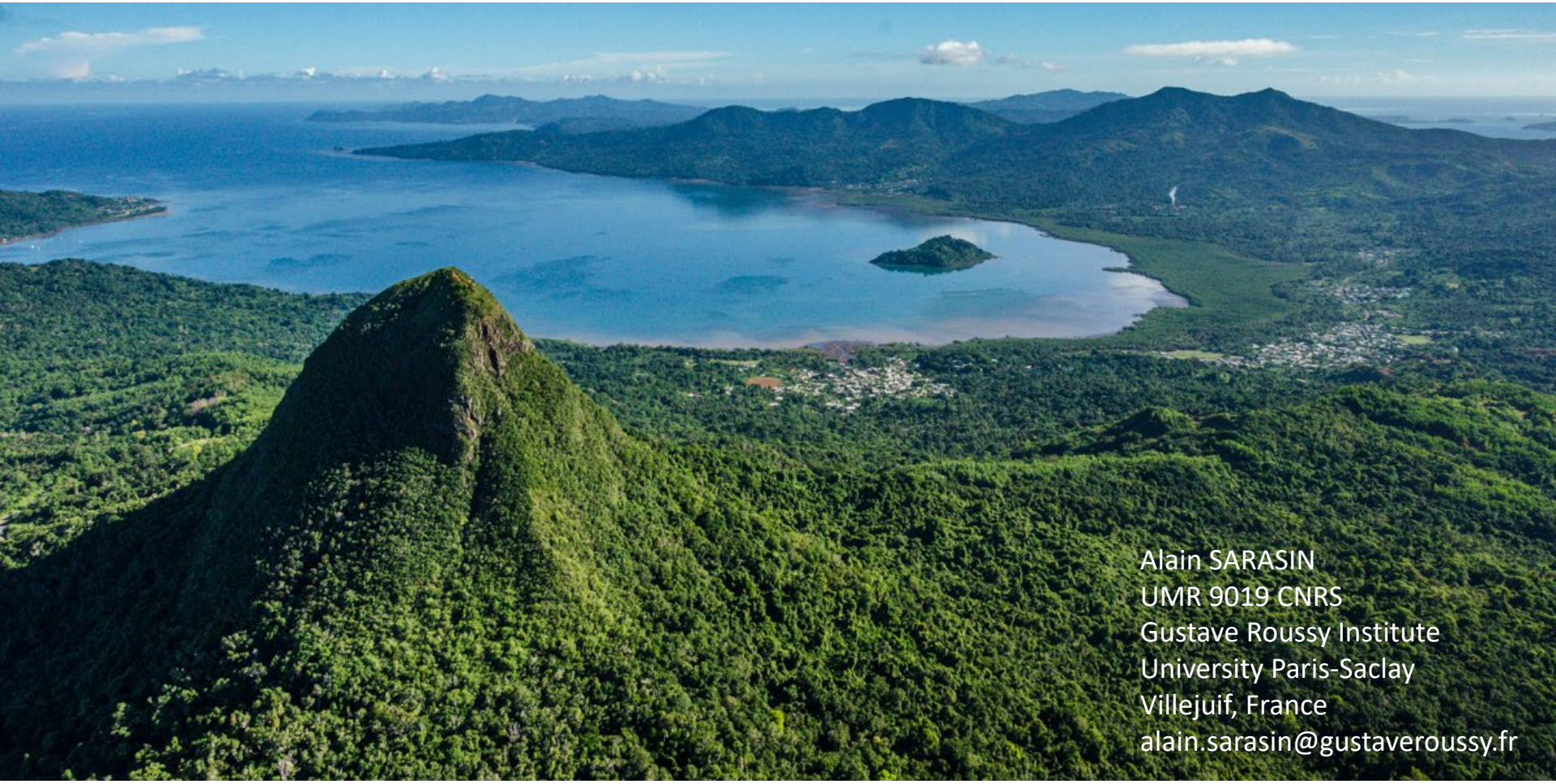
Xeroderma pigmentosum:
High risk of internal tumors and mutational landscape of XP leukemia

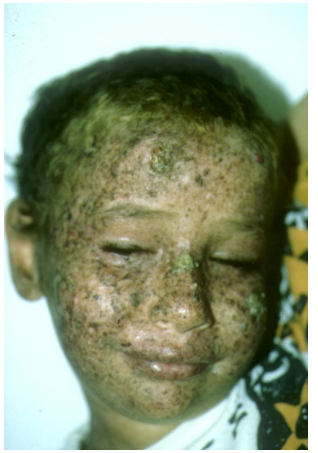


Alain SARASIN
UMR 9019 CNRS
Gustave Roussy Institute
University Paris-Saclay
Villejuif, France
alain.sarasin@gustaveroussy.fr

Outline

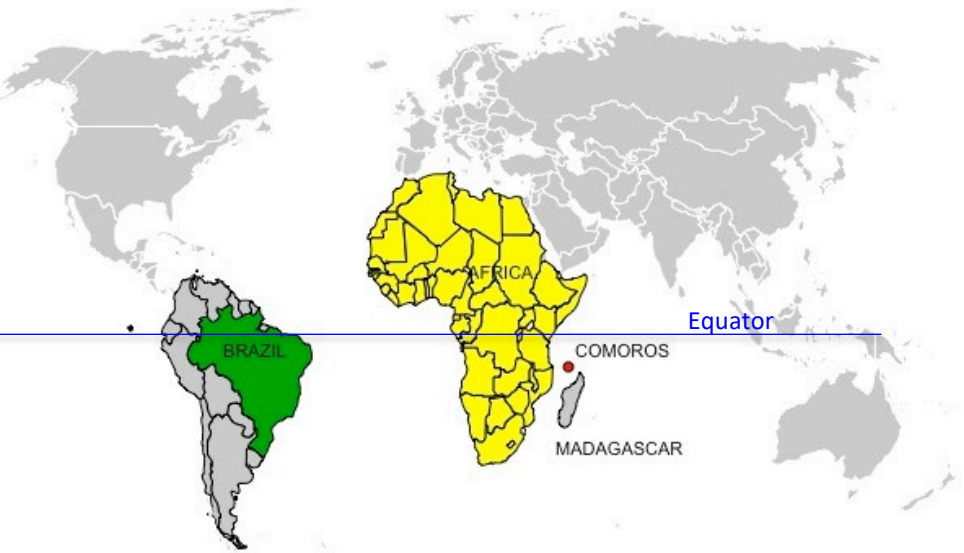
- Xeroderma Pigmentosum (XP) and Nucleotide Excision Repair (NER)
- Following an *XPC* gene mutation: from Comoros to East Africa, then to Pakistan and Brazil
- What are the risk factors of developing internal tumors in XP patients?
- Mutational landscape of hematological malignancies of XP-C patients
- Conclusions

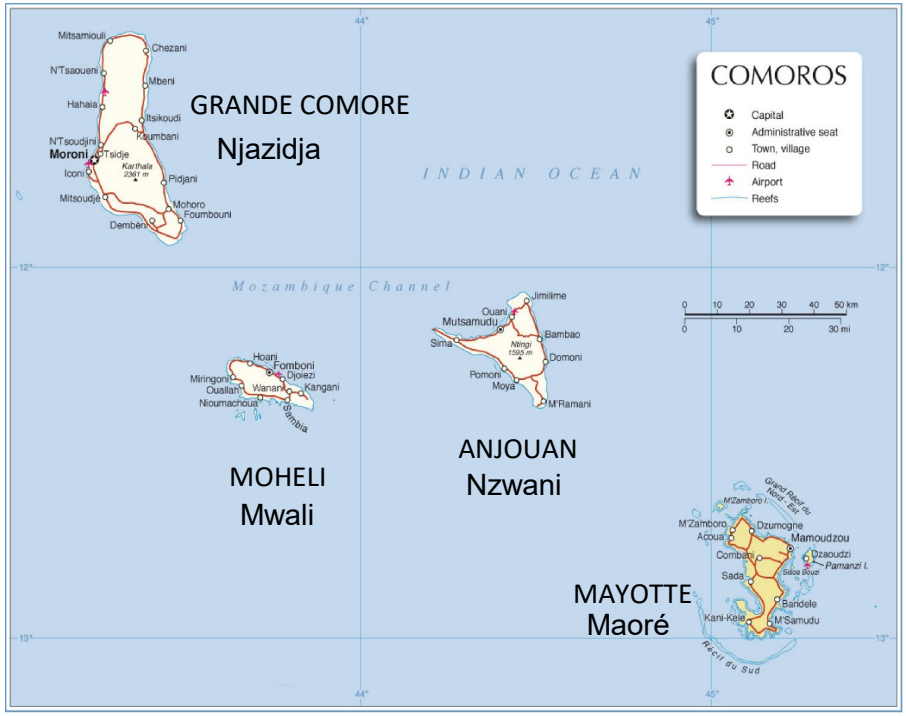
Xeroderma Pigmentosum



- Genetic autosomal recessive disease
- Rare
  - 1/500,000-1/1,000,000 in USA and Europe
  - >1/10,000-1/50,000 in North Africa, Japan, Comoros
- UV-sensitivity, skin disorders
- DNA repair deficiency:
  - Nucleotide Excision Repair (7 genes *XPA* to *XPG*)
  - Polη translesion polymerase (XP variant)
- High frequency of UV-induced skin cancers
- Short lifetime if unprotected from sun exposure

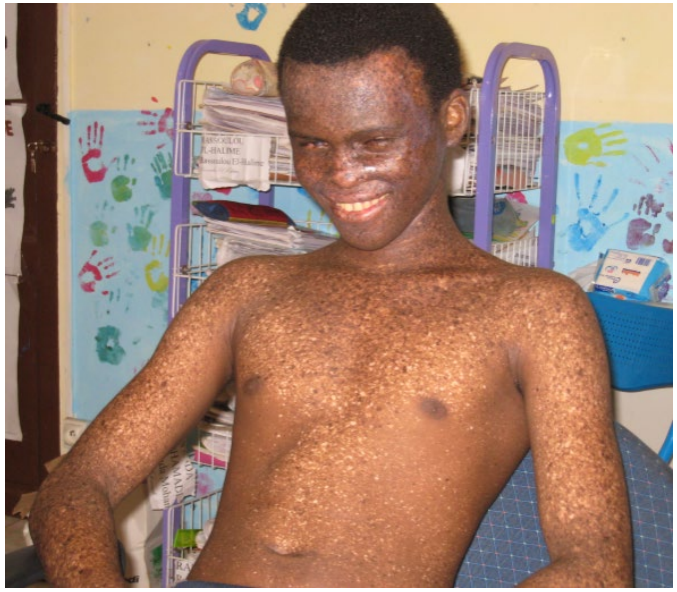
The Comorian Archipelago in the Indian Ocean

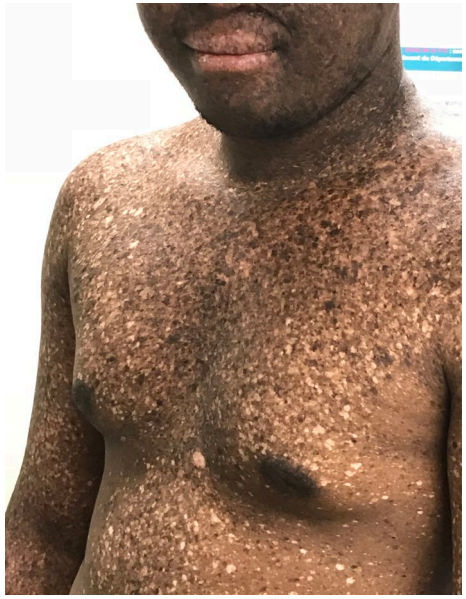




- Very high incidence (around 1/5000) of black-skinned XP patients in the Comorian population.
- XP patients with a unique *XPC* mutation: IVS 12-1G>C; c.2251-1G>C: "Comorian mutation"
- We calculated the age of the mutation inside the islands to be 800 years old
- Islands: closed environment and remarkable degree of inbreeding with still polygamy

Cartault et al., 2011, DNA Repair, 10, 577-585

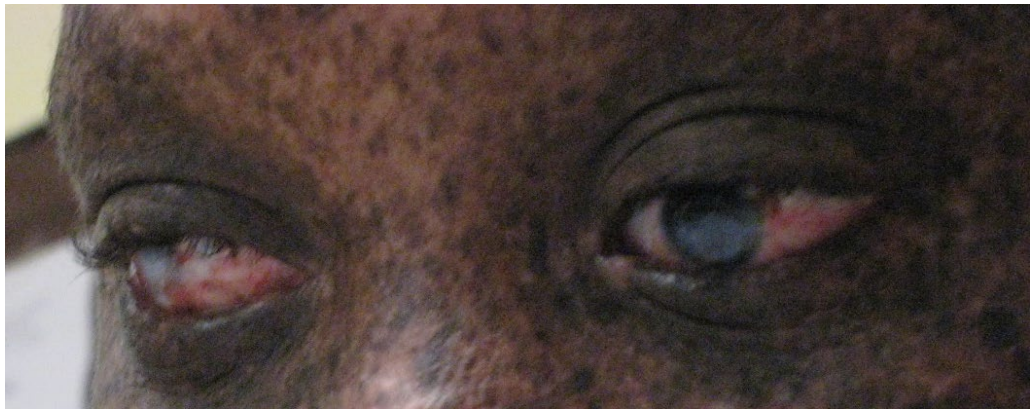




Patient XP845VI 15 and 23 years old

Patient XP-C
7 years old

Patient XP845VI 23 years old



Patient XP844VI 15 years old

Photographs shown with authorization

LATER, we found this “Comorian” mutation among XP patients from Kenya, Mozambique, Zimbabwe and in the north of South Africa (Pretoria).
We calculated the age of the *XPC* mutation in this population to be around 1100 years. So the “Comorian” mutation from East Africa is older than the one found in Comoros. So, we should talk about the “East-African” mutation.

