# TAGC — Implementation of whole genome longreads sequencing to decode somatic genotypes in T-ALL

07 November 2024

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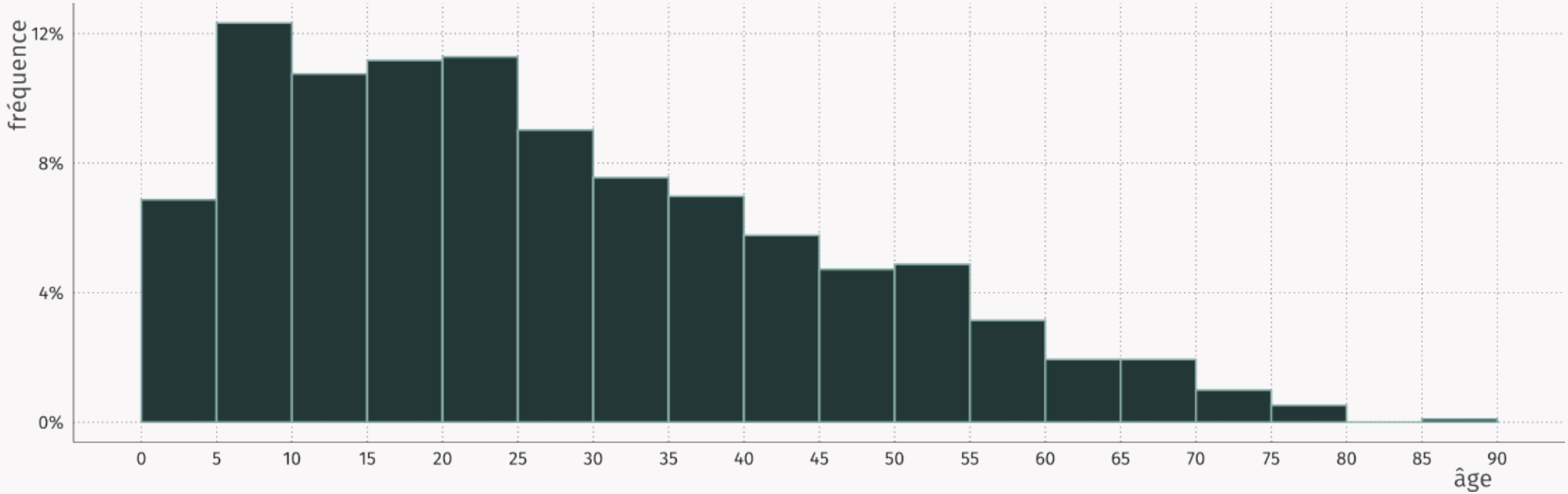
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

# ❓ Main Hypothesis

**Tumor proliferations harbor within their genome intergenic somatic mutations that disrupts the expression of oncogenes.**

# 👨 Model — T-ALL

Acute lymphoblastic leukemia is a rare disease (≃ 120/yr in Fr) resulting from a tumoral process driven by the clonal **proliferation of immature T lymphocytes**.




Internal unpublished data

# 👨‍⚕️ Model — T-ALL

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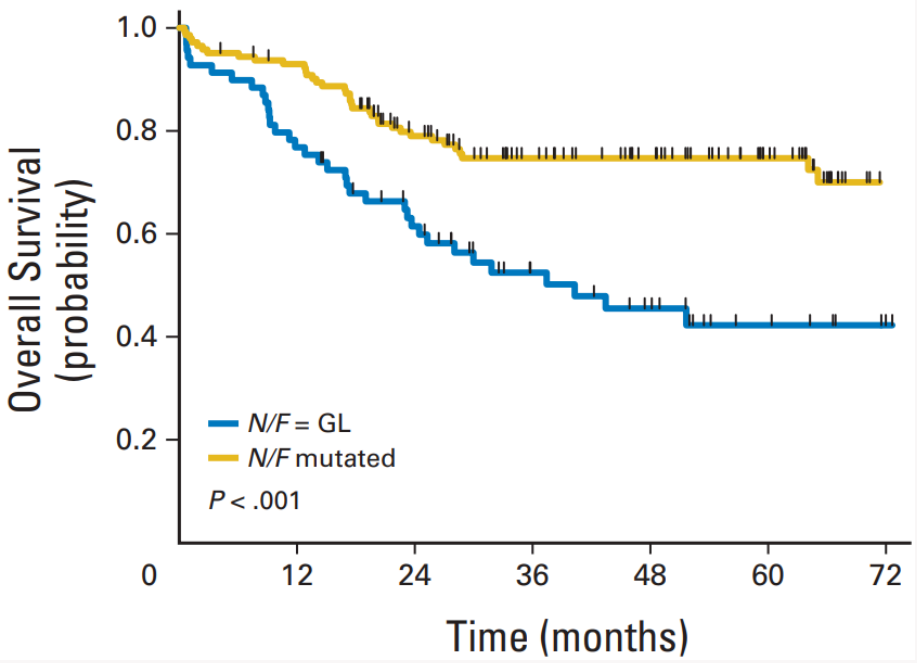
- Adults OS 3 years: 67% (GRAALL-2005)




Trinquand A et al. J Clin Oncol. 2013;31(34):4333-4342

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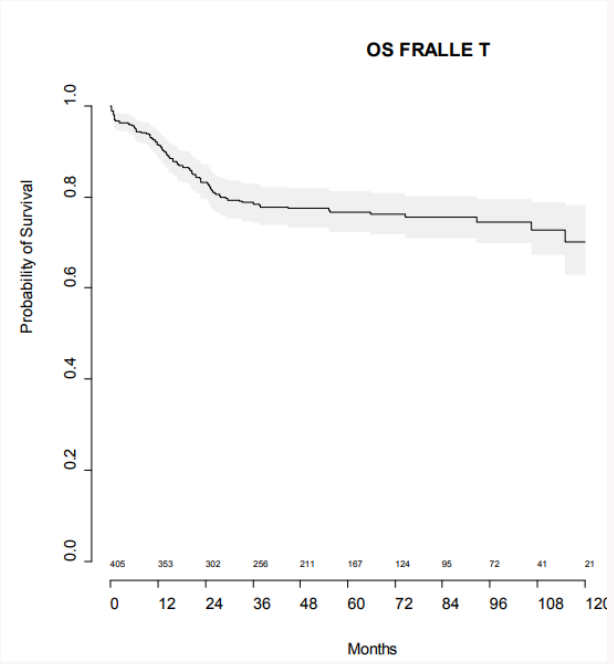
- Youths OS 5 years: 77% (FRALLE)




Petit A et al. Blood. 2018;131(3):289-300.

# 👨‍⚕️ Model — T-ALL

**Refractory** cases to standard chemotherapy as well as **relapses** (UKALL12: adults at 5 years 42%) have a poor prognosis.

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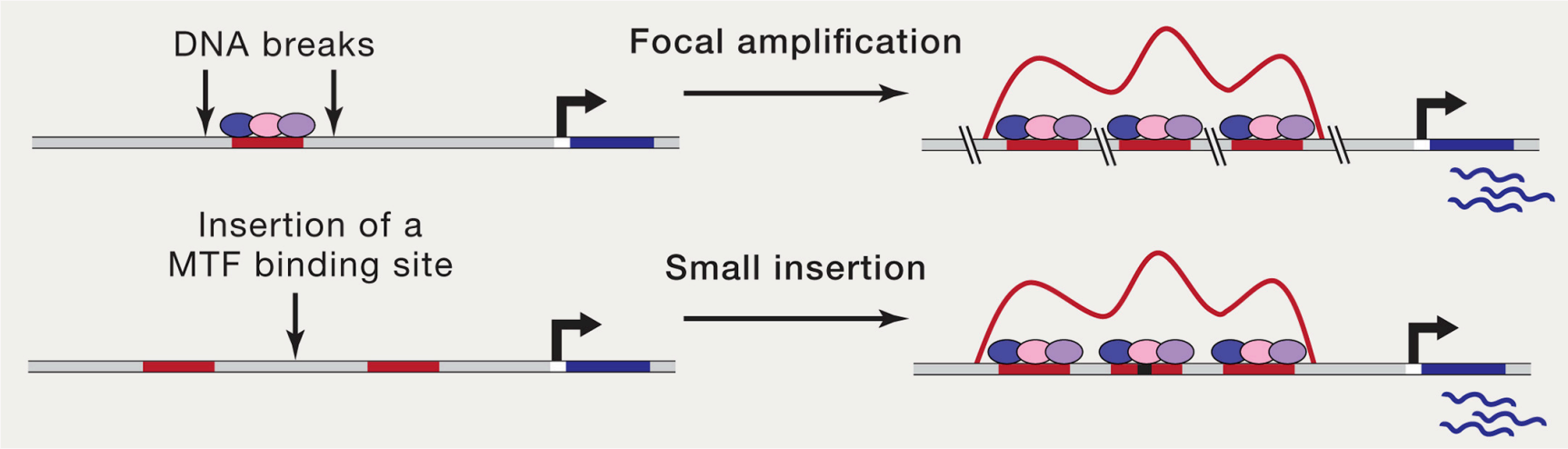
**We need new treatments to address these cases !**

The tumor phenotype emerges from alterations in their genotype.

