Molecular basis of canine histiocytic sarcoma in dogs:

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Histiocytic Sarcoma (HS)

In human

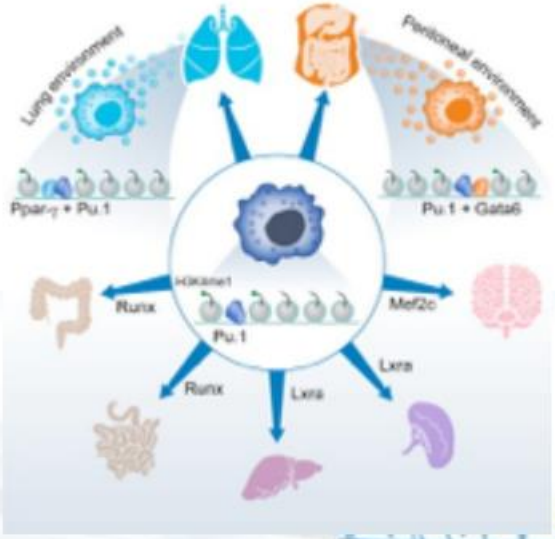
(Credit : J Donadieu)

In dogs

tumor from histiocytic cells :
monocyte/macrophage or dendritic cells

localized or disseminated tumors:
joint, skin, spleen, liver, lymph node, lung

limited response to chemotherapy



Benoît Hedan 2022

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- half of « secondary » cases

- No consensus on prognostic factors and on standard treatment

- rare in whole population but frequent in predisposed breeds (BMD, Rottweilers, Retrievers)

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=> unique model to identify genetic bases of this cancer

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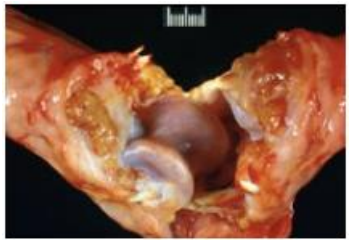
Clinical presentations

in dogs

localized HS (LHS):
skin/joint



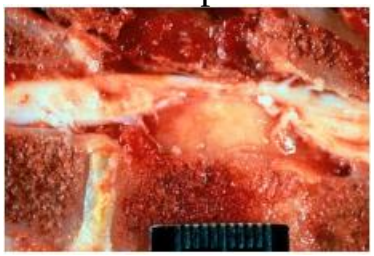
www.histiocytosis.ucdavis.edu/sarcoma.html

Retrievers

disseminated HS (DHS)

BMD

subdural HS:
Cerebrum/spinal cord



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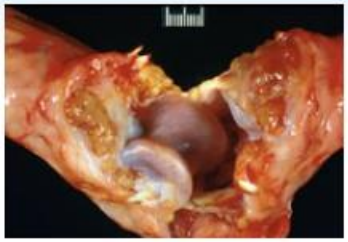
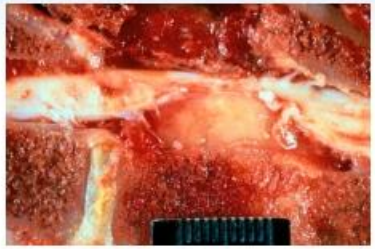
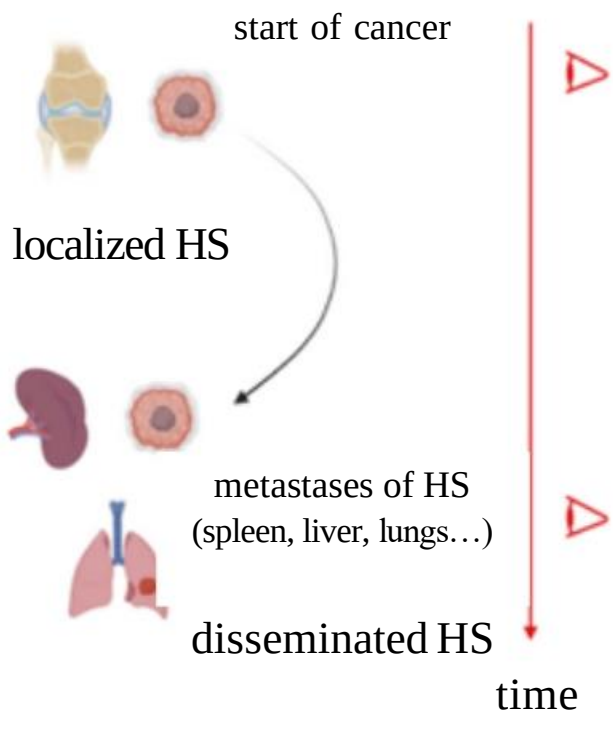
Pembroke Welsh Corgi