Heterozygous XP-C individuals (males and females) should have been moving from East Africa to Comoros around the 7th to the 10th centuries.
“Accumulation” of XP-C patients in Comoros is probably due to the specific Geographical and Historical isolated situation.

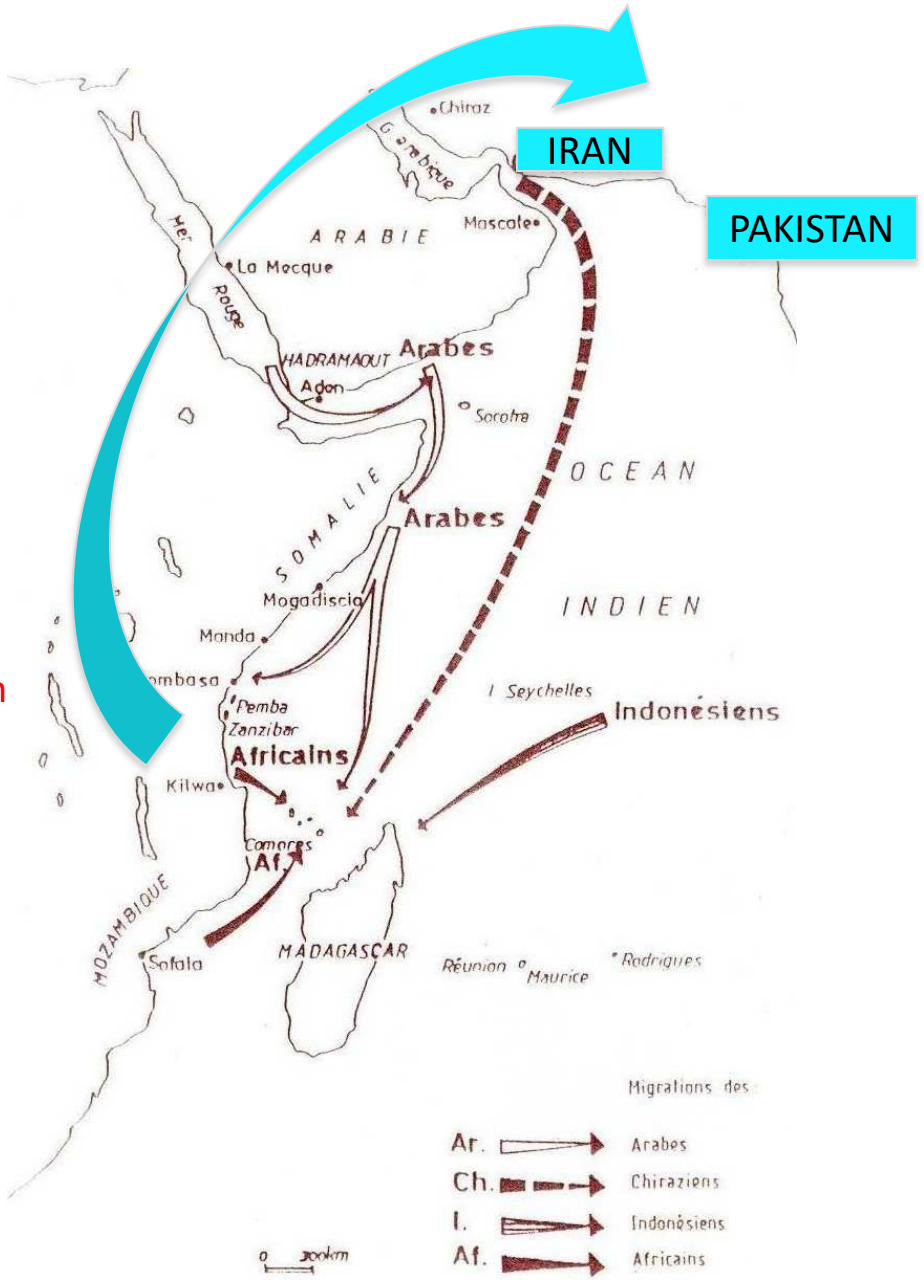
Kgokolo et al., Br. J. Dermat., 2019, 181, 1070-1072
Sarasin et al., Genet. Mol. Biol., 2019, 43, e20190046

Origins of the XP-C patients described in Pakistan?

-Arrival of the Arabs from the Arabic Peninsula and from Chirazia (South Iran) around 11-13th centuries (700-800y from now)

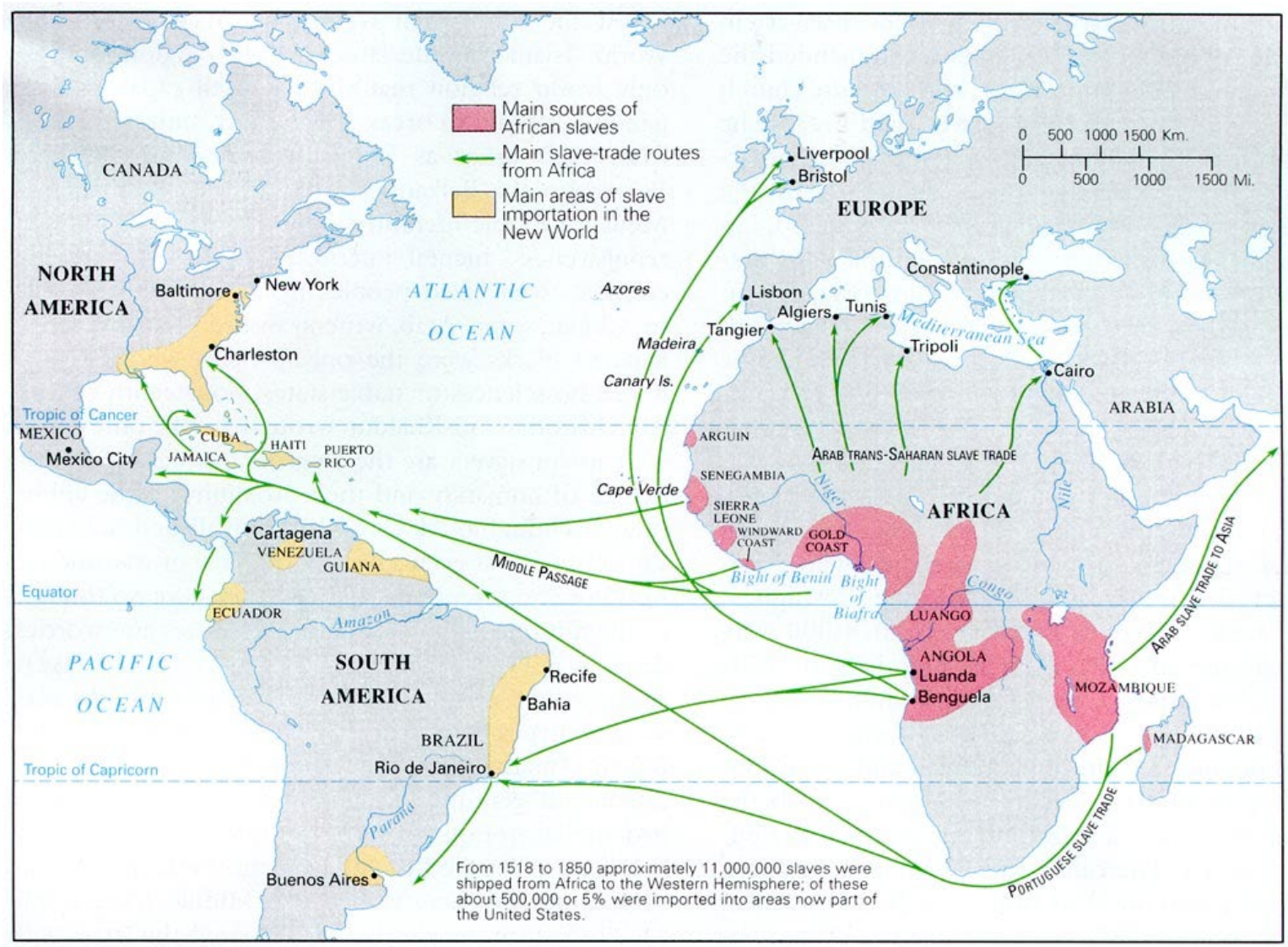
-The Arabs were going back home with black slave men and with black women as "spouse"

-The described XP-C from Pakistan have the same mutation and partly same haplotypes than the Comorian XPs. They live as nomad tribes in between Pakistan and Iran



Ijaz et al., Congenit. Anom. (Kyoto) 2019, 59, 18-21

Seaborne slave trades between Africa and South America



During the 17-19th centuries, 20% of black African slaves in Brazil came from East Africa, mainly from the Portuguese colony Mozambique. These slaves were called ("The Moçambicos"). They have the same ethnic origins as the population of Comoros. In 1850, 40% of Rio de Janeiro inhabitants were black slaves.

From "Moçambiques" in Brazil, Edwards A. Alpers (UCLA)

Distribution of the *XPC* Comorian mutation in Brazil

(Gift of Dr. Maria-Isabel ACHATZ and Dr. Ligia PEREIRA-CASTRO)

AS2735 New Eng.

Ethnogeny of the African *XPC* mutation

Sarasin *et al.*, Genet. Mol. Biol., 2019, 43, e20190046

What about internal tumors in XP?

- XP patients live longer in developed countries due to better protection and education
- All cells of XP patients are deficient in repair (NER or *POLH*)
  - Skin cells: XP are known to develop skin carcinomas and melanomas (>10,000 fold than normal)
  - What about internal organs?
- Some reports in the past showed a 10-20 higher risk of CNS tumors in XP (Kraemer's lab)
- Recent reports of hematological malignancies in XPs
  - Sarasin et al., Blood, 2019, 133, 2718-2724
  - Oetjen et al., Haematologica, 2020, 105, e144-e146

