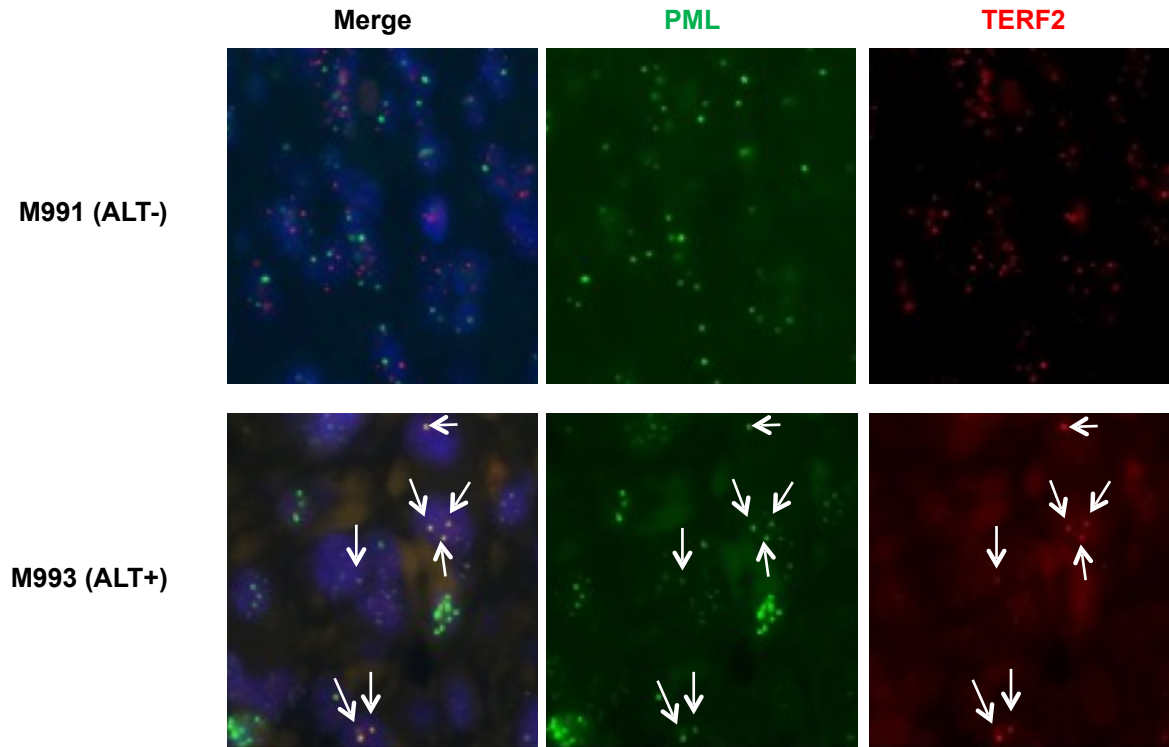


ATRX & ALT phenotype



ATRX mutated cases: 97% ALT+

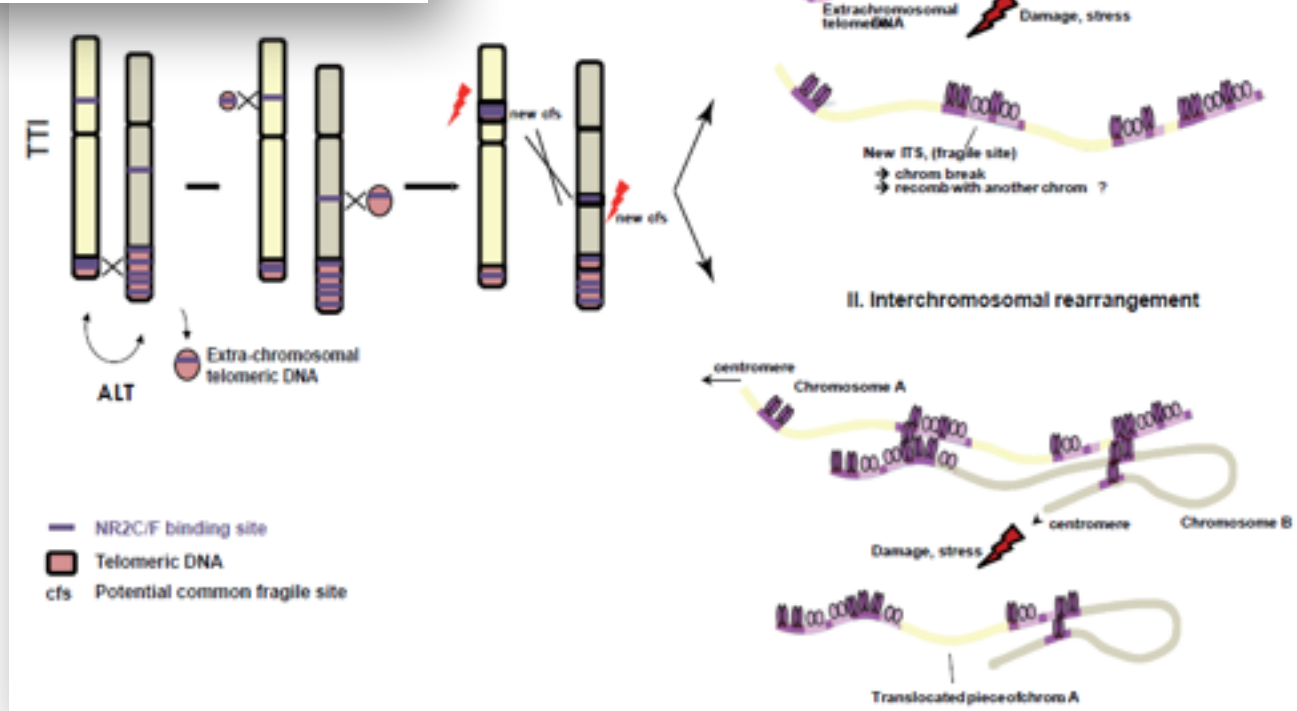
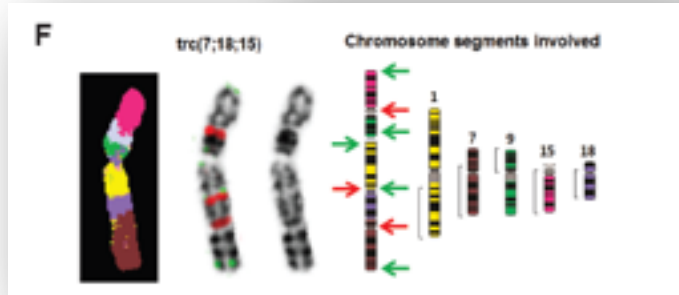
ATRX & ALT phenotype



Marzec et al; Cell 2015

ATRX mutated cases: 97% ALT+

ALT & Chromosomal instability Targeted Telomere Insertion



ATRAX, ALT & Therapies ?