hypotheses
regarding these different clinical presentations

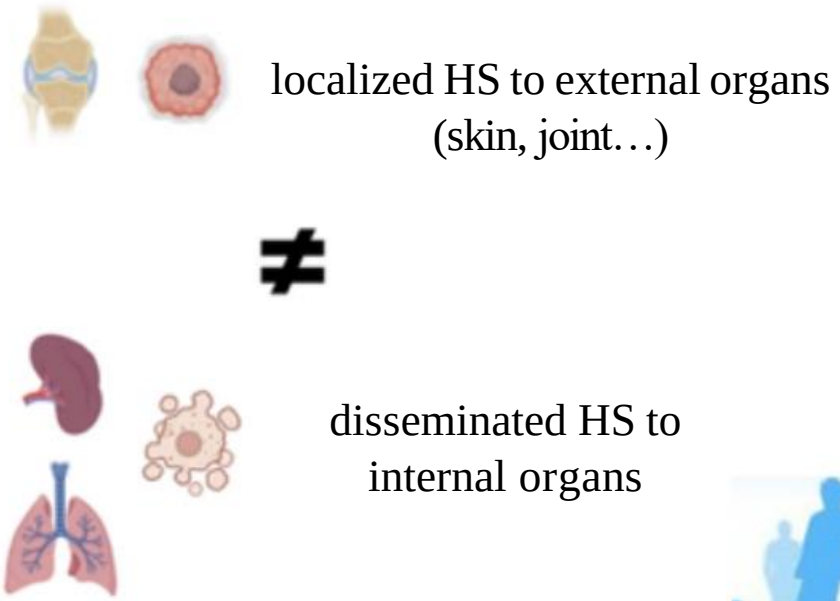




Hypothesis 1: same cancers observed at different time