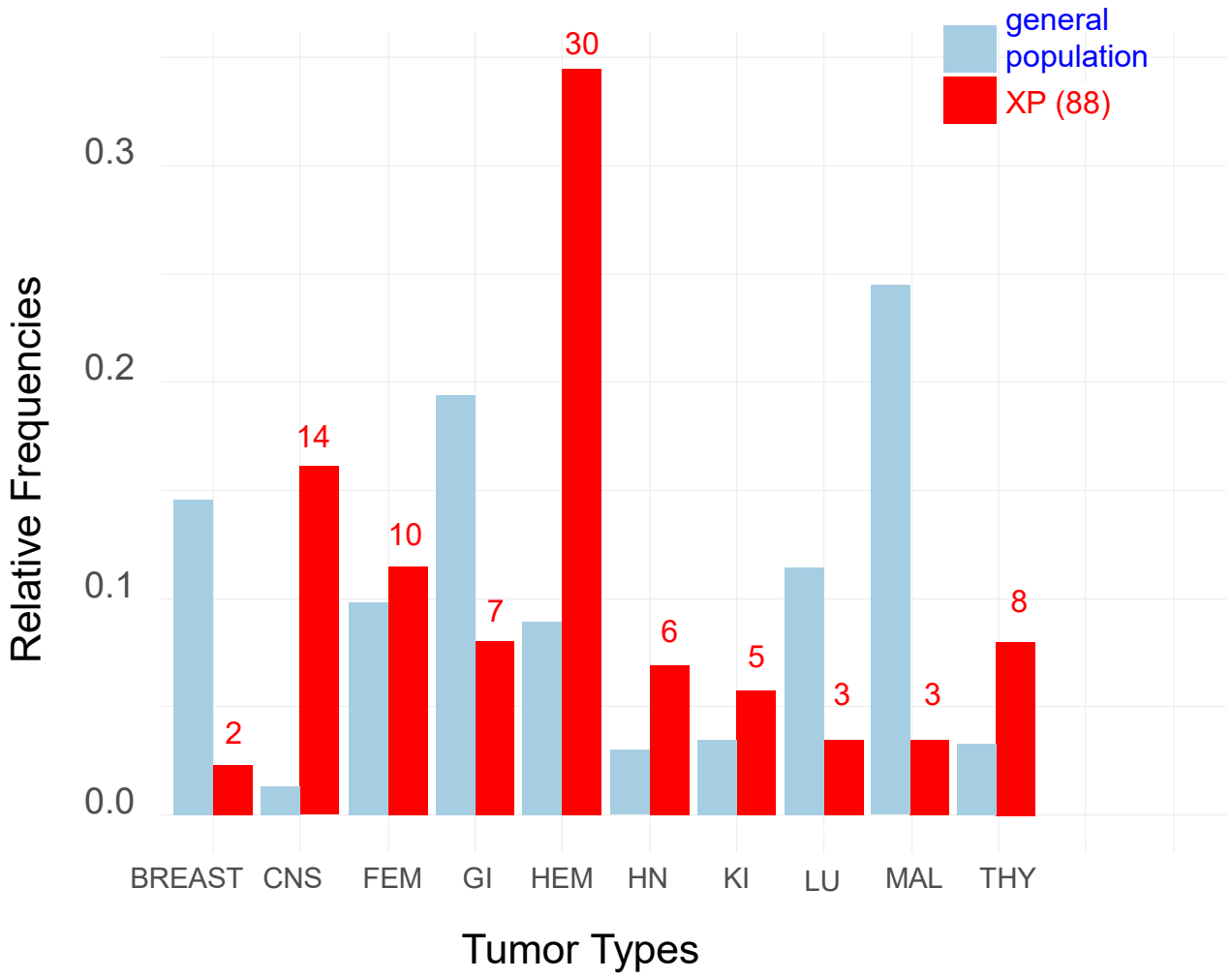
Internal tumors in XP patients

- We have collected 89 XP internal tumors (non-skin cancers, not UV-associated cancers) reported in the literature between 1958 and 2020 (88 XP patients)

  - Aggressive and mostly lethal tumors
  - Occur 50 years earlier than in the general population
  - Almost 50% of these XP are originated from North Africa, 22% from USA and 8% from Japan
  - We know the complementation group for only 65 patients (74%):
    83% XP-C, 9% XP-V, 3% XP-A, 1.5% for XP-D, XP-E, XP-F

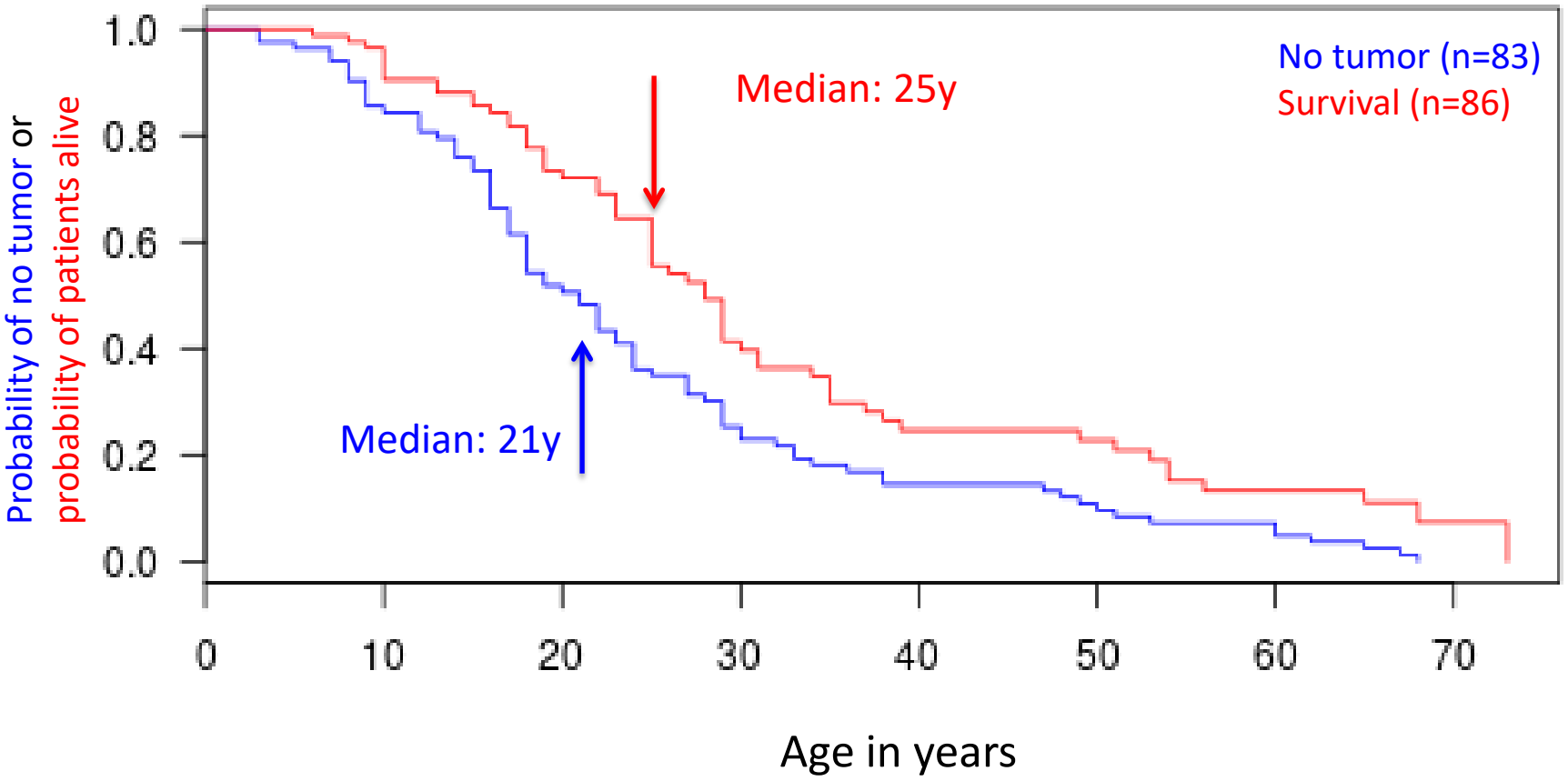
    The vast majority of the XP-C originated from North Africa bears the founder mutation (at least 1200y old):
    c.1643_1644 delTG; p.Val548AlafsX572 (delTG, exon 9)

Relative frequencies of internal tumor types between the American general population and the xeroderma pigmentosum patients



The CNS tumors are 10 times more frequent in XP and the hematological malignancies 3.5 times more frequent in XP than in the general population. Inversely, breast cancers and male-reproductive system tumors are 7 times less frequent in XP than in the general population.

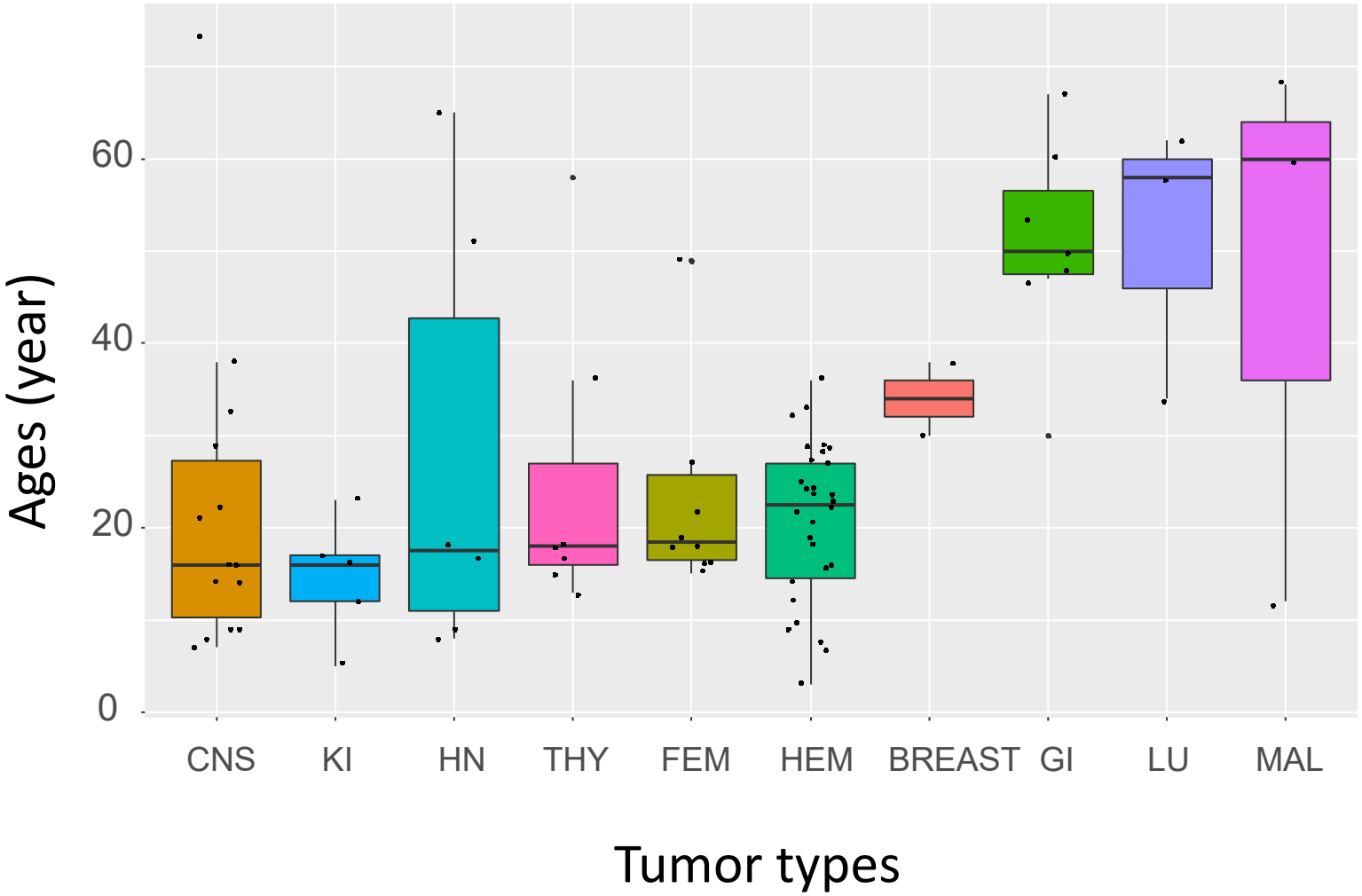
Internal tumor occurrence and survival in XP patients



Two classes of XP internal tumors: the majority occurred before 20-25 years and death occurred before 30 years. A smaller number occurred later (>50y) at an age similar to what is found in the general population

Nikolaev, Yurchenko and Sarasin, Orphanet J Rare Dis. 2022, In Press.

Median ages of XP patients (88) at diagnosis of internal tumors grouped by tumor types



Nikolaev, Yurchenko and Sarasin, Orphanet J Rare Dis. 2022, In Press.