⇨ A better description of the genotype will lead to a better understanding of oncogenic mechanisms and to the discovery of more **effective therapies tailored to specific alterations**.

# Intergenic alterations

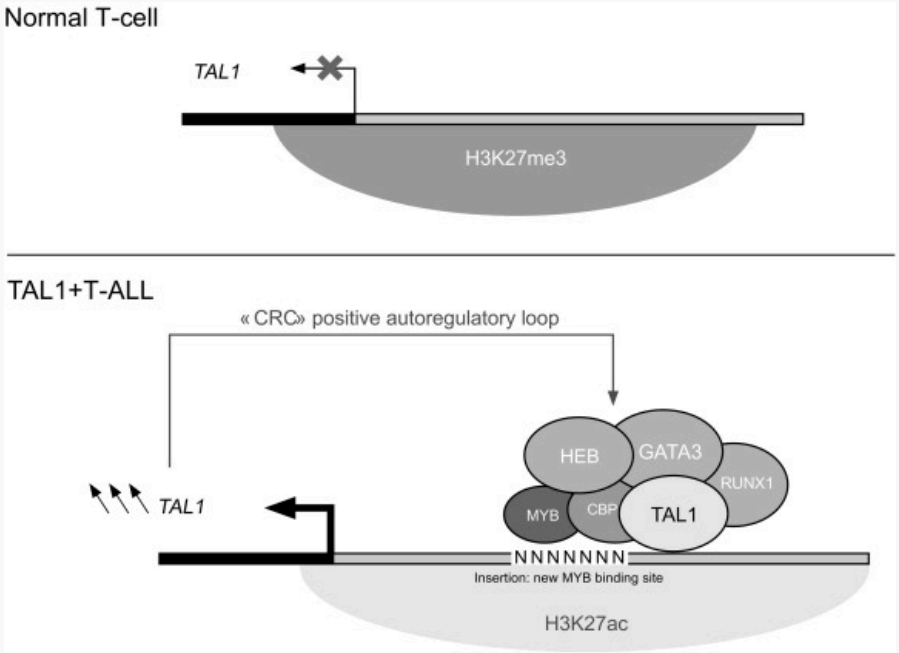
**Somatic intergenic alterations are responsible of the deregulation of oncogenes.**




Bradner JE, et al. Cancer. Cell. 2017 Feb9 ;168(4):629-643

# Preliminary results

- Our laboratory has shown that a somatic insertion upstream of *TAL1* leads to the formation of a **neo-enhancer** and thus leads to the overexpression of *TAL1*.




Smith, C et al. "TAL1 activation in T-cell acute lymphoblastic leukemia: a novel oncogenic 3′ neo-enhancer." Haematologica vol. 108,5 1259-1271. 1 May. 2023

# 🎯 Goals

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⇨ Implementation of the **Oxford Nanopore sequencing method** and integrate multi-omics data.

# Infrastucture

**Computer A** for processing and analyzing the signal generated by the sequencer (MAD).

- 2x Intel Xeon Platinum 8380 CPU 2.30GHz
- 160 cores
- 503 GB RAM
- 4 nVidia A100 GPU

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**Computer B** for data archiving and sharing.

- 52 To fo HD in raidz1 mode (redundancy)
- LTO: magnetic tape drive (raw data archiving)

# 🧪 Wet lab

- Intermediate difficulty level.
- 3 half-days of work.

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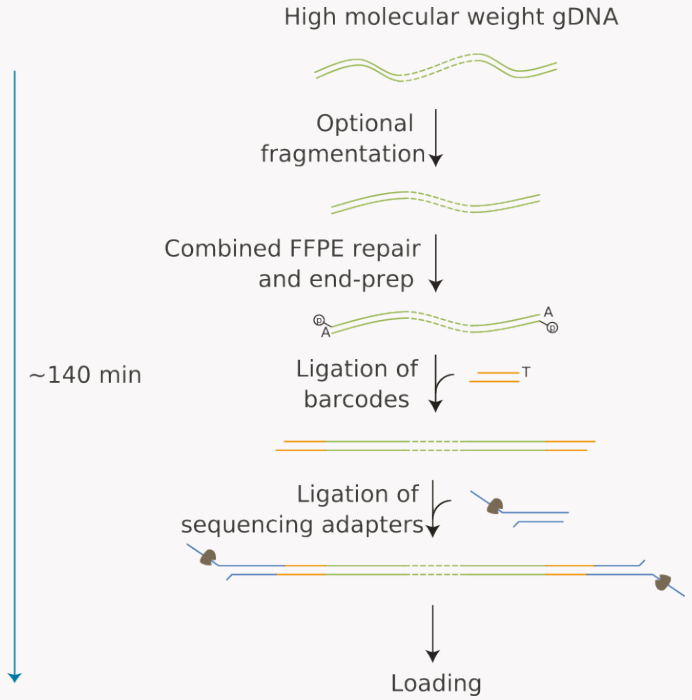
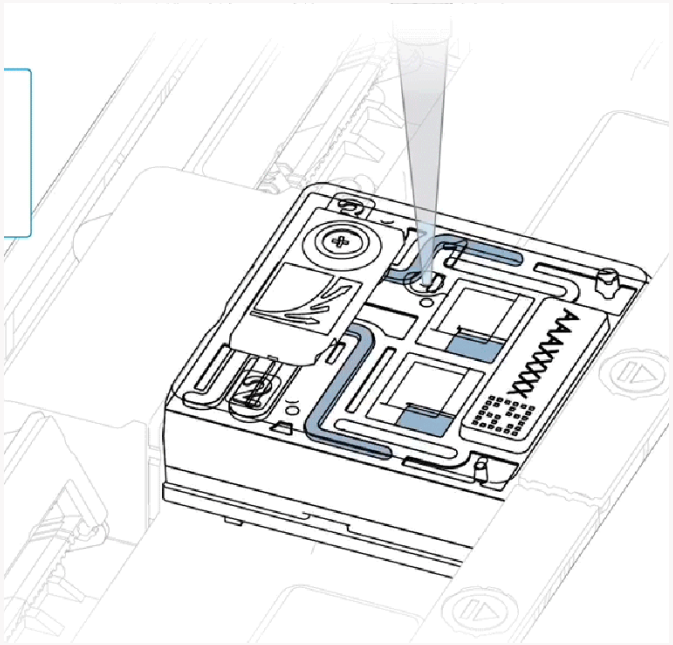
1. Genomic DNA **shearing** Covaris g-TUBE (3µg, 8,000rpm, 1min)

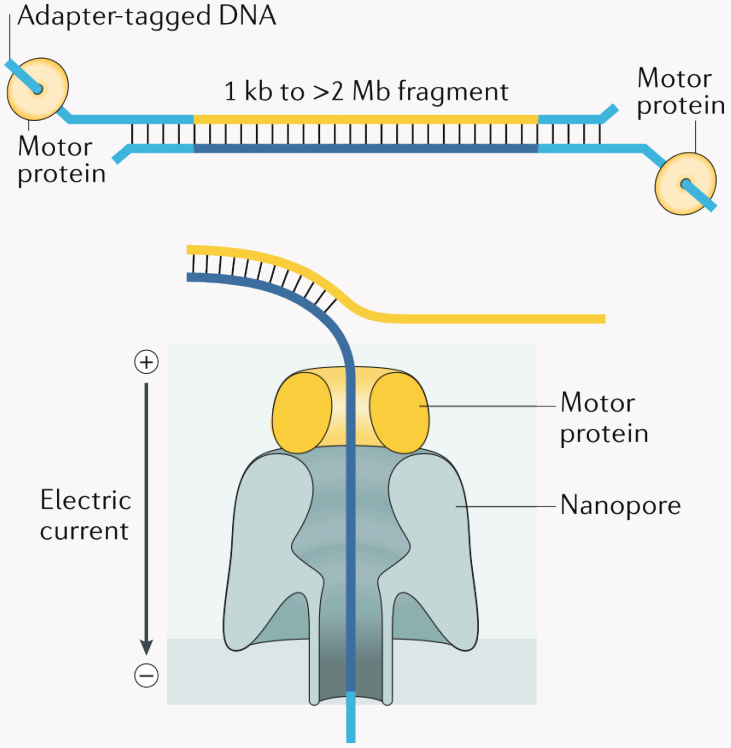
## 2. Preparation of the sequence library

DNA **repair** and end-prep ⇨ **barcode** ligation ⇨ **adapter** ligation



## 3. Flowcells **loading** and **sequencing run**

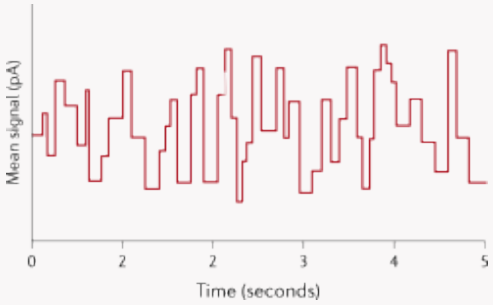




# Bioinformatic Pipeline

1. **Base calling** and **Alignement** on hs1 (T2T).

Latest version of Dorado 0.8.1 with latest AI model of 5mC 5hmC modified basecalling (v5.0).