REFERENCES AND NOTES

1. Li H, et al. *Nature* 435: 68-72 (2006).
2. Li H, et al. *Nature* 435: 68-72 (2006).
3. Li H, et al. *Nature* 435: 68-72 (2006).
4. Li H, et al. *Nature* 435: 68-72 (2006).
5. Li H, et al. *Nature* 435: 68-72 (2006).
6. Li H, et al. *Nature* 435: 68-72 (2006).
7. Li H, et al. *Nature* 435: 68-72 (2006).
8. Li H, et al. *Nature* 435: 68-72 (2006).
9. Li H, et al. *Nature* 435: 68-72 (2006).
10. Li H, et al. *Nature* 435: 68-72 (2006).
11. Li H, et al. *Nature* 435: 68-72 (2006).
12. Li H, et al. *Nature* 435: 68-72 (2006).
13. Li H, et al. *Nature* 435: 68-72 (2006).
14. Li H, et al. *Nature* 435: 68-72 (2006).
15. Li H, et al. *Nature* 435: 68-72 (2006).
16. Li H, et al. *Nature* 435: 68-72 (2006).
17. Li H, et al. *Nature* 435: 68-72 (2006).
18. Li H, et al. *Nature* 435: 68-72 (2006).
19. Li H, et al. *Nature* 435: 68-72 (2006).
20. Li H, et al. *Nature* 435: 68-72 (2006).
21. Li H, et al. *Nature* 435: 68-72 (2006).
22. Li H, et al. *Nature* 435: 68-72 (2006).
23. Li H, et al. *Nature* 435: 68-72 (2006).
24. Li H, et al. *Nature* 435: 68-72 (2006).
25. Li H, et al. *Nature* 435: 68-72 (2006).
26. Li H, et al. *Nature* 435: 68-72 (2006).
27. Li H, et al. *Nature* 435: 68-72 (2006).
28. Li H, et al. *Nature* 435: 68-72 (2006).
29. Li H, et al. *Nature* 435: 68-72 (2006).
30. Li H, et al. *Nature* 435: 68-72 (2006).
31. Li H, et al. *Nature* 435: 68-72 (2006).
32. Li H, et al. *Nature* 435: 68-72 (2006).
33. Li H, et al. *Nature* 435: 68-72 (2006).
34. Li H, et al. *Nature* 435: 68-72 (2006).
35. Li H, et al. *Nature* 435: 68-72 (2006).
36. Li H, et al. *Nature* 435: 68-72 (2006).
37. Li H, et al. *Nature* 435: 68-72 (2006).
38. Li H, et al. *Nature* 435: 68-72 (2006).
39. Li H, et al. *Nature* 435: 68-72 (2006).
40. Li H, et al. *Nature* 435: 68-72 (2006).
41. Li H, et al. *Nature* 435: 68-72 (2006).
42. Li H, et al. *Nature* 435: 68-72 (2006).
43. Li H, et al. *Nature* 435: 68-72 (2006).
44. Li H, et al. *Nature* 435: 68-72 (2006).
45. Li H, et al. *Nature* 435: 68-72 (2006).
46. Li H, et al. *Nature* 435: 68-72 (2006).
47. Li H, et al. *Nature* 435: 68-72 (2006).
48. Li H, et al. *Nature* 435: 68-72 (2006).
49. Li H, et al. *Nature* 435: 68-72 (2006).
50. Li H, et al. *Nature* 435: 68-72 (2006).