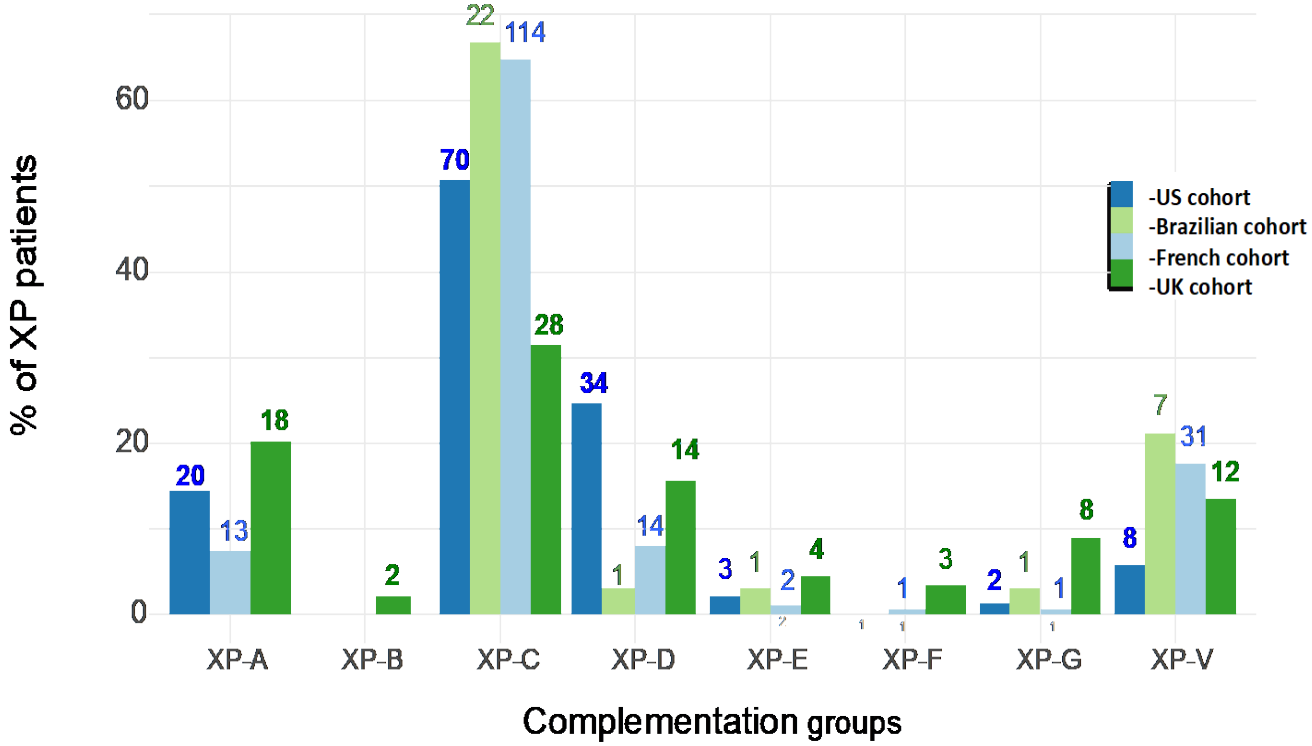
Median ages of XP patients at diagnosis of internal tumors grouped by tumor types and complementation groups

54 tumors with known groups (74 XPs)
91% XP-C

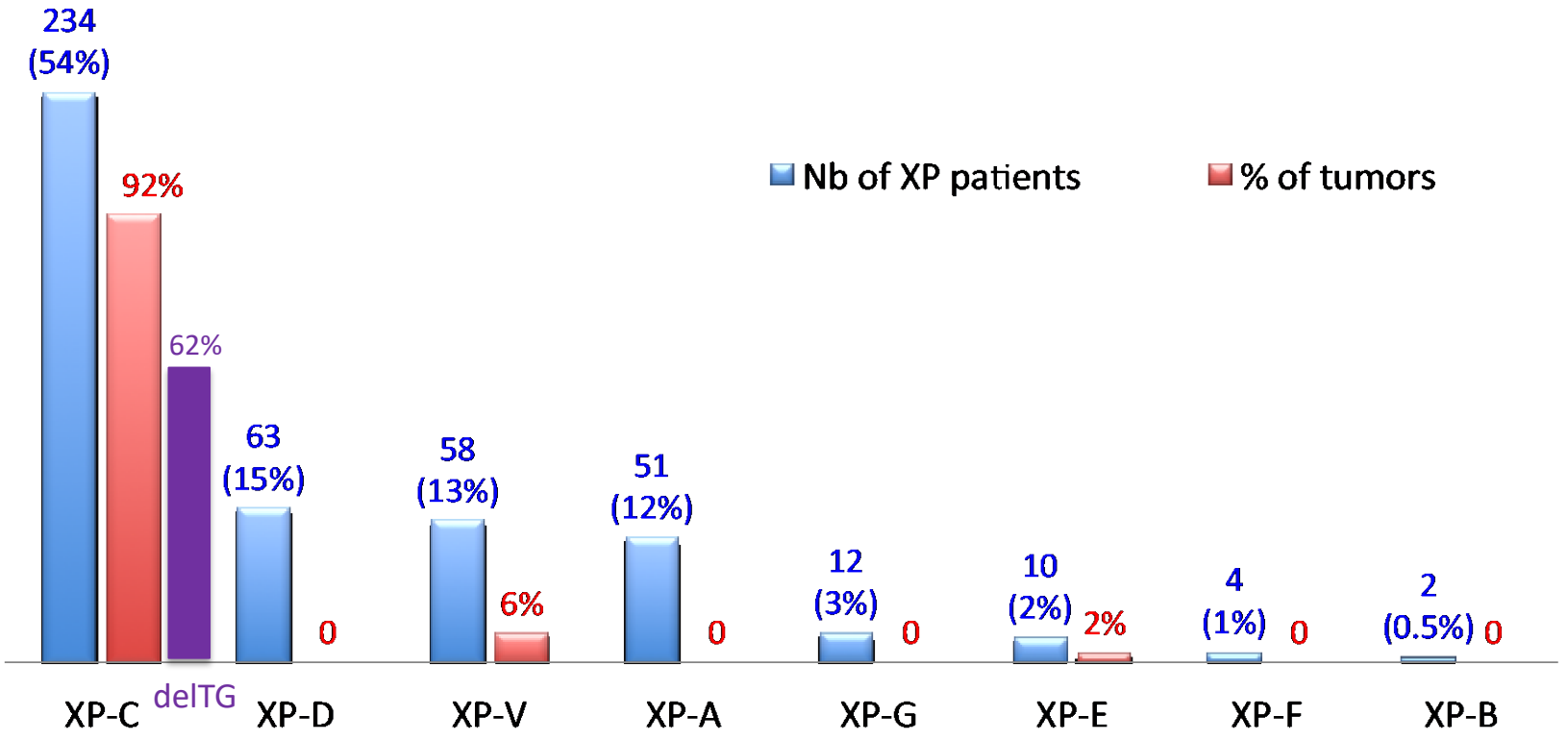
9 tumors (13 XPs)
33% XP-C
56% XP-V
11% XP-F

Four international and independent XP cohorts

- To calculate the risk factor of XP patients to develop internal tumors, we analyzed 4 XP cohorts: 50 tumors among 434 XP patients:
  - 137 NIH American XP with 14 internal tumors (10.2%)
  - 176 French XP with 32 internal tumors (18.2%)
  - 32 Brazilian XP with 2 internal tumors (6.3%)
  - 89 English XP with 2 internal tumors (2.2%)
- The majority of the 434 XP belongs to XP-C (54%) but the distribution of complementation groups is different between the 4 cohorts



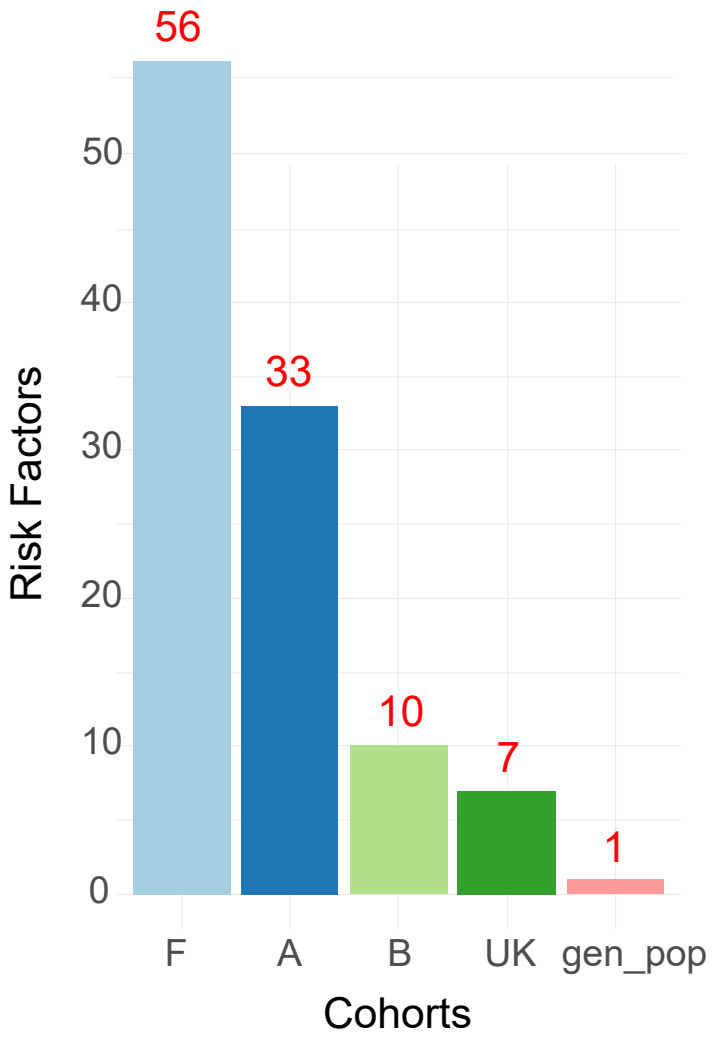
Distribution of internal tumors (50) according to complementation groups among the 4 independent XP cohorts (434 XPs)



Odds ratio for XP-C patients to develop internal tumors: OR= 9.8 (CI95%: 3.5 to 38.1)
as compared to all other complementation groups. 62 % of XP-C patients have the delTG mutation from North Africa

Nikolaev, Yurchenko and Sarasin, Orphanet J Rare Dis. 2022, In Press.

Risk factors (Odds Ratio) for the 4 independent XP cohorts to develop internal tumors

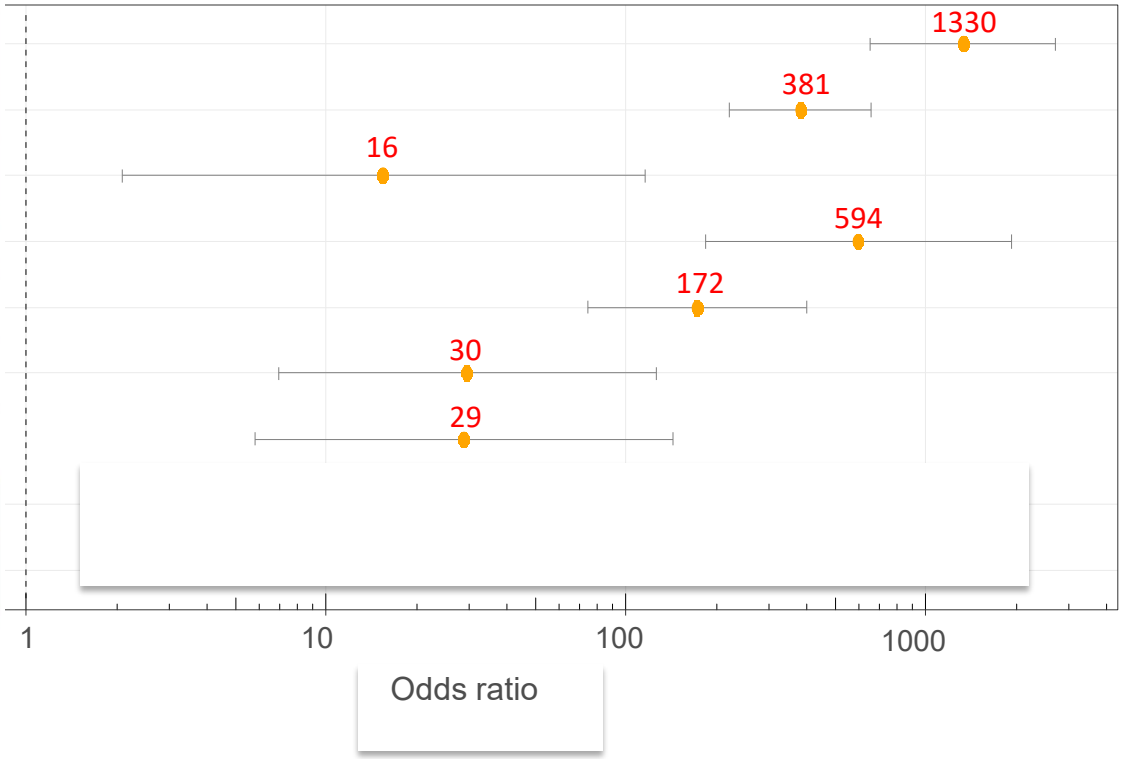


French: OR= 56 (CI 95%: 37-84)
American: OR= 33 (CI 95%: 19-59)
Brazilian: OR= 10 (CI 95%: 1.4-73)
English: OR= 7 (CI 95%: 1.8-30)

Nikolaev, Yurchenko and Sarasin, Orphanet J Rare Dis. 2022, In Press.