→

```
059b2db8-416c-4fdf-b7eb-3cdd3390a3bd 0 chr1 2 1
1075S5M1D8M1D36M1D4M1D116M1D111M1I485M3I595M...69M1I12M1I16M1D48M1641S * 0 0
AAGGTTAAAACCAAGACTCGCTGTGC 8;88;;<<<:::98...87877676645654456798997 qs:i:22 du:f:14.
              137 ns:i:70685 ts:i:904 mx:i:2 ch:i:1307 st:Z:2023-12-14T18:30:59.187+00:00
rn:i:36917 fn:Z:PAS34492_pass_barcode
              09_2e65ae3a_a7dfa7d7_4228.pod5 sm:f:-764.172 sd:f:0.00798761 sv:Z:pa dx:i:0
RG:Z:a7dfa7d727c0e04ecb6e9c5c3dac8080fe7cffaa_dna_r10.4.1_e8.2_400bps_sup@v4.3.0 MN:i:5392
MM:Z:C+h.,1,0,1,0,0,4,5,117,36,0,93,99,56,0,65...; ML:B:C,152,3,5,3,3,1,...; NM:i:1
              71 ms:i:4485 AS:i:4438 nn:i:0 de:f:0.0502045 tp:A:P cm:i:11 s1:i:82 s2:i:148
MD:Z:5^A8^C36^C4^A116^C...; rl:i
              :3576 SA:Z:chr8,146252402,-,1604M1D3788S,36,10;
```

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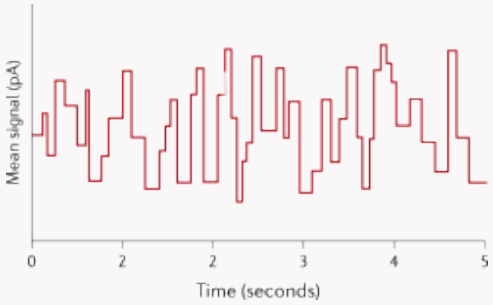