51. Li H, et al. *Nature* 435: 68-72 (2006).
52. Li H, et al. *Nature* 435: 68-72 (2006).
53. Li H, et al. *Nature* 435: 68-72 (2006).
54. Li H, et al. *Nature* 435: 68-72 (2006).
55. Li H, et al. *Nature* 435: 68-72 (2006).
56. Li H, et al. *Nature* 435: 68-72 (2006).
57. Li H, et al. *Nature* 435: 68-72 (2006).
58. Li H, et al. *Nature* 435: 68-72 (2006).
59. Li H, et al. *Nature* 435: 68-72 (2006).
60. Li H, et al. *Nature* 435: 68-72 (2006).
61. Li H, et al. *Nature* 435: 68-72 (2006).
62. Li H, et al. *Nature* 435: 68-72 (2006).
63. Li H, et al. *Nature* 435: 68-72 (2006).
64. Li H, et al. *Nature* 435: 68-72 (2006).
65. Li H, et al. *Nature* 435: 68-72 (2006).
66. Li H, et al. *Nature* 435: 68-72 (2006).
67. Li H, et al. *Nature* 435: 68-72 (2006).
68. Li H, et al. *Nature* 435: 68-72 (2006).
69. Li H, et al. *Nature* 435: 68-72 (2006).
70. Li H, et al. *Nature* 435: 68-72 (2006).
71. Li H, et al. *Nature* 435: 68-72 (2006).
72. Li H, et al. *Nature* 435: 68-72 (2006).
73. Li H, et al. *Nature* 435: 68-72 (2006).
74. Li H, et al. *Nature* 435: 68-72 (2006).
75. Li H, et al. *Nature* 435: 68-72 (2006).
76. Li H, et al. *Nature* 435: 68-72 (2006).
77. Li H, et al. *Nature* 435: 68-72 (2006).
78. Li H, et al. *Nature* 435: 68-72 (2006).
79. Li H, et al. *Nature* 435: 68-72 (2006).
80. Li H, et al. *Nature* 435: 68-72 (2006).
81. Li H, et al. *Nature* 435: 68-72 (2006).
82. Li H, et al. *Nature* 435: 68-72 (2006).
83. Li H, et al. *Nature* 435: 68-72 (2006).
84. Li H, et al. *Nature* 435: 68-72 (2006).
85. Li H, et al. *Nature* 435: 68-72 (2006).
86. Li H, et al. *Nature* 435: 68-72 (2006).
87. Li H, et al. *Nature* 435: 68-72 (2006).
88. Li H, et al. *Nature* 435: 68-72 (2006).
89. Li H, et al. *Nature* 435: 68-72 (2006).
90. Li H, et al. *Nature* 435: 68-72 (2006).
91. Li H, et al. *Nature* 435: 68-72 (2006).
92. Li H, et al. *Nature* 435: 68-72 (2006).
93. Li H, et al. *Nature* 435: 68-72 (2006).
94. Li H, et al. *Nature* 435: 68-72 (2006).
95. Li H, et al. *Nature* 435: 68-72 (2006).
96. Li H, et al. *Nature* 435: 68-72 (2006).
97. Li H, et al. *Nature* 435: 68-72 (2006).
98. Li H, et al. *Nature* 435: 68-72 (2006).
99. Li H, et al. *Nature* 435: 68-72 (2006).
100. Li H, et al. *Nature* 435: 68-72 (2006).