Risk factors (OR, 95%CI) for developing internal tumors in XPs stratified by ages

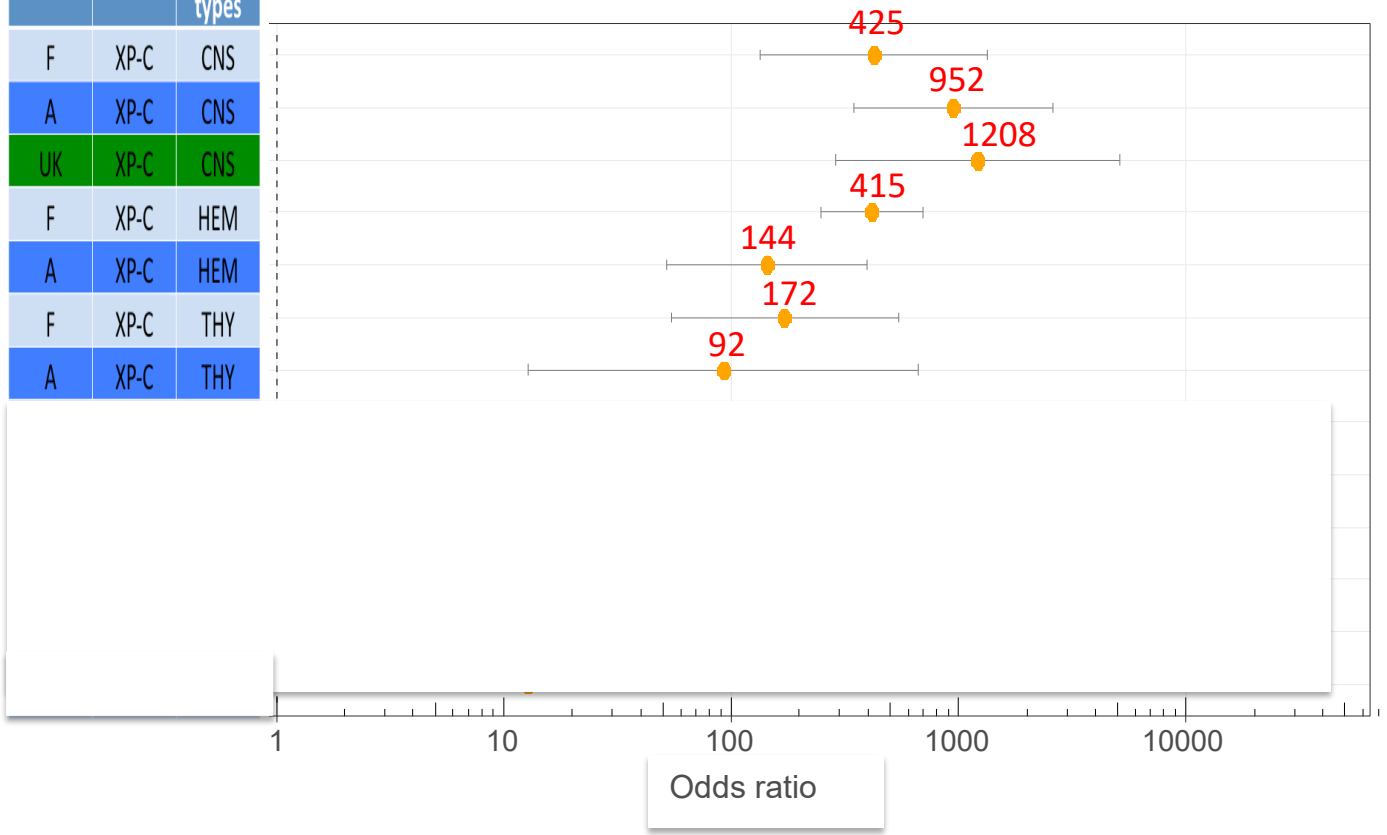
Cohorts	Ages (Y)
F	0-20
F	20-40
F	40-60
A	0-20
A	20-40
A	40-60
A	60-80



The risk factor is very high for the young XPs in all cohorts.

Risk factors to develop internal tumors in XPs stratified by tumor types and complementation groups

Cohorts	Groups	Tumor types
F	XP-C	CNS
A	XP-C	CNS
UK	XP-C	CNS
F	XP-C	HEM
A	XP-C	HEM
F	XP-C	THY
A	XP-C	THY



The XP-C patients exhibit the highest risk to develop CNS and thyroid tumors, and hematological malignancies

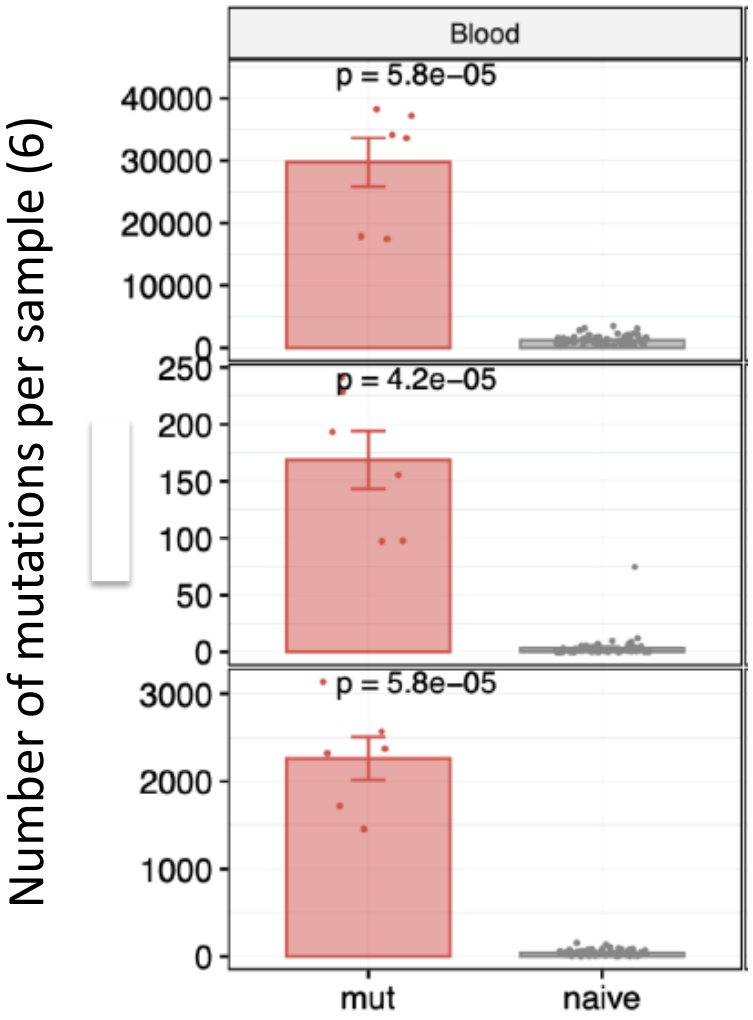
Mutational landscape of haematological malignancies XP-C patients

- **Rational of the study**
  - Is the increased risk of cancer in XP associated with a mutator phenotype?
  - Are the mutational patterns in XP internal cancers the same as in cancer-type matched sporadic tumors?
  - What are the genetic mechanisms of leukemia in XP?

- **Sampling**
  - 6 XP-C Acute Myeloid Leukemia (15-26y) (delTG)
  - 1 XP-C breast cancer (30y)(Comorian mutation)
  - 1 XP-C uterine rhabdomyosarcoma (16y)(delTG)

- **Methods**
  - Whole Genome Sequencing of tumoral DNA and germline patient DNA
  - BioInformatics analysis: A. Yurchenko and S. Nikolaev (Gustave Roussy Institute)

Mutational burden of XP-C leukemia (6)



Single Base Substitutions: 176 538

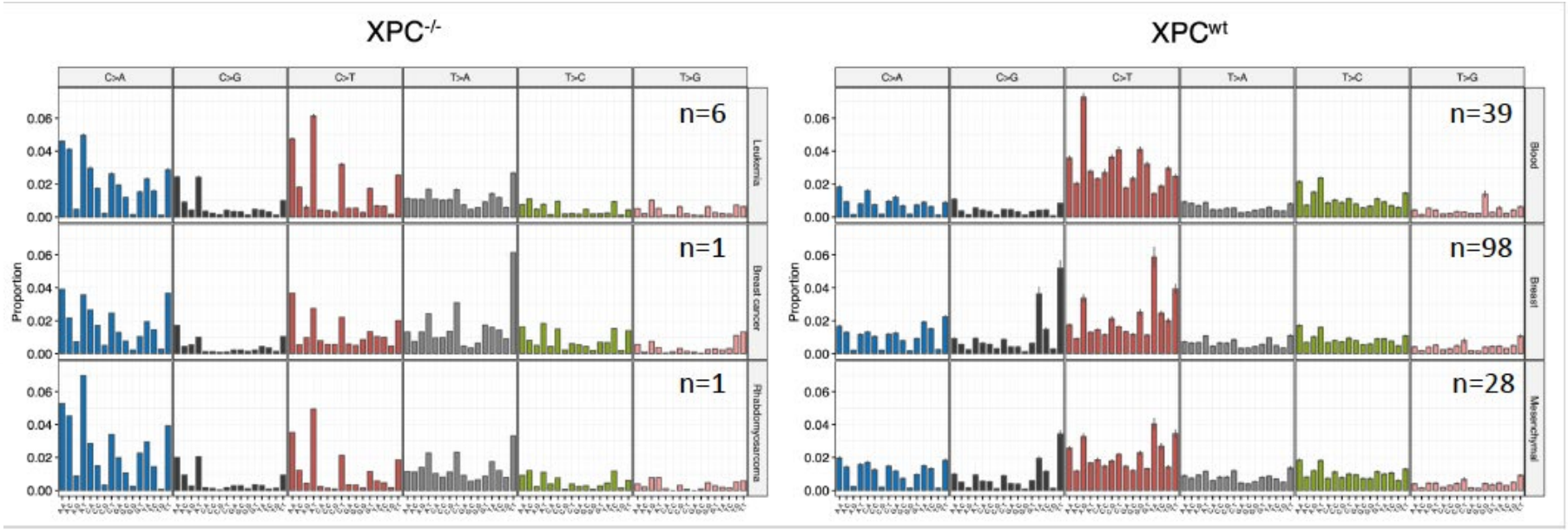
Double Base Substitutions: 1 035

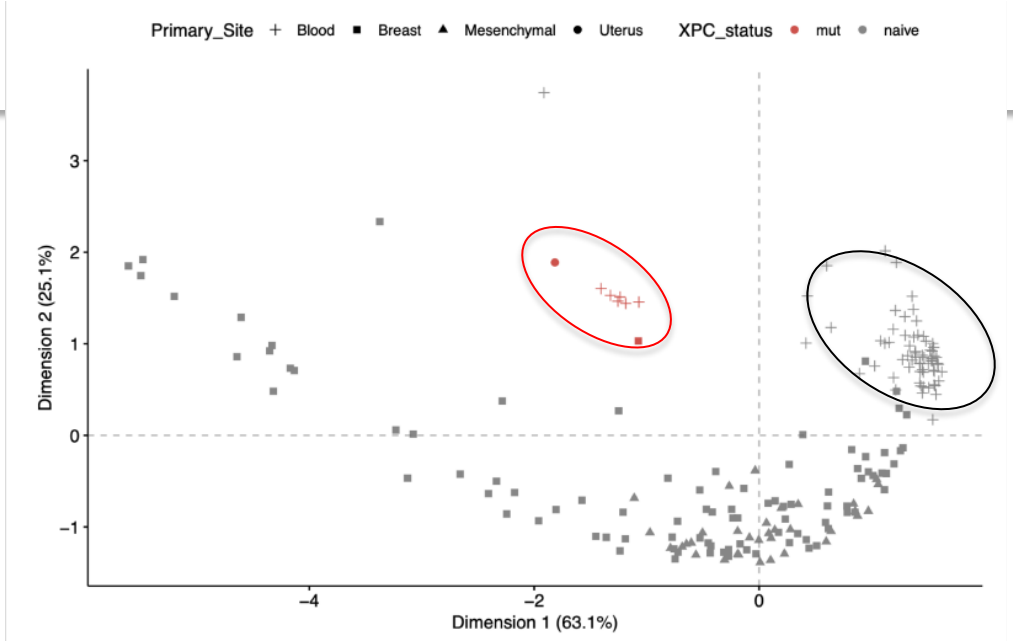
Indels: 13 607

We identified a 25 fold (14.5-31.2) increase in somatic mutations in XP-C leukemia relative to sporadic myeloid leukemia ($P= 5.8x10^{-5}$)

Yurchenko et al., Nature Comm., 2020, **11**, 5834.