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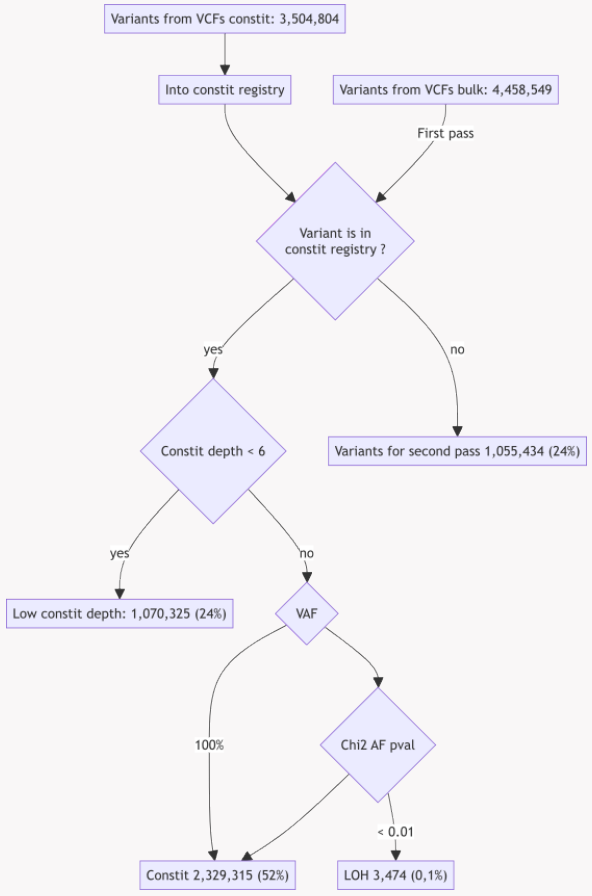
059b2db8-416c-4fdf-b7eb-3cdd3390a3bd 0 chr1 2 1
1075S5M1D8M1D36M1D4M1D116M1D111M1I485M3I595M...69M1I12M1I16M1D48M1641S * 0 0
AAGGTTAAAACCAAGACTCGCTGTGC 8;88;;<<<:::98...87877676645654456798997 qs:i:22 du:f:14.
                137 ns:i:70685 ts:i:904 mx:i:2 ch:i:1307 st:Z:2023-12-14T18:30:59.187+00:00
rn:i:36917 fn:Z:PAS34492_pass_barcode
                09_2e65ae3a_a7dfa7d7_4228.pod5 sm:f:-764.172 sd:f:0.00798761 sv:Z:pa dx:i:0
RG:Z:a7dfa7d727c0e04ecb6e9c5c3dac8080fe7cffaa_dna_r10.4.1_e8.2_400bps_sup@v4.3.0 MN:i:5392
MM:Z:C+h.,1,0,1,0,0,4,5,117,36,0,93,99,56,0,65...; ML:B:C,152,3,5,3,3,1,...; NM:i:1
                71 ms:i:4485 AS:i:4438 nn:i:0 de:f:0.0502045 tp:A:P cm:i:11 s1:i:82 s2:i:148
MD:Z:5^A8^C36^C4^A116^C...; rl:i
                :3576 SA:Z:chr8,146252402,-,1604M1D3788S,36,10;
```

2. **Variant calling**
   - DeepVariant (Google v1.6.1) on constit and tumoral BAMs.
   - ClairS (HK-UBAL v0.1.7) takes both constit and tumoral BAMs.
   - ~~Sniffles (Fritz Sedlazeck v2.2) on constit and tumoral BAMs.~~
   - NanoMonSV (Yuichi Shiraishi v0.7.2) takes both constit and tumoral BAMs.
   - Exogene (Z. Stephens v15) viral integration.

# Bioinformatic Pipeline — Aggregation

## 1. VCF filter



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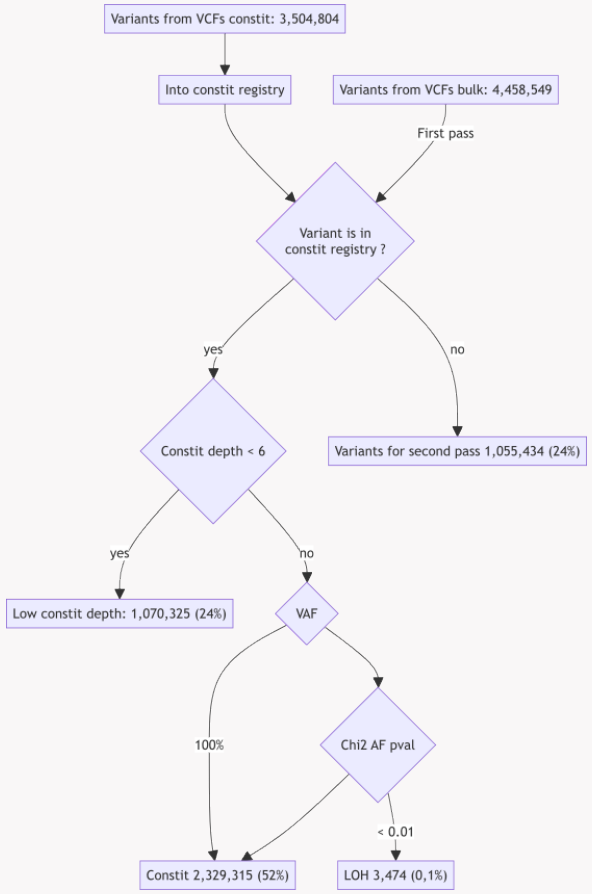
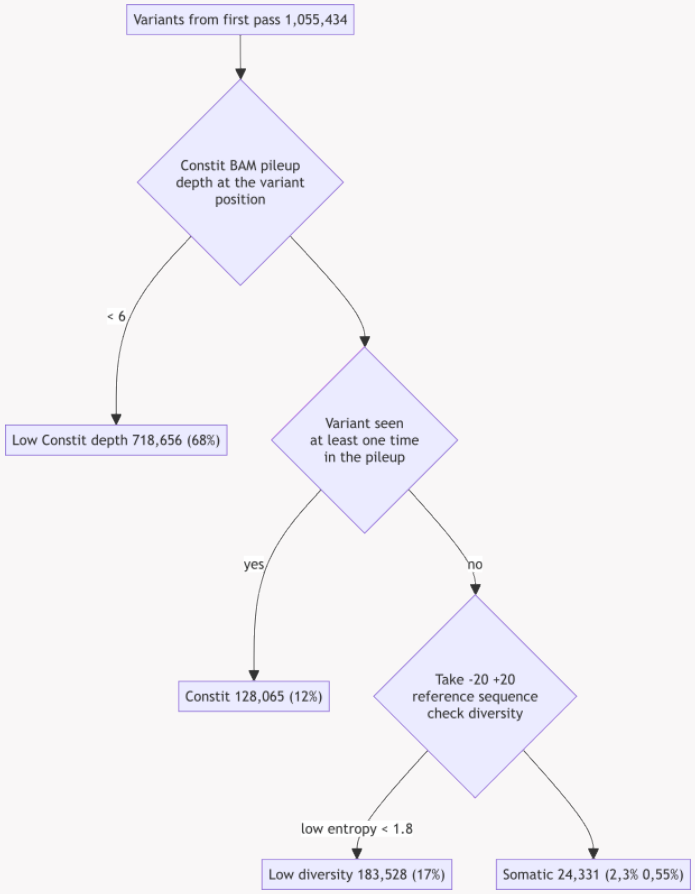
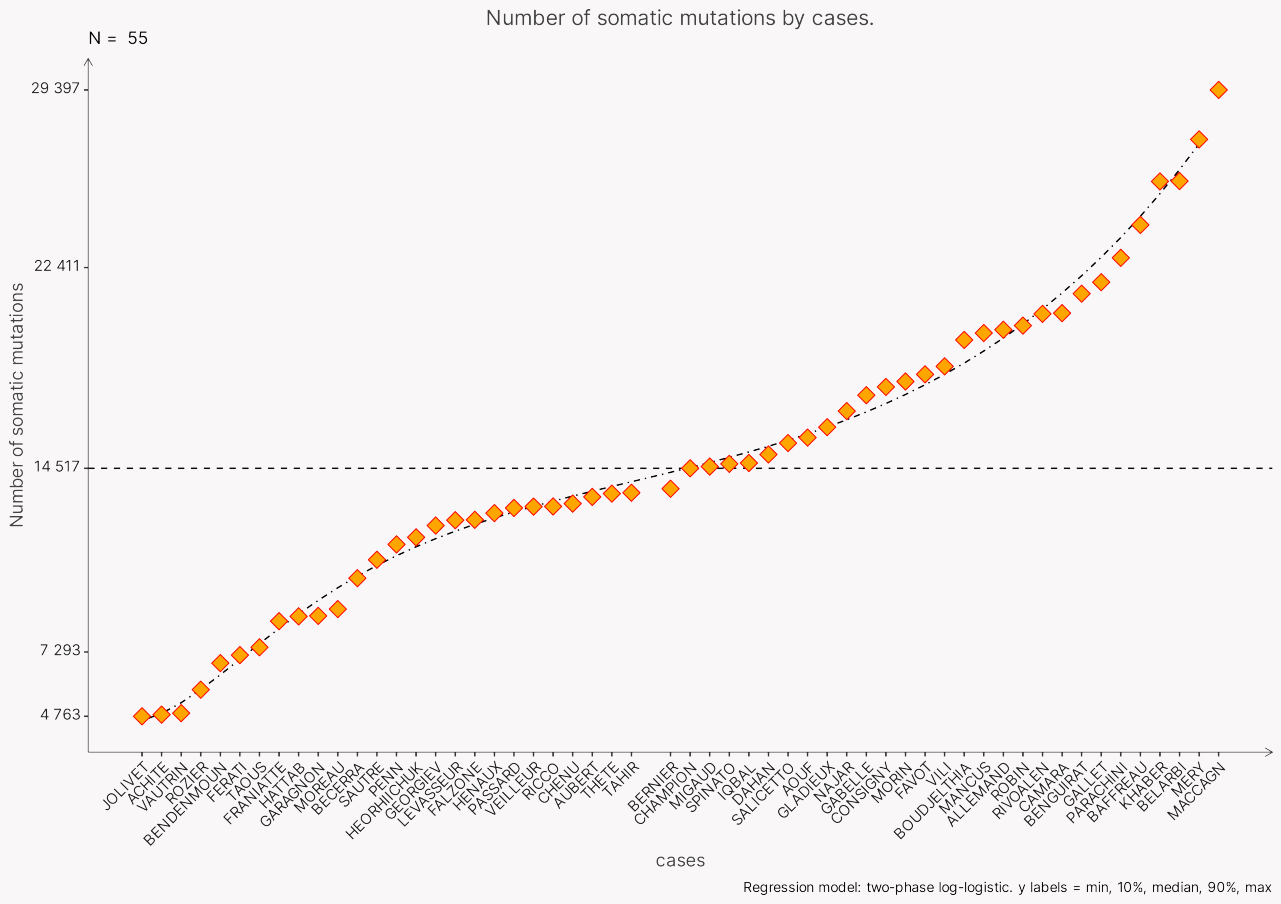



## 2. BAM filter



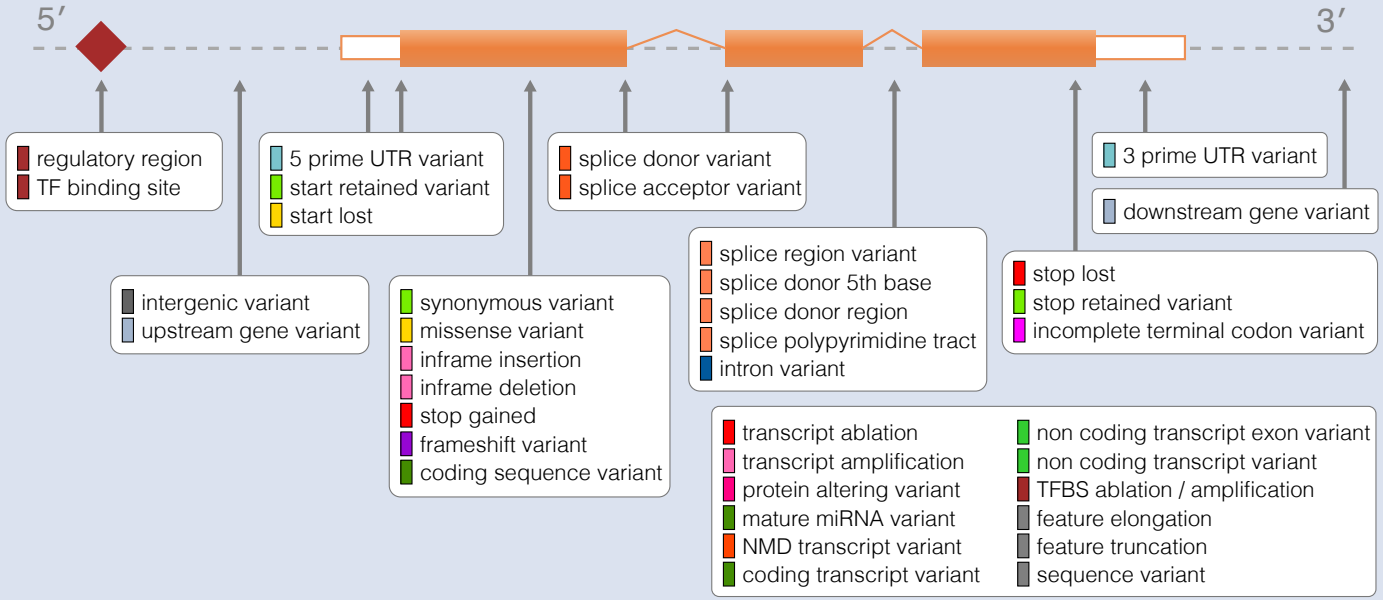
# Results — Number of somatic alterations



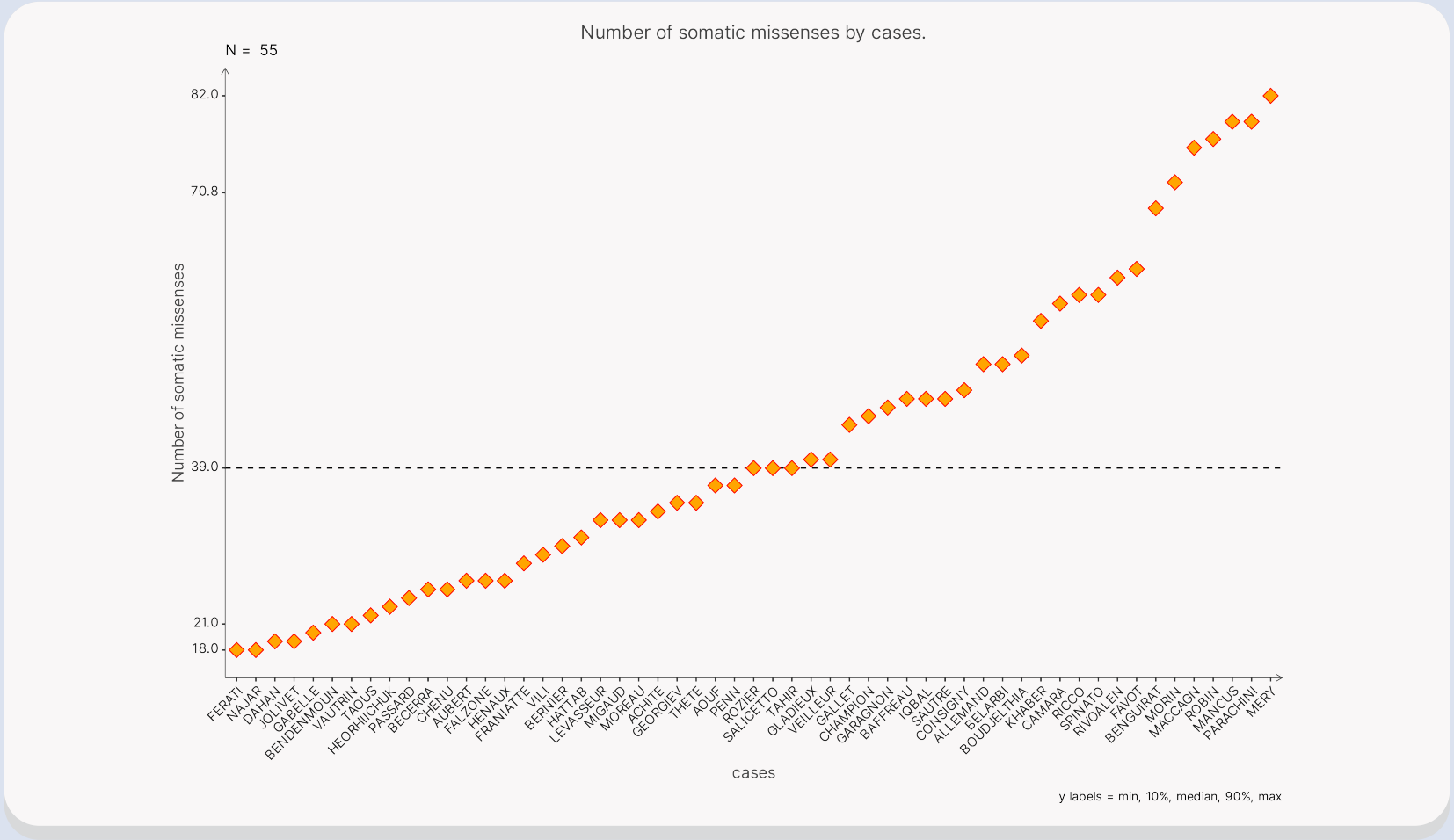
# Bioinformatic Pipeline — Annotation

## 3. Annotations

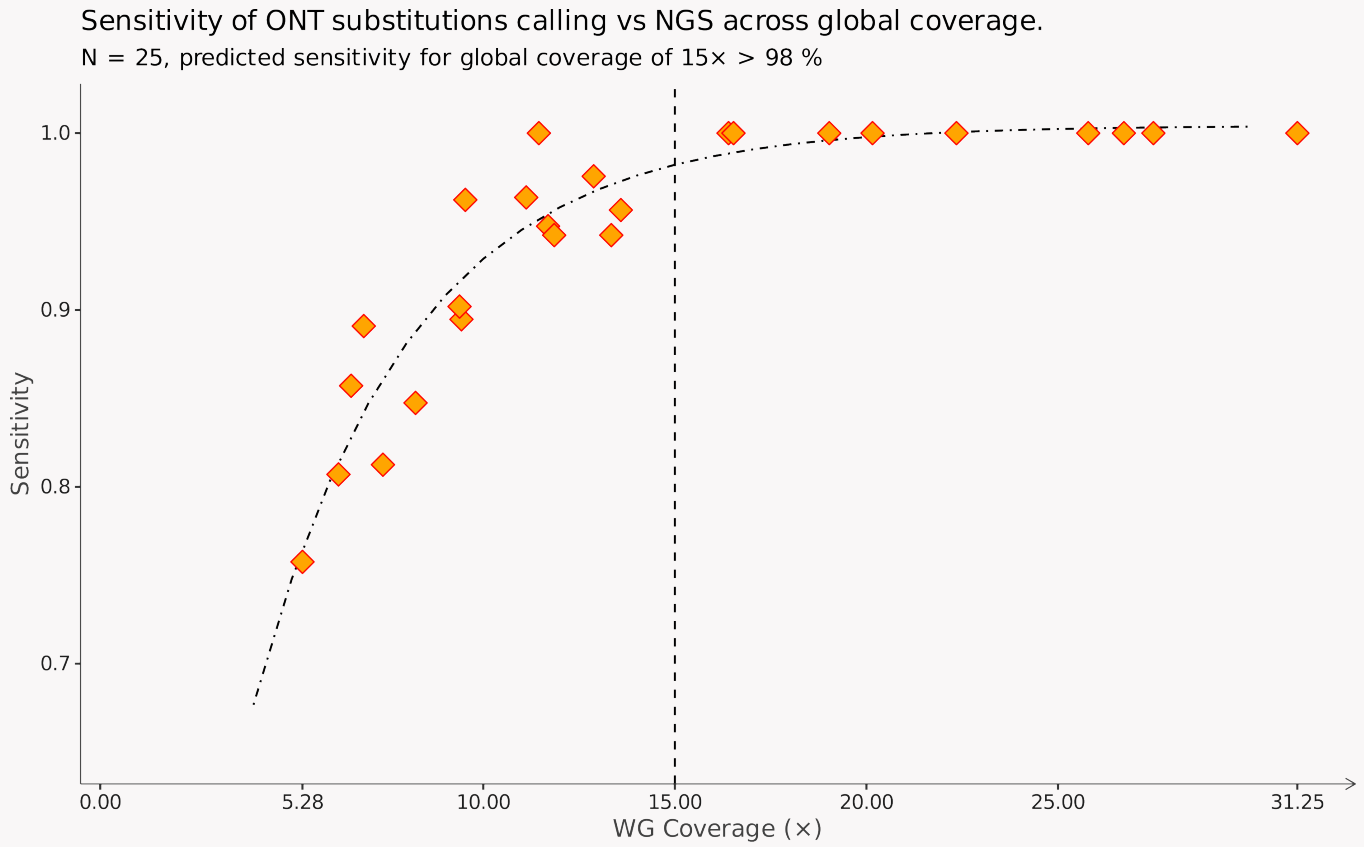
- VEP for variant consequence prediction (ensembl v112)