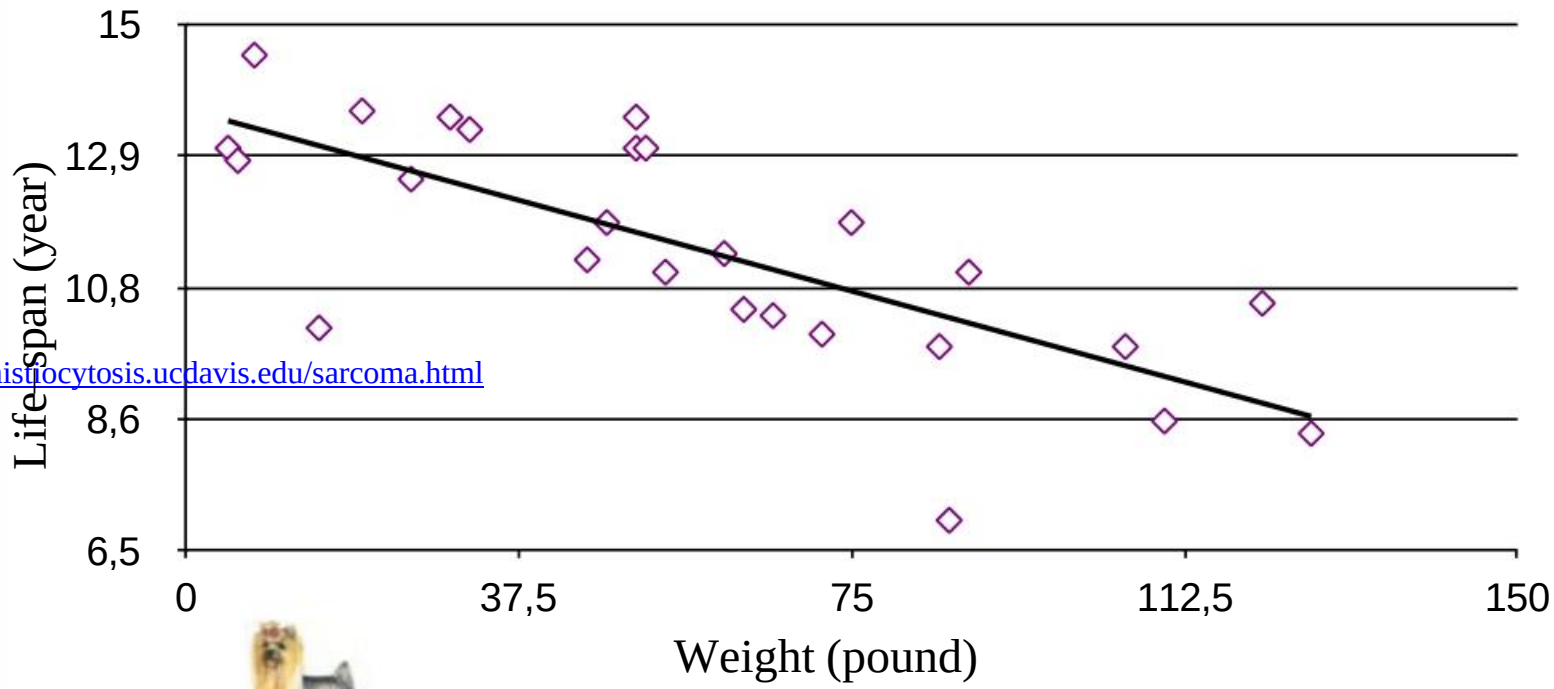


Hypothesis 2: different subtypes of Histiocytic cells



Clinical presentations

in dogs



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