Mutational profiles in XP-C internal tumors and and sporadic cancers

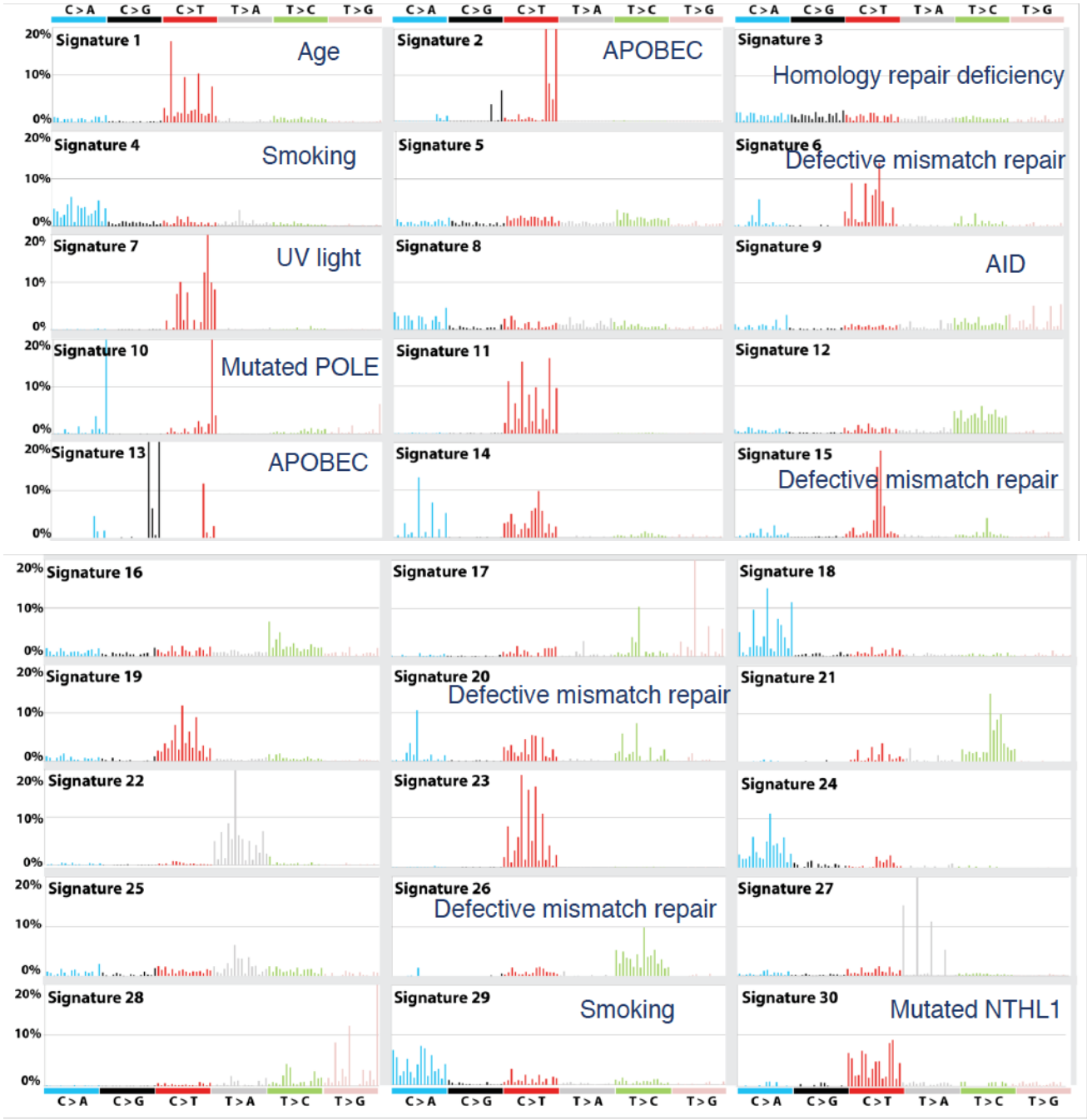




Multidimension scaling plot of the distance between the different mutational profiles

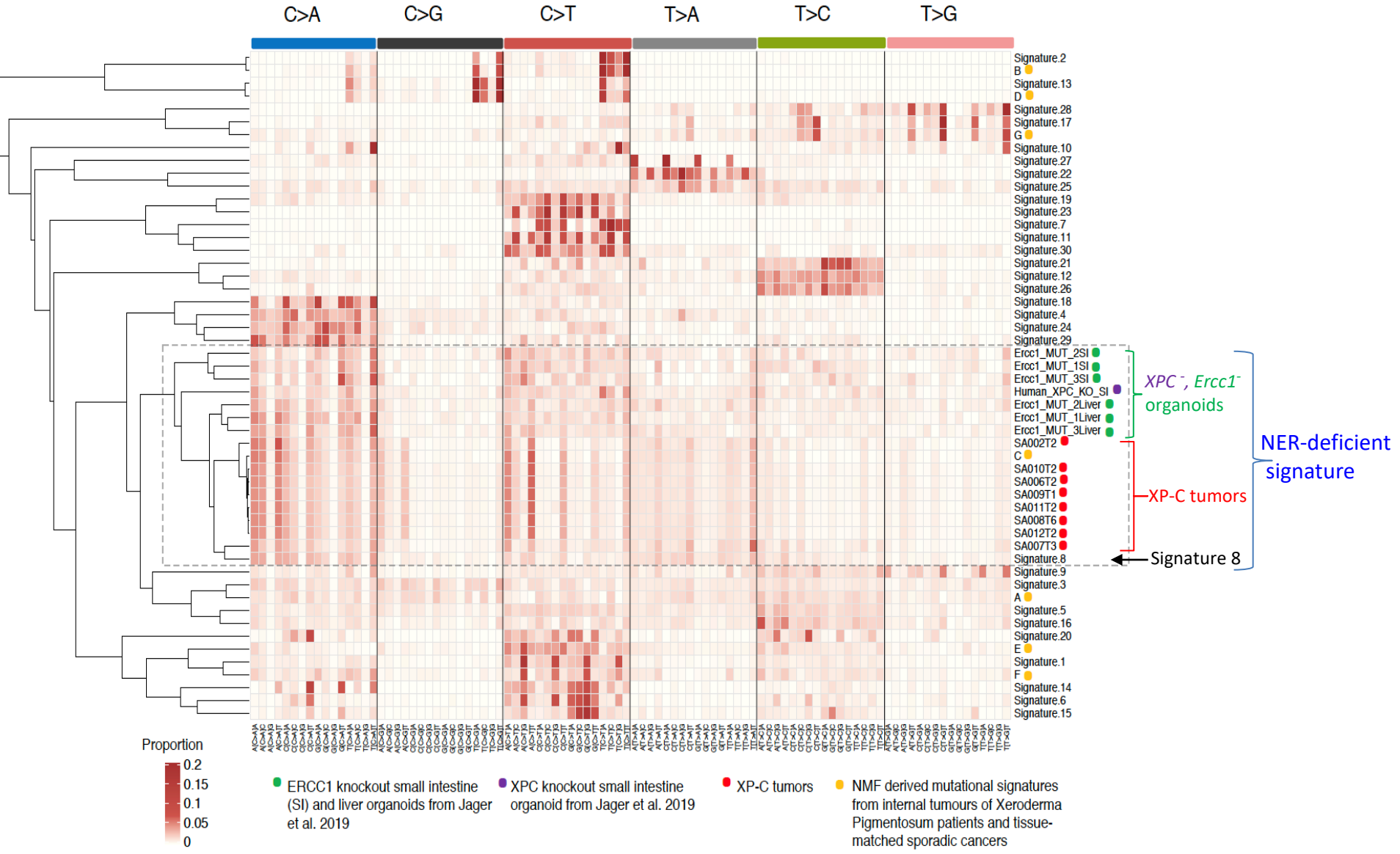
Different trinucleotide-context mutational profiles and dissimilarity between XP-C and sporadic tumors

30 mutational signatures extracted from thousands of cancers



Alexandrov et al., Nature, 2013, **500**, 415-421

Unsupervised clustering of the XP tumor mutational profiles with COSMIC signature 8



The 8 XP-C tumors cluster together, and the *XPC* KO and *Ercc1*⁻ organoid cultures (Jager et al., 2019) cluster only with signature 8

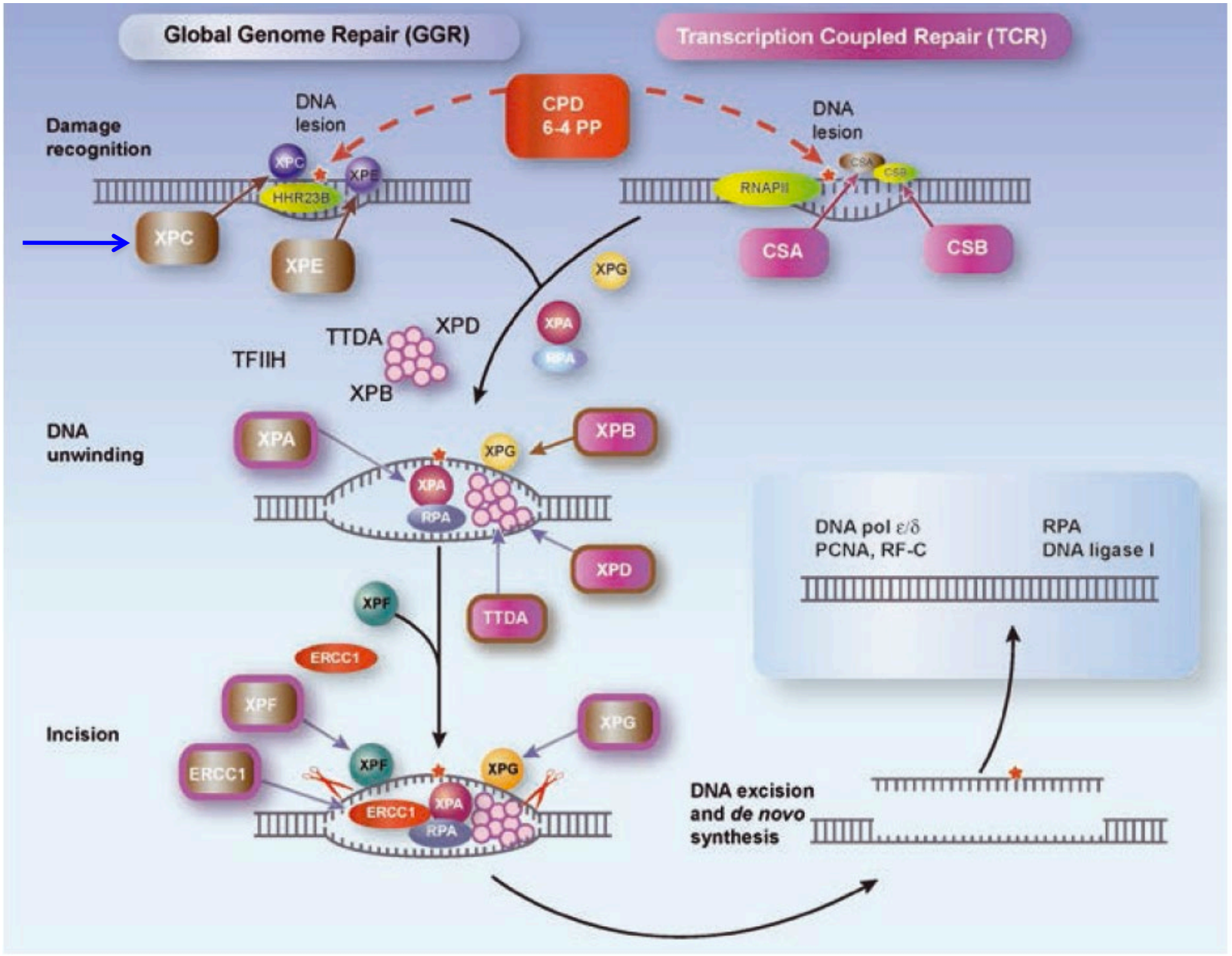
Yurchenko et al., Nature Comm., 2020, **11**, 5834.

What is the signature 8 of the Catalog Of Somatic Mutations In Cancer (COSMIC)?