- Cosmic DB (latest)
- dbSNP
- NCBI genomic regions (latest)



# Results — Number of somatic missense alterations



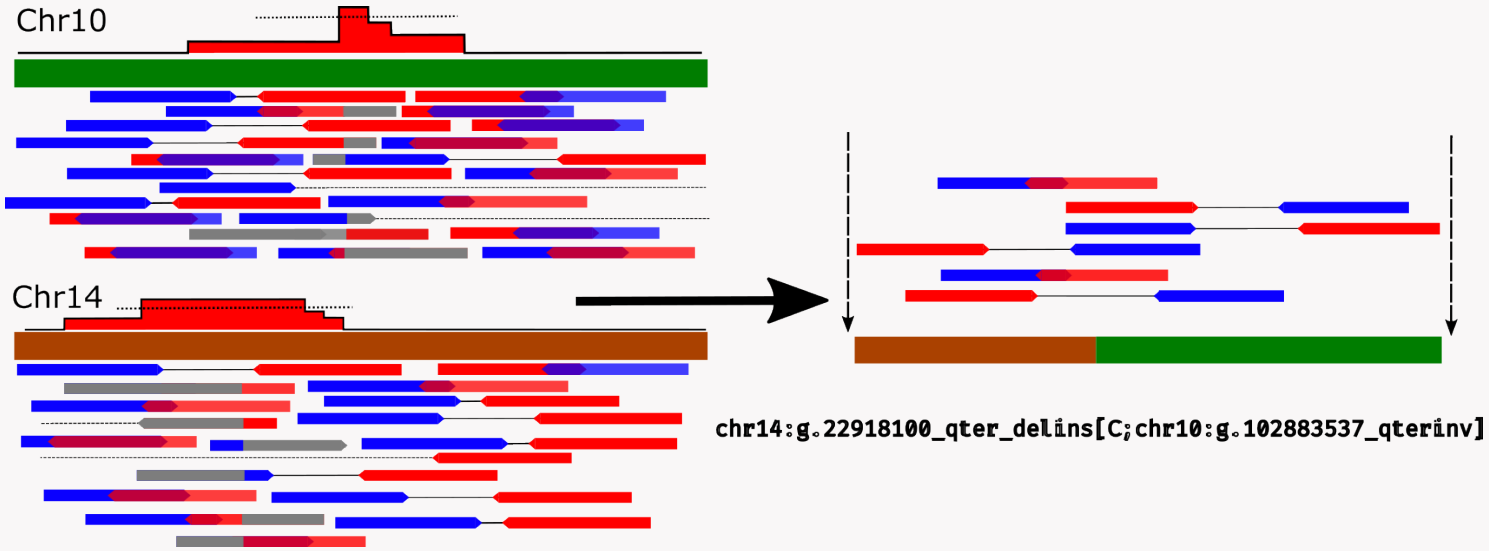
# Performances — Substitution calling



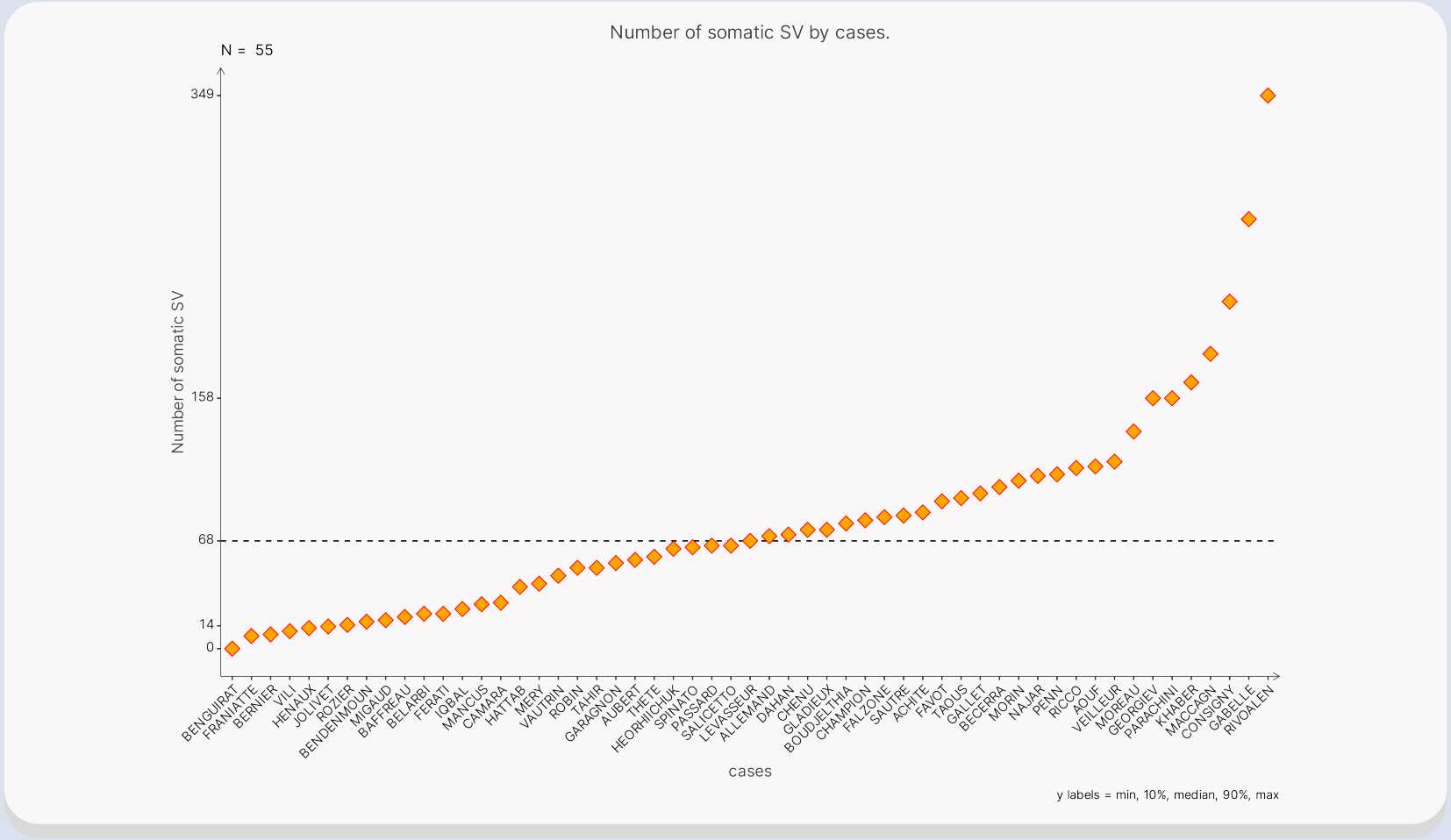
# Bioinformatic Pipeline — *de novo*

Implementation of *de novo assemblage* for LRS:
Inspired by **SV-finder** (local de novo assembly)

- **Scan** alignements and select locally misaligned reads (outliers detection).
- **Assemble** them together (wtdbg2 v2.5 and spades v4.0).
- **Describe** the resulting consensus sequence (Blast, minimap2).



# Results — Number of somatic SV



# 🔬 Visualization and interpretation of results

Development of a web service (HTMX + Bun) for **sharing, visualization, and interpretation of the results**.

**DEMO** (C.... ex.: *PHF6* et *AEBP2*)

# Reporting

After the manual interpretation of the results and the redaction of a conclusion.

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Example...

# 👥 Cohorts

Initially, to investigate our hypothesis, we decided to sequence T-ALLs harboring deregulation of the expression of known frequent oncogenes in T-ALL:

- ***TAL1*** (cis-deregulated),
- ***HOXA9*** (RT-MLPA neg),
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We also decided to sequence a cohort of pediatric T-ALLs and an adult one with deregulation of *TLX3* (Pediac/Manon project). As well as a cohort of T-ALLs **< 3 years**.

# 👥 Bilan des flowcells

## Bilan WGS Nanopore par projet

| | Fait WGS Nanopore | |
|---|---|---|
| projet | patients | echantillions |
| <3ans | 20 | 40 |
| | 20 D+M | |
| TLX3 ped | 13 | 24 |
| | 11 D+M; 2 D | |
| TLX3 ad | 13 | 25 |
| | 12 D+M; 1 D | |
| TAL1 | 14 | 20 |
| | 6 D+M; 8 D | |
| TLX1 | 17 | 28 |
| | 11 D+M; 6 D | |
| HOXA | 17 | 34 |