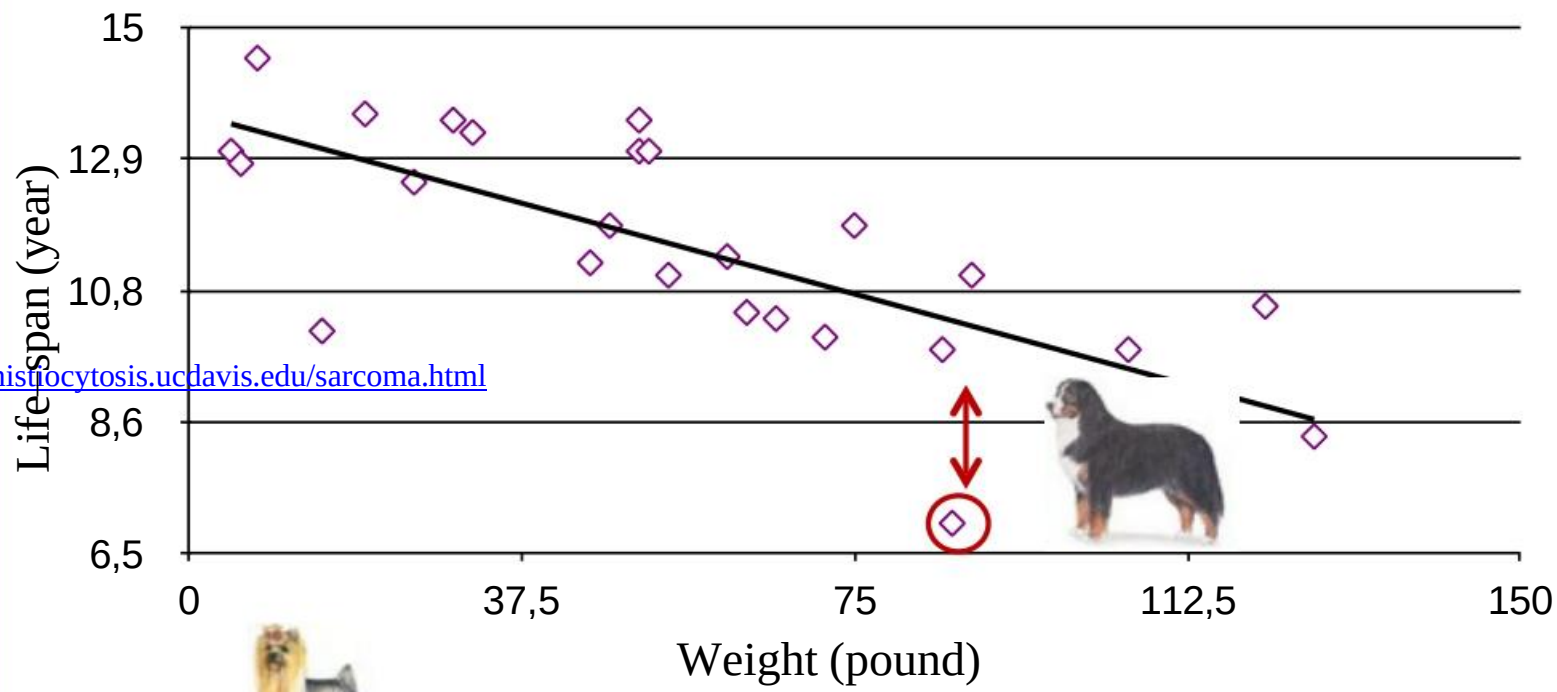

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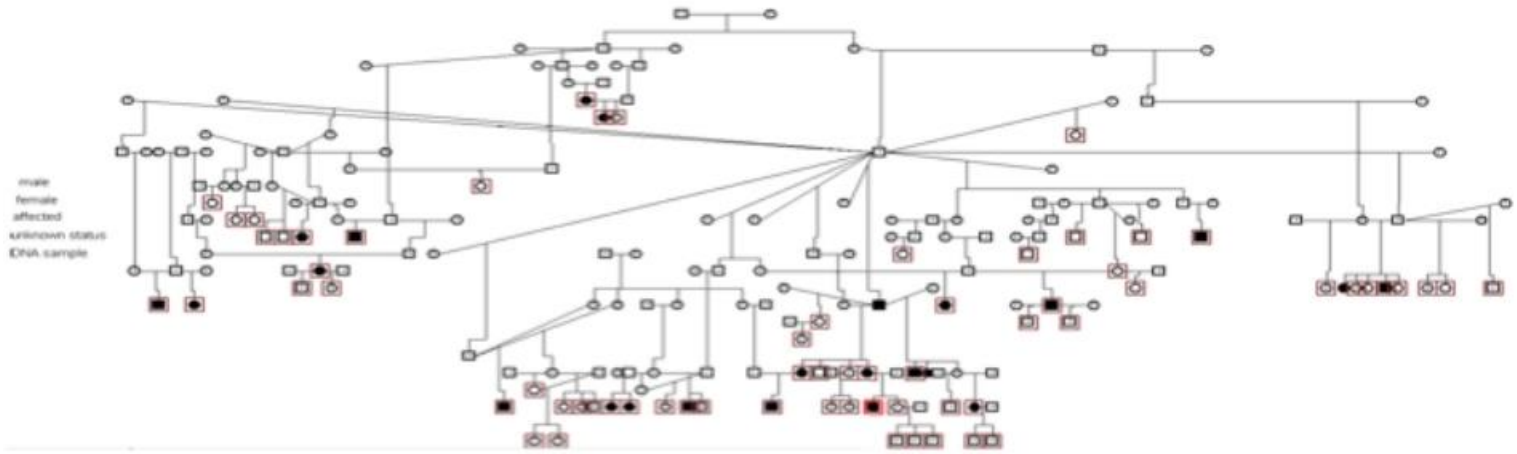
How explain these breed predispositions?