- No attribution to any molecular mutagenesis pathway (Alexandrov)
- We clearly showed it is a marker of NER-deficiency
- Signature 8 is 700 fold more implicated in XP-C leukemia as compared to sporadic leukemia
- It is characterized by mutations mainly G to T and at GpG

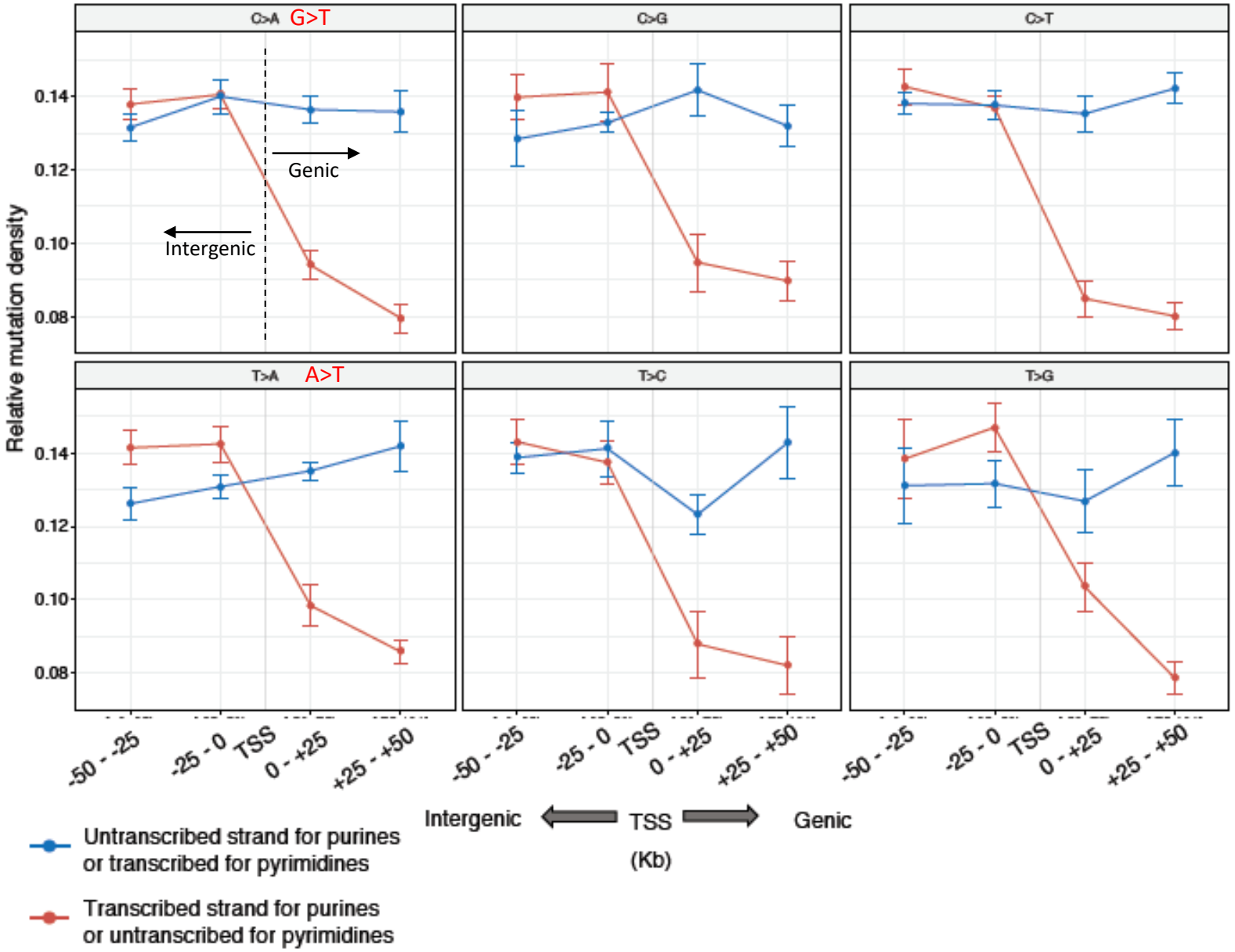
- Mutations are caused by unrepaired Purines on the non-transcribed strands in XP-C tumors

The Nucleotide Excision Repair pathway



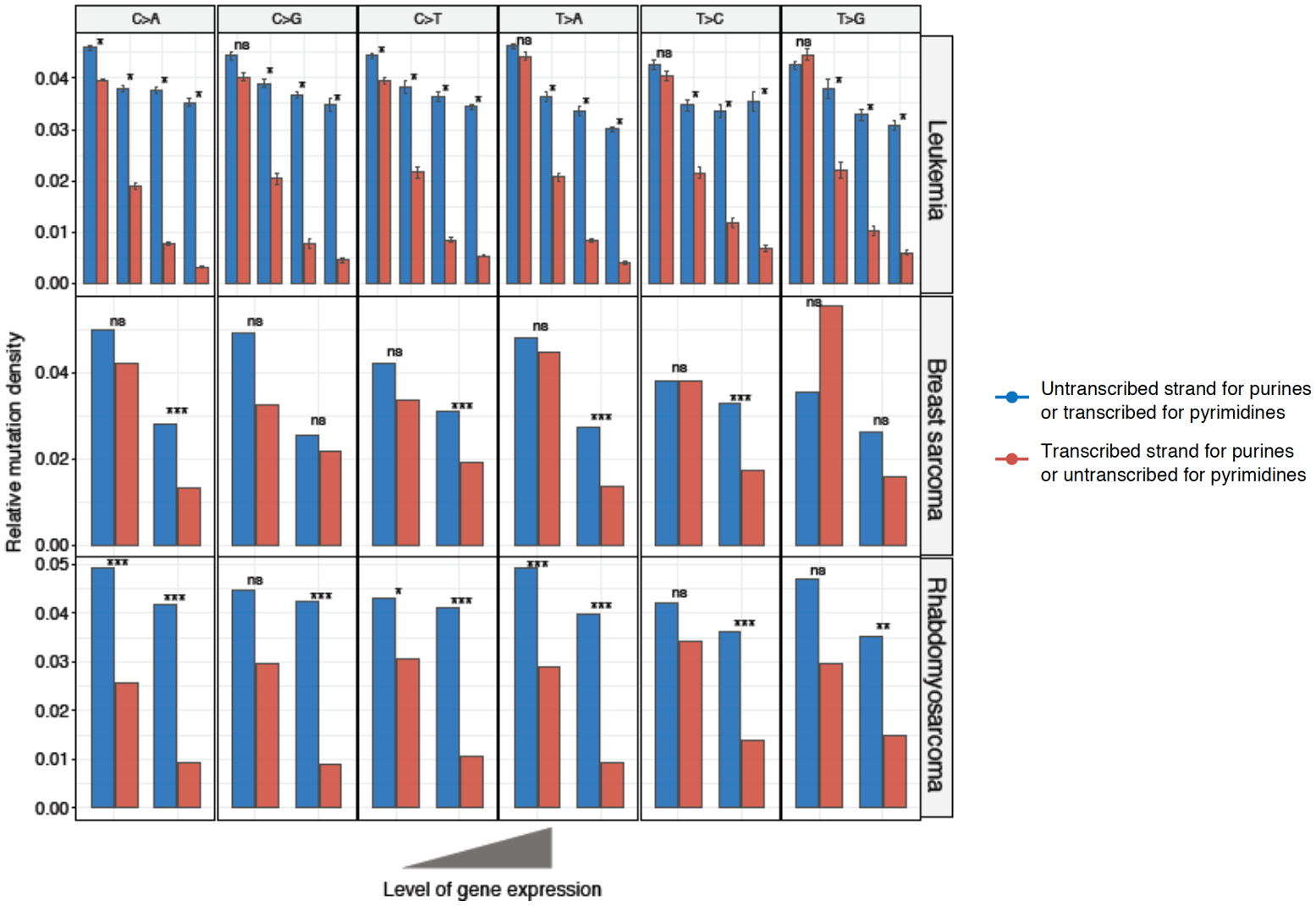
Kraemer K.H. and Sarasin A., 2018

Transcription bias is caused by TCR of Purines on the transcribed strands of XP-C leukemia



Strong decrease of mutation density on the transcribed strands for Pu (red) but not on the untranscribed for Pu (blue) as compared to intergenic regions (left of TSS) ($P = 10^{-13}$)

Transcriptional bias is the strongest in highly expressed genes



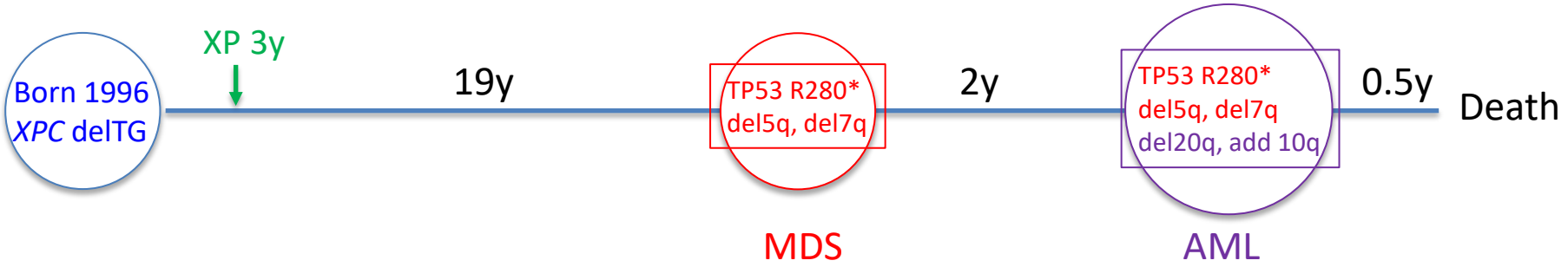
Transcriptional bias for mutation density is observed with all mutation classes, in the 3 types of XP-C tumors and essentially in intensively transcribed genes: up to 7.4 fold ($P > 10^{-11}$)

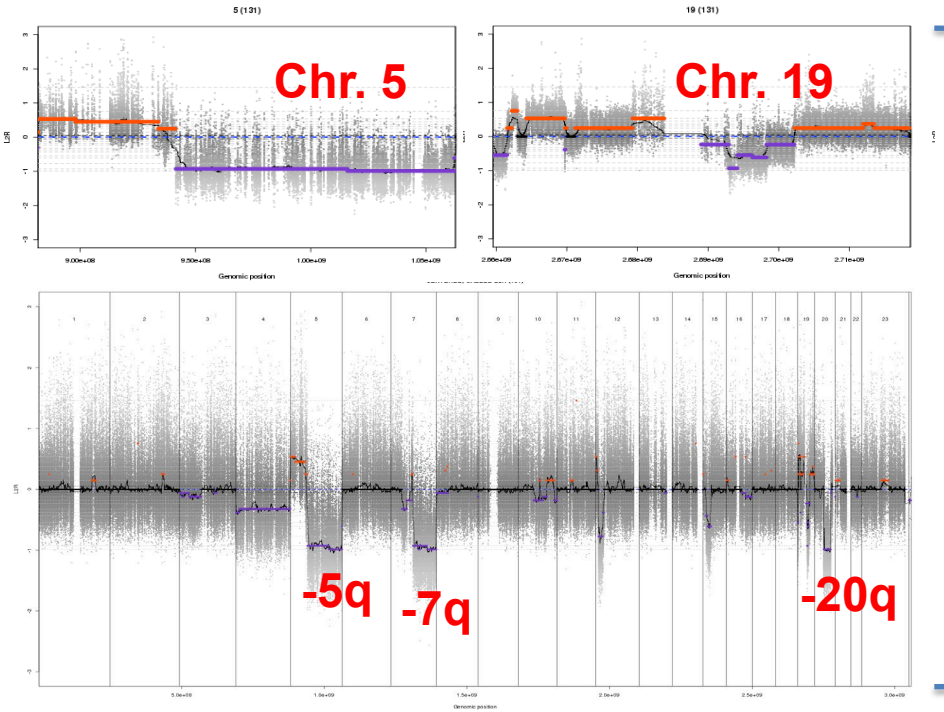
Yurchenko et al., Nature Comm., 2020, **11**, 5834.

When the mutator phenotype is expressed in XP patient?

- Along the all life?
- Only during the carcinogenesis process?