| | 17 D+M | |
| LBL-T | 9 | 16 |
| | 7 D+M; 2 D | |
| ZFP36L2 | 1 | 2 |
| | 1 D+M | |
| TOTAL | 104 | 189 |
| TOTAL* | 98 | 179 |
| | 81 D+M; 17 D | |

* 6 patients comuns entre les projets

| | Reste a faire | |
|---|---|---|
| projet | patients | echantillions |
| <3ans | 0 | 0 |
| TLX3 ped | 1 | 2 |
| | 1 D+M | |
| TLX3 ad | 0 | 0 |
| TAL1 | 3 | 5 |
| | 2 D+M ; 1 D | |
| TLX1 | 0 | 0 |
| HOXA | 8 | 16 |
| | 8 D+M | |
| LBL-T | 8 | 16 |
| | 8 D+M | |
| ZFP36L2 | 1 | 2 |
| | 1 D+M | |
| TOTAL | 21 | 41 |

commandes FC WGS :

1- Commande en Mai de 40 pack de FC (soit 160 FC)

a. Réception de 5 pack (20 FC) en Juin – reste 0

b. Réception de 4 pack (16 FC) en septembre - reste 16

2- Réception prévu en décembre : 13 pack (52 FC) – reste 52

3- Réception prévu en Mars 2025 : 17 pack ( 68 FC) – reste 68

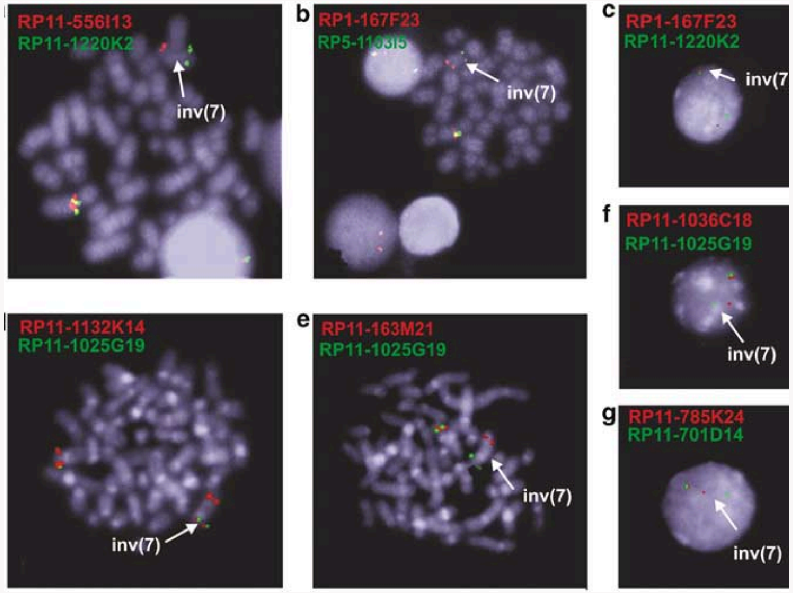
# 👥 Cohort *HOXA* — n = 14

| id | X | mécanisme | interpretation | info |
|---|---|---|---|---|
| ALLEMAND | 11 | Multiples mutations de *MLLT3* + hotspot *CSF3R* | ❓ | |
| BELARBI | 13 | Altération de *GATA3* + *KMT2C* | ❓ | |
| BERNIER | 15 | DPP10::SET | ✅ | |
| BOUDJELTHIA | 13 | *KMT2E* ter | ❓ | JAK/STAT |
| GARAGNON | 13 | TRB::HOXA **inv7** | ✅ | JAK/STAT |
| HATTAB | 11 | AFDN::KMT2A **t(6;11)** | ✅ | |
| KENNOUCHE | *en cours* | | | |
| LAVIDALE | *en cours* | | | |
| MANCUSO | 10 | TNRC18::KMT2A | ✅ | |
| MERY | 16 | TRB::HOXA **inv7** | ✅ | JAK/STAT |
| MICHELAS | *en cours* | | | |
| MIGAUD | 26 | mutation *EN1* (homeobox) | ❓ | |
| MORIN | 22 | TRB::HOXA **inv7** | ✅ | JAK/STAT |
| SAUTRE | 28 | AFDN::KMT2A **t(6;11)** | ✅ | |

# Interesting results — *HOXA9*

**ME**

- **Inv(7)(p15q34)** TRB/HOXA10
- `chr7:27,287,813_delins[ATGGGGGGGG_chr7:144,128,326inv]`
- `chr7:27,313,165_delins[GATGG_chr7:144,161,537inv]`