Breed practices could increase transmission of risk alleles:

- Breed = isolated population <-> reduced diversity

- Consanguinity and sire effect increase frequency of inherited diseases



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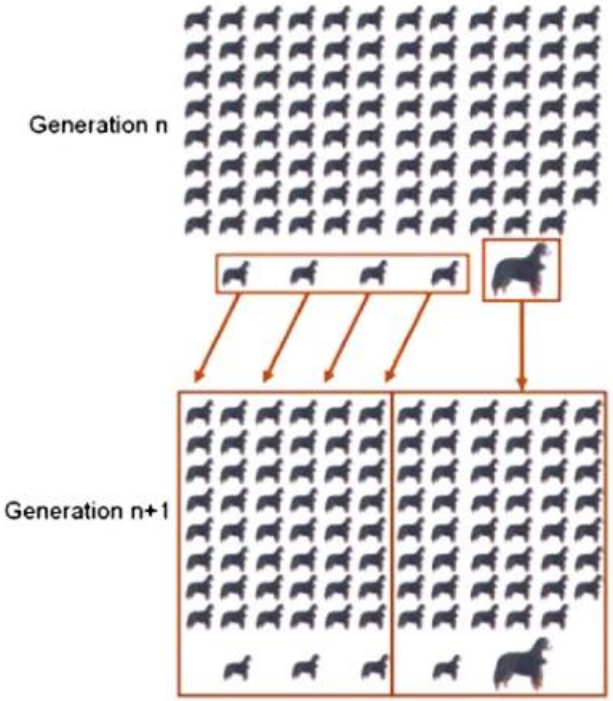
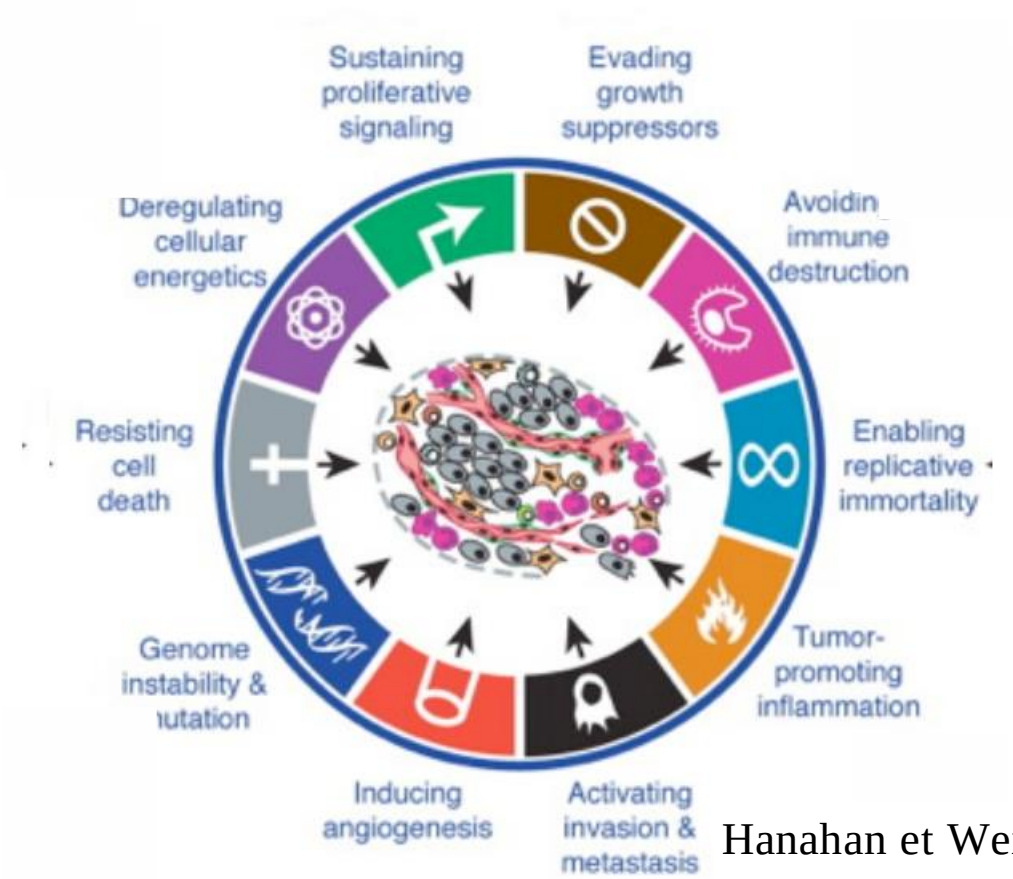