Somatic Copy Number Aberrations in the AML of the XP185VI patient



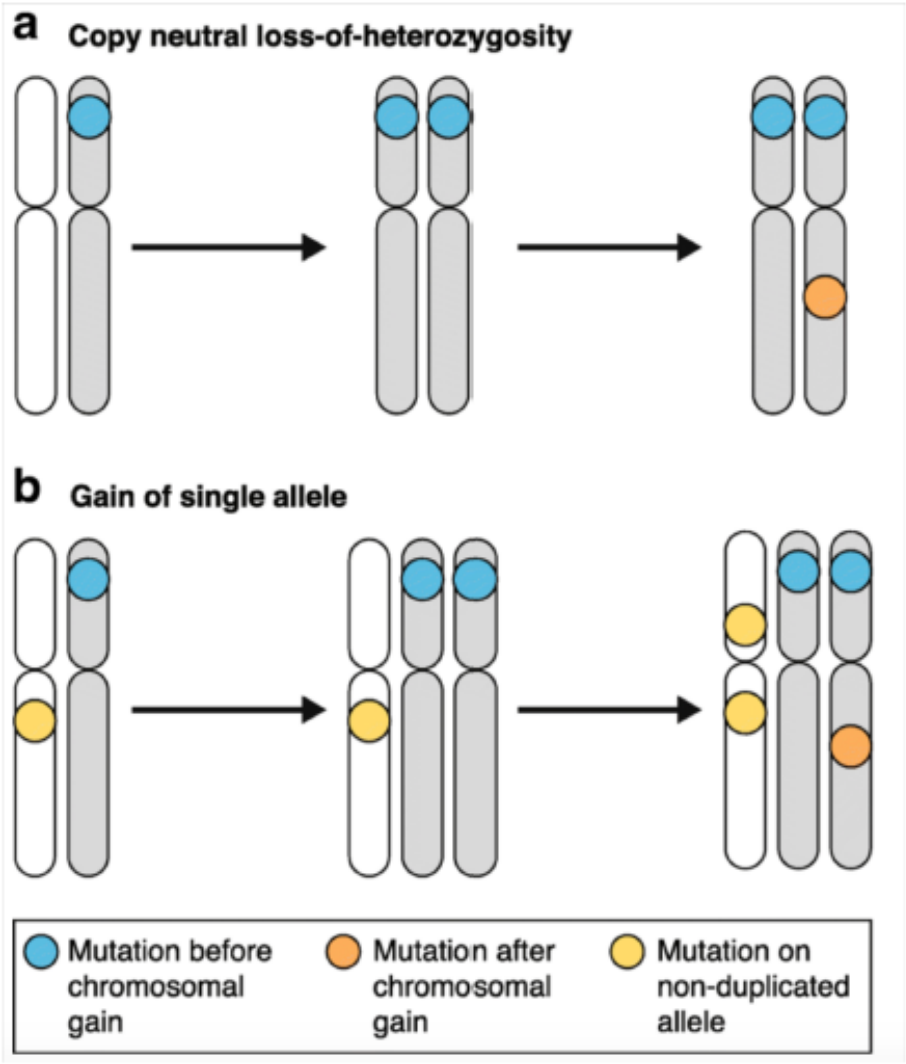


Cytogenetics: -5q, -7q11, add(11)(q21), der(12), -19, -20, -21, …….

Sarasin et al., Blood, 2019, 133, 2718-2724

AS2749 bis

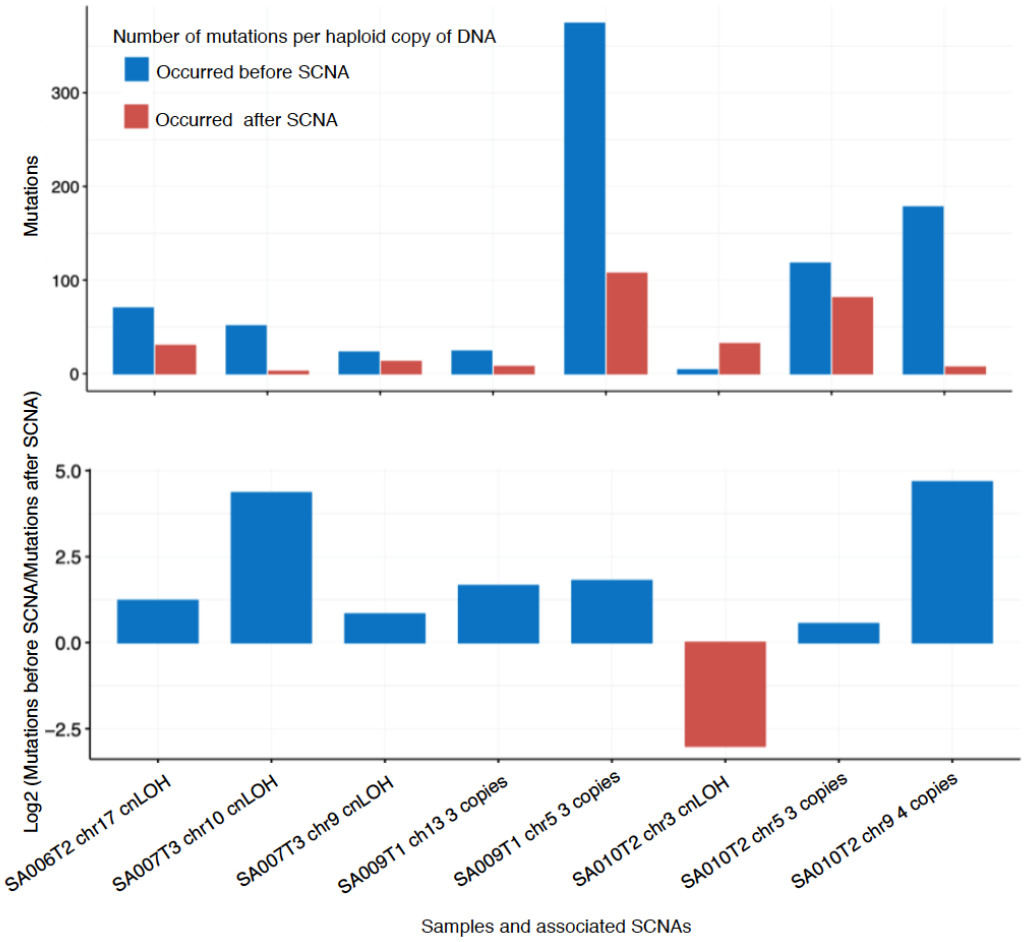
Mutation accumulation before and after tumorigenesis



Jolly and Van Loo 2015

The analysis of mutations that occur on duplicated alleles allows us to determine if the mutations occurred before or after Somatic Copy Number Aberrations

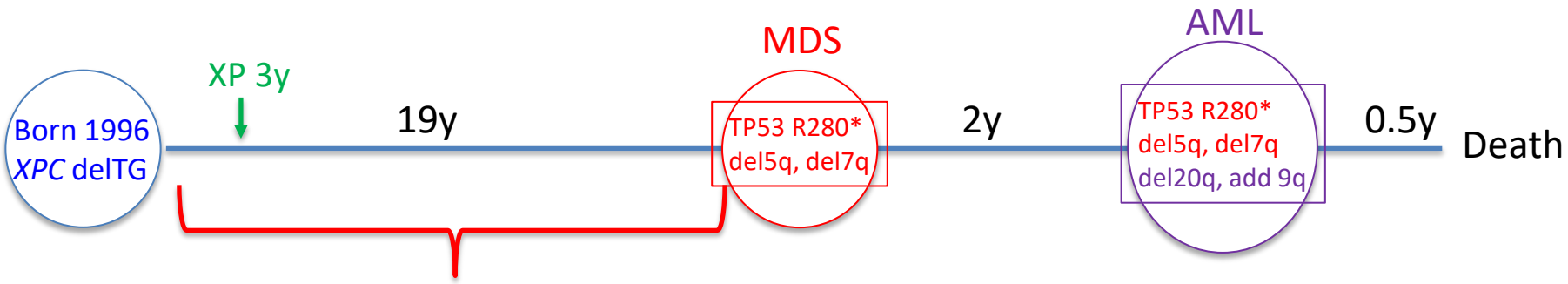
Number of mutations in selected regions of Somatic Copy Number Aberrations where one allele is duplicated



The majority of mutations (75%) occurred before SCNA suggesting they accumulated along the life of the patients or in early tumorigenesis in progenitor blood cells (P=0.04)

Yurchenko et al., Nature Comm., 2020, **11**, 5834.

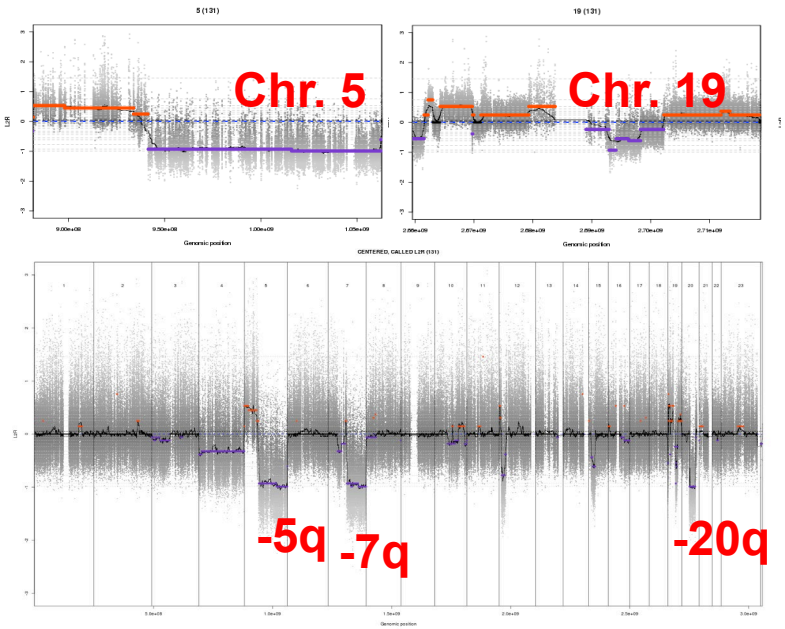
Accumulation of DNA damage and mutations all along the life of XP patients



Most of leukemia in XP resemble
"Secondary therapy-related MDS/AML"
observed in the general population of
patients who develop firstly a cancer,
then are treated by chemo- and/or radio-therapy
and then later develop a MDS and AML:
In both cases, there is accumulation of
DNA damage and mutations with time.

- -TP53 mutations
- -del5q, del7q
- -Complex karyotypes
- -Bad prognosis

Of course, the XP patients were not treated by anti-tumoral drugs before the discovery of the MDS/leukemia!!



Cytogenetics: -5q, -7q11, add(11)(q21), der(12), -19, -20, -21, …….

Sarasin et al., Blood, 2019, 133, 2718-2724

Summary

- XP patients are at high risk of internal tumors
  - Leukemia, CNS, gynecological and thyroid tumors
  - Young XP-C patients are at the highest risk, particularly the delTG patient (France, USA, Brazil)

- We observed a huge excess of mutations in XP leukemia
  - Mutations should accumulate all along the life, at least in some XP patients
  - XP leukemia resemble leukemia in the general population treated by chemo- and/or radio-therapy for a previous cancer

- Transcriptional bias is clearly observed with all types of mutations proportionally to the amount of gene expression as expected for TCR-proficient cells

- Cosmic signature 8 is caused by NER deficiency

- The mutations in internal XP tumors are caused by unrepaired purine-lesions produced by exogenous (food genotoxins) or endogenous genotoxins. These lesions should also be produced in the general population but are repaired by NER

- Endogenous: ROS-induced purine lesions, aldehydes, formaldehydes, cyclopurines?

- Medical doctors following XP patients should be aware of the high risk of internal tumors. Prevention and early detection of brain, gynecological and thyroid tumors, and hematological malignancies are needed

Thanks to

Genetic Stability CNRS-UMR 8200 Oncogenesis

GUSTAVE ROUSSY CANCER CAMPUS GRAND PARIS

Hematology people

Jean Soulier (St Louis hospital, Paris)
Stephane De Botton (IGR, Villejuif)
Eric Solary (IGR)
Thierry Leblanc (Robert Debré hospital, Paris)

Bio informatics

Andrey Yurchenko (IGR)
Sergey Nikolaev (IGR)
Mourad Sahbatou (CEPH, Paris)
Nathalie Droin (sequencing platform IGR)

Cytogenetics/Pathologists

Véronique Saada (IGR)
Nathalie Auger (IGR)

XP Dermatologists

Caroline Robert (IGR)
Alain Taïeb (Bordeaux CHU Hospital)
Smail Hadj-Rabia (Necker hospital, Paris)

Chikako Nishigori (Kobe, Japan)

- The NIH XP cohort (Bethesda, USA)
  - Ken Kraemer, John DiGiovanna, Deborah Tamura, Sikandar Khan, Elizabeth Rizza
- The Brazilian connection (University of Sao Paulo)
  - Carlos Menck
  - Ligia Castro
  - Maria-Isabel Achatz
- The Comorian and African Connection
  - François Cartault (La Réunion)
  - Mahlatse Kgokolo (South Africa)
  - Cécile Ged (Bordeaux)
- XP French Association des “Enfants de la Lune”

UNIVERSITÉ PARIS SUD

Enfants de la Lune
Association pour le Xeroderma Pigmentosum