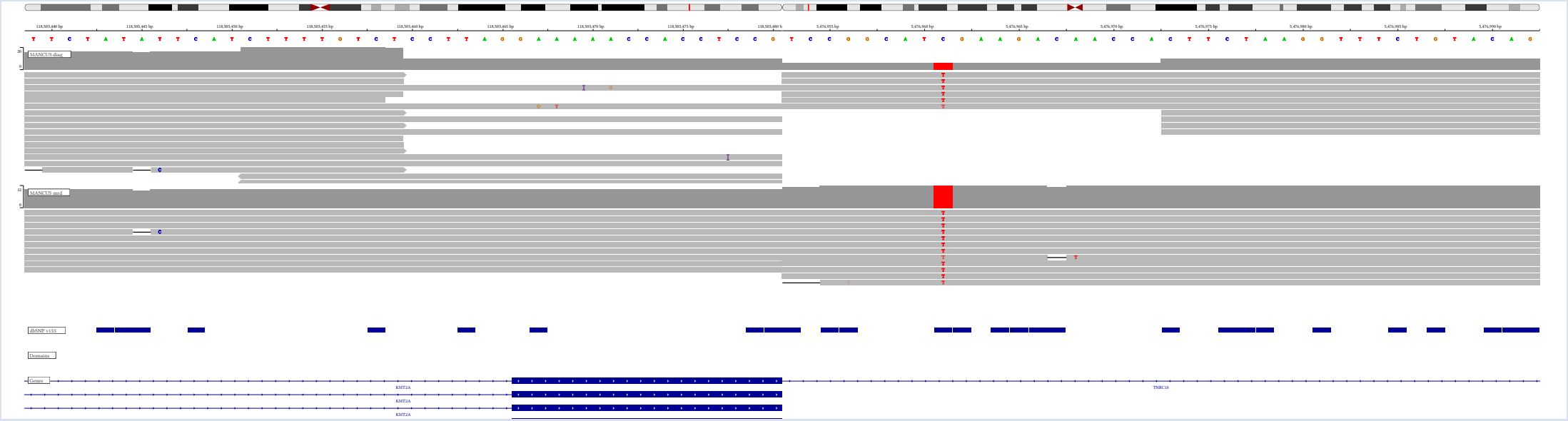

Résultats FISH break apart.

Speleman, F et al. "A new recurrent inversion, inv(7)(p15q34), leads to transcriptional activation of HOXA10 and HOXA11 in a subset of T-cell acute lymphoblastic leukemias." Leukemia vol. 19,3 (2005): 358-66.

# Interesting results — *HOXA9*

## MA

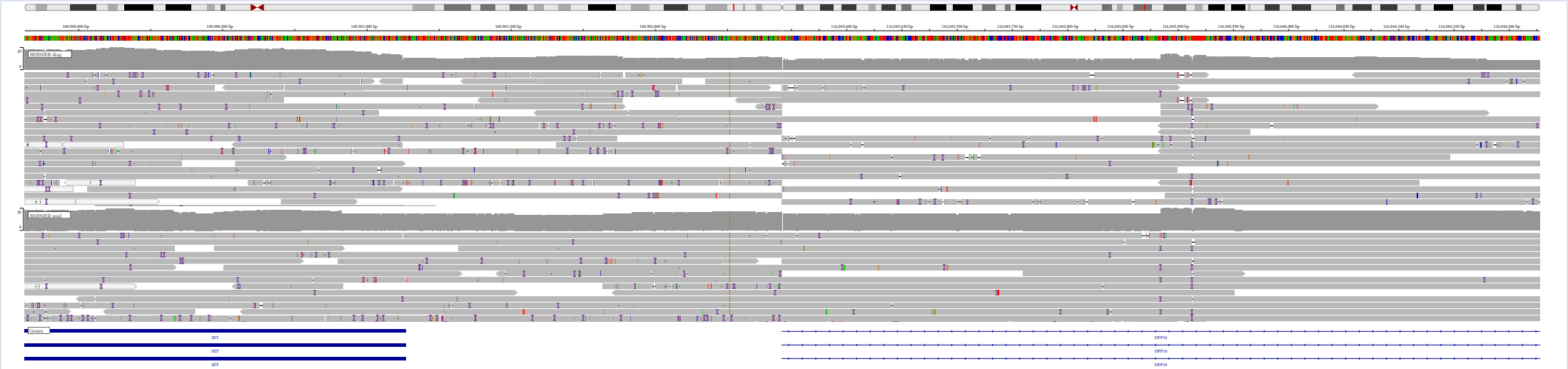
- t(7;11)(p22;q23) likely ***KMT2A::TNRC18***
- `chr11:118,503,451(KMT2A)_delins[CCCC_chr7:5476973(TNRC18)]`
- Known fusion transcript (2 pediatric cases / 759 LAL. Meyer, C et al. Leukemia 2009)
- RT-MLPA probes w/o TNRC18



# Interesting results — *HOXA9*

**BE**

- Translocation t(2;9)(q14.1;q34.11) likely fusion transcript ***SET::DPP10***
- `chr9:140,901,114(SET)_delins[GAACATAAAGAAAAAAA_chr2:116,043,899(DPP10)]`
- **New fusion transcript in T-ALL** (only described one time in CRC: Xia, Li C et al. “Identification of large rearrangements in cancer genomes with barcode linked reads.” Nucleic acids research vol. 46,4 2018)



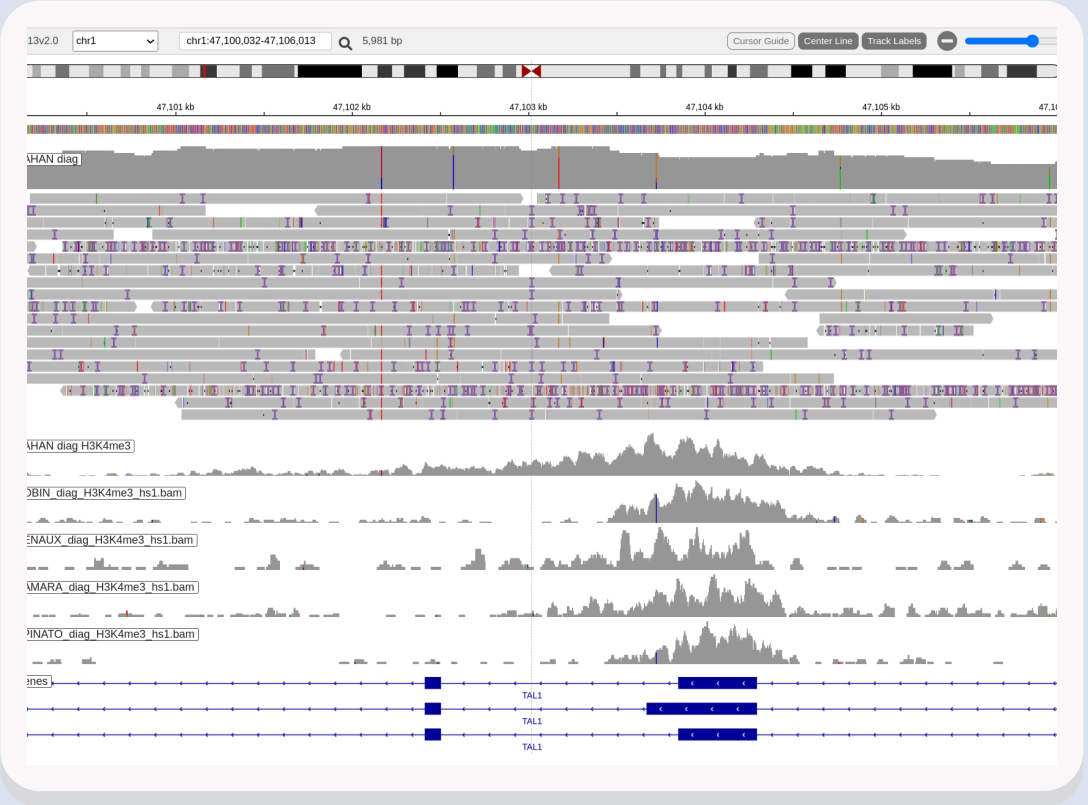
# 👥 Cohort *TAL1* — n = 12

| id | X | mécanisme | interpretation |
|---|---|---|---|
| ACHITE | 12 | TAL1::RPTOR t(1;17) | ✅ |
| BECERRA | 12 | TCR::LMO2 | ❓ |
| CAMARA | 20 | insertion intronique *MYB* | ❓ |
| COLLE | 7 | | |
| DAHAN | 13 | duplication intronique en tandem *TAL1* (ex2-3) | ❓ |
| DOUYERE | 22 pas mrd | (BCL11B::TLX3) | ❓ |
| FALLE | *en cours* | | |
| GILLOUX | 15 pas mrd | pas d'altération locus *TAL1* | ❓ |
| HENAUX | 12 | insertion intronique de 9nt dans *TAL1* (ex3-4) | ❓ |
| JEANSELME | 14 | duplication inversée intronique de 19nt dans *DAB1* | ❓ |
| ROBIN | 14 | LMO2::RAG1 del 11q | ❓ |
| SCHOLZ | 18 pas mrd | Probable dup inversée chr2:26,269,840-32,135,324 | ❓ |

# Interesting results — *TAL1*

**DAH**

- Mono-allelic surexpression of TAL1.