TELOMERE IN CANCER

Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors

Richard Wilson¹, Kelli A. Cox¹, Maya Fakhry¹, Shiroshi Wakimoto¹, Akhila B. Bhat¹, Paul S. Casam¹, Francesca Belmont¹, Paul M. Flanagin¹, Scott M. C. Lee¹, James M. Roberts¹, Francisco M. Martinez¹, Leo Liu¹

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

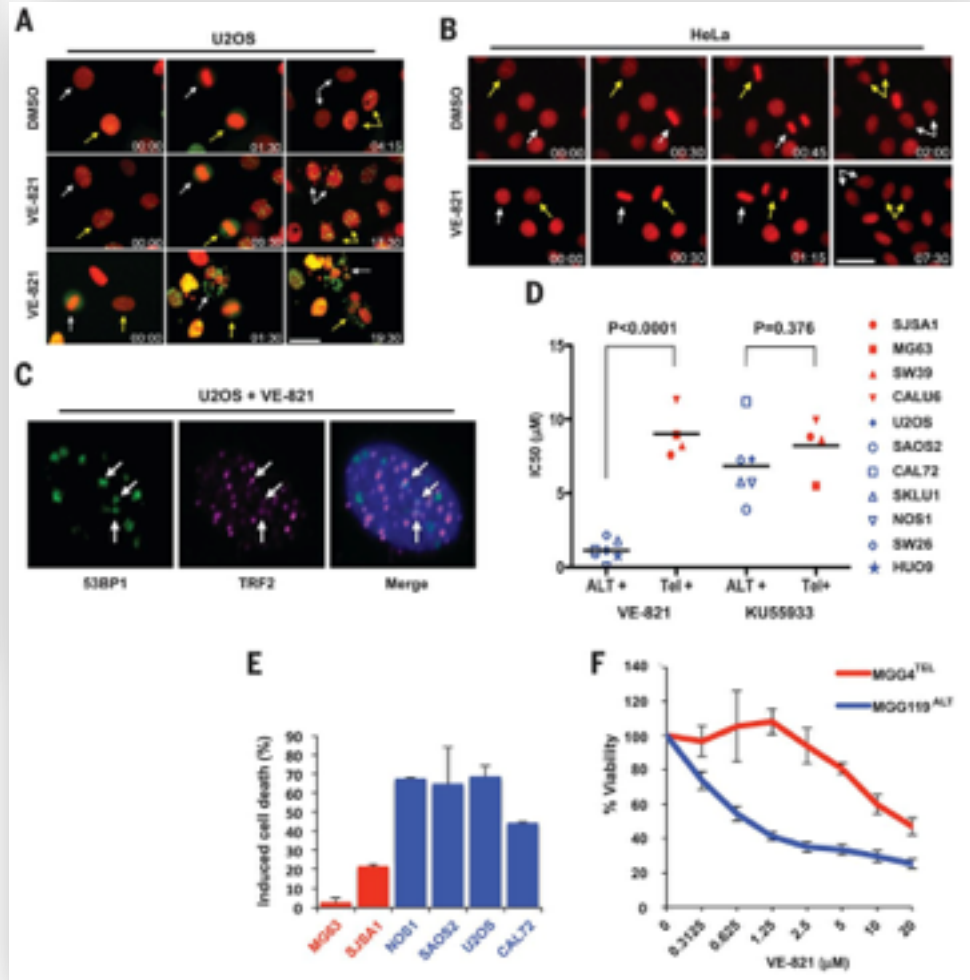
Cancer cells overcome replicative senescence by utilizing telomerase or the alternative lengthening of telomeres (ALT) pathway (1-5). ALT is used in cells of all human tissues and is present in specific cancer types, including sarcomas and glioblastomas (6). Currently, there are no therapies specifically targeting ALT. ALT relies on recombination to elongate telomeres (6), but how the recombination gene state of ALT telomeres is established remains unclear. To address this question, we reconstituted human telomerase (hTERT) and telomeric repeat amplification protocol (TRAP) telomerase (7, 8). ALT-positive cells are TRAP proficient (9). Thus, the reliance of ALT on recombination was the question as to whether recombination can be exploited in ALT-positive cancers as a route for targeted therapy.

Single-strand DNA (ssDNA) created by replication protein A (RPA) is a key intermediate in both DNA replication and HR (10). RPA transiently associates with telomeres during DNA replication, but is released from telomeres after S phase (11, 12). The release of RPA may be an important mechanism to suppress HR at telomeres. The association of RPA with telomeres is

regulated by telomerase (13), which is also present at telomeres during the prophase II (P-II) to I transition. To investigate how ALT is established, we determined whether the association of TERCRA with telomeres is altered in ALT cells. TERCRA colocalized with the telomeric-binding protein TRF2 in telomerase-positive HeLa control cells (Fig. 1A, B). However, in both HeLa and telomerase-positive U2OS telomerase cells (Fig. 1B), the number of TERCRA foci declined from S phase to G2 (Fig. 1A and B, and Fig. S1, S2, S3). Although in ALT-positive U2OS telomerase cells TERCRA also colocalized with the telomeric marker TRF2 (Fig. 1A, A and B), neither the levels of TERCRA, nor the colocalization of TERCRA and TRF2, declined from S to G2 (Fig. 1A and B, and Fig. S1, S2, S3). Thus, in contrast to telomerase-positive cells, ALT cells are deficient in the cell-cycle regulation of TERCRA.

We next explored why TERCRA persistently associates with telomeres in ALT cells. Recent studies have revealed a contribution of ATR to recombination in the ATRX gene and loss of the chromosome-repairing protein ATRX is cancer (14, 15). ATRX was detected in HeLa but not U2OS cells (Fig. 1B) and TRF2 (16), prompting us to investigate whether the dysregulation of TERCRA in ALT cells is a result of ATRX loss. Indeed, knockdown of ATRX in HeLa cells resulted in persistent TERCRA foci and elevated TERCRA levels (Fig. 1B, Fig. S1, S2, S3, and Fig. S4 and S5). Furthermore, the levels of TERCRA declined from S phase to G2 in cells in which HeLa

Downloaded from www.sciencemag.org on June 16, 2015



Acknowledgments