- Somatic tandem duplication between exon 2 and 3 of TAL1.
- Also observed in ChIP-seq data showing broad H3K4me3 coverage.



# 👥 Cohort *TLX1* — n = 8

| id | X | mécanisme | interpretation |
|---|---|---|---|
| AUBERT | 17 | del 10q (10mb) *TLX1* | ✅ |
| CHAMPION | 32 | inv10 | ✅ |
| LEVASSEUR | 20 | inv10 (avec 5' UTR ralongé) | ✅ |
| PARACHINI | 17 | t(10;14) | ✅ |
| PASSARD | 18 | chromothripsis du 10 | ✅ |
| PAYSAN | *en cours* | | |
| RIVOALEN | 19 | inv10 | ✅ |
| SALICETTO | 12 pas mrd | inv10 | ✅ |

# Interesting results — *TLX1*

**LEV**

- Surexpression of TLX1.
-
-

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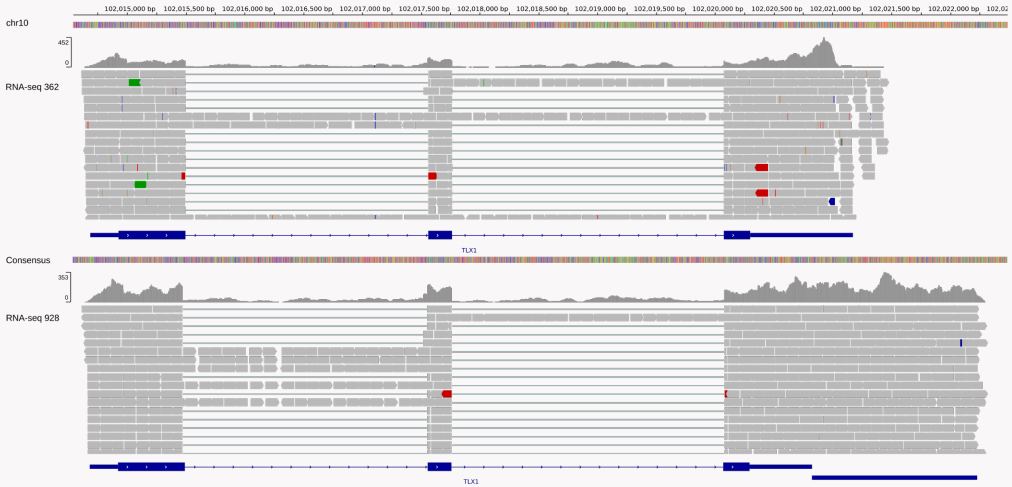
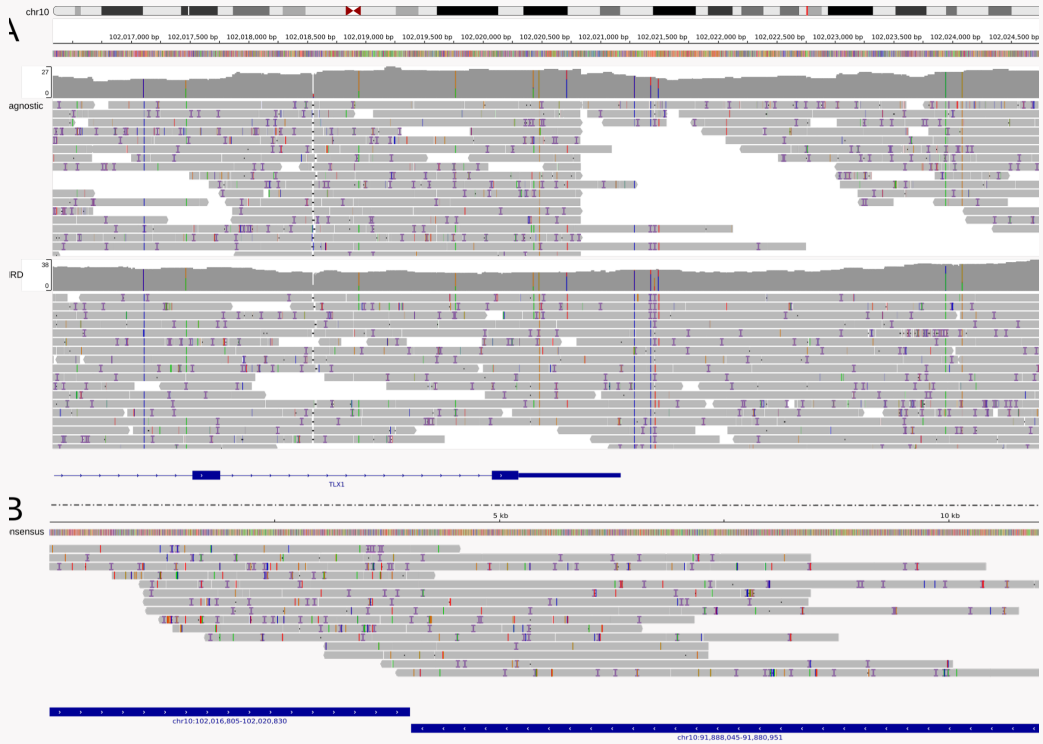
- Inv10
-

# Interesting results — *TLX1*

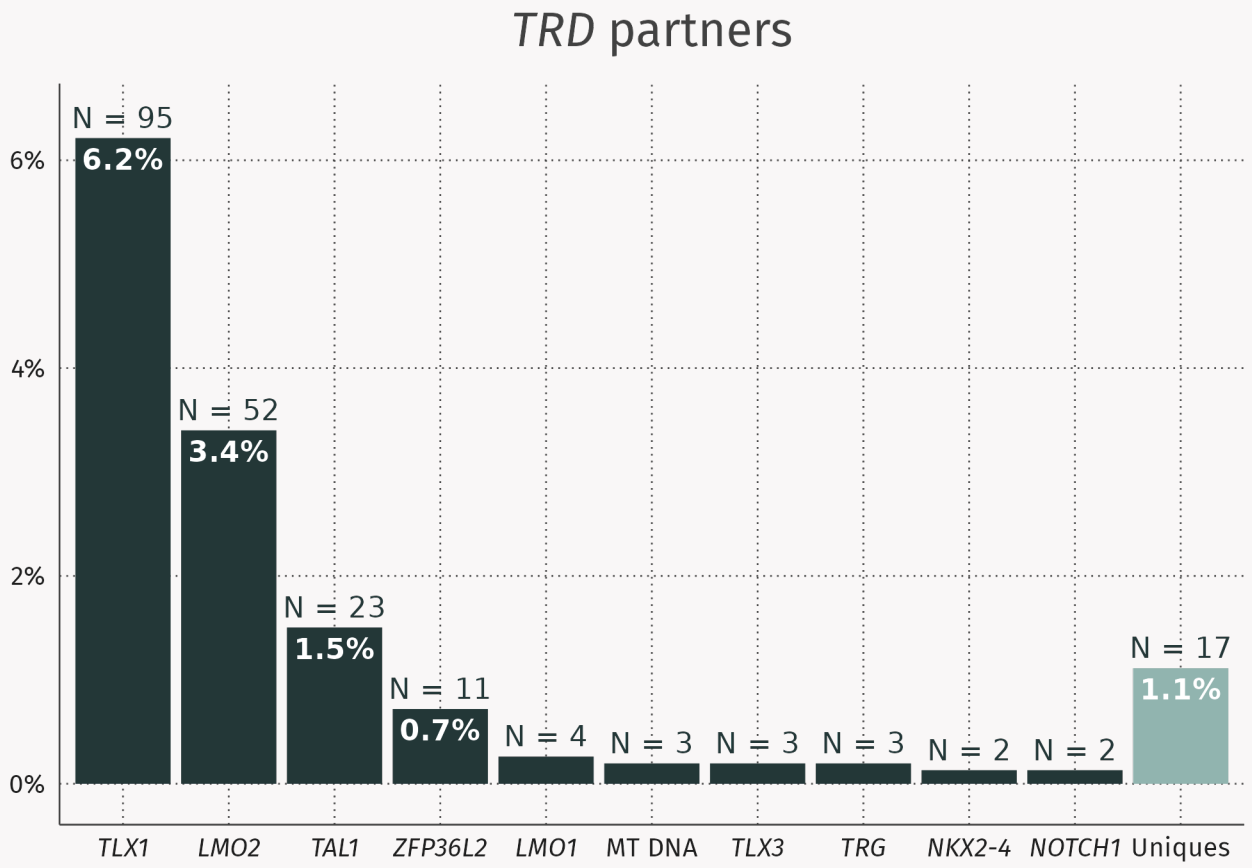
## LEV

- Surexpression of TLX1.
- Inv10
- Modification of 3′ UTR.



# Discussion — Poisson distribution & rare variants strategy



# 🏁 Conclusions

What is **done**

- ✅ The implementation of a robust and more informative whole-genome sequencing method.
- ✅ The development of a pipeline for detecting somatic alterations (SNV, SV, viral insertion) as well as a simple way to visualize and interpret the results.
- ✅ Compare missense/indels variant calling with NGS panel.
- ✅ The integration, if available, of RNAseq / ChIPseq.
- ✅ Develop a de novo assembly pipeline (better accuracy and visualization of SV).
- ✅ Automate reporting.

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## TODO

- ❌ Functional experiments ?
- ❌ Complete sequencing of cases (> 200).
- ❌ Aggregate results by cohorts and tag recurrency (at gene and mutation levels).

# Remerciements


**The Necker team**

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