Abadie, Hedan et al. 2009

A long term goal :
Identify genetic mechanisms involved in HS

Cancer = genetic disease



Hanahan et Weinberg. Cell. 2011

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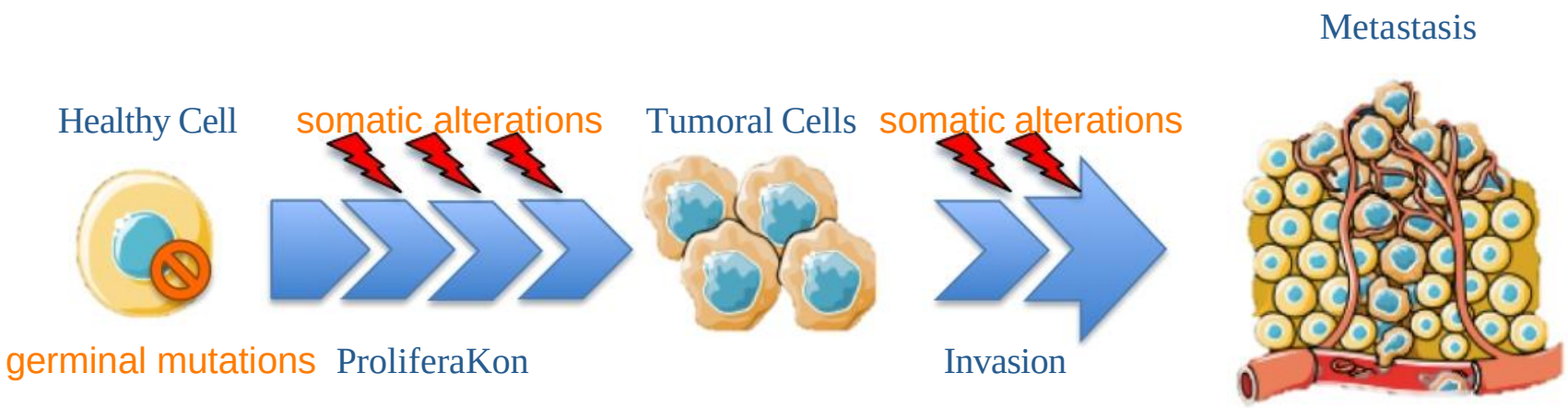
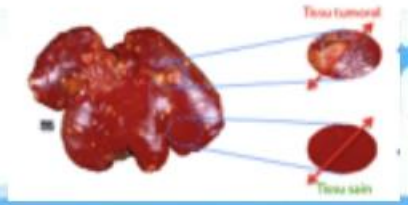


- Identify predisposing genes and risk alleles

-> Blood collection

- Identify recurrent somatic alterations associated with tumor progression

-> Tissue collection



Looking for predisposing genes: Research project

➢ Since 2002 - Cani-DNA biobank

- > 4000 blood samples and > 400 tissue samples
- > 450 HS cases with histopathological report

➢ Characterization of the Disease:
Abadie, Hedan et al. 2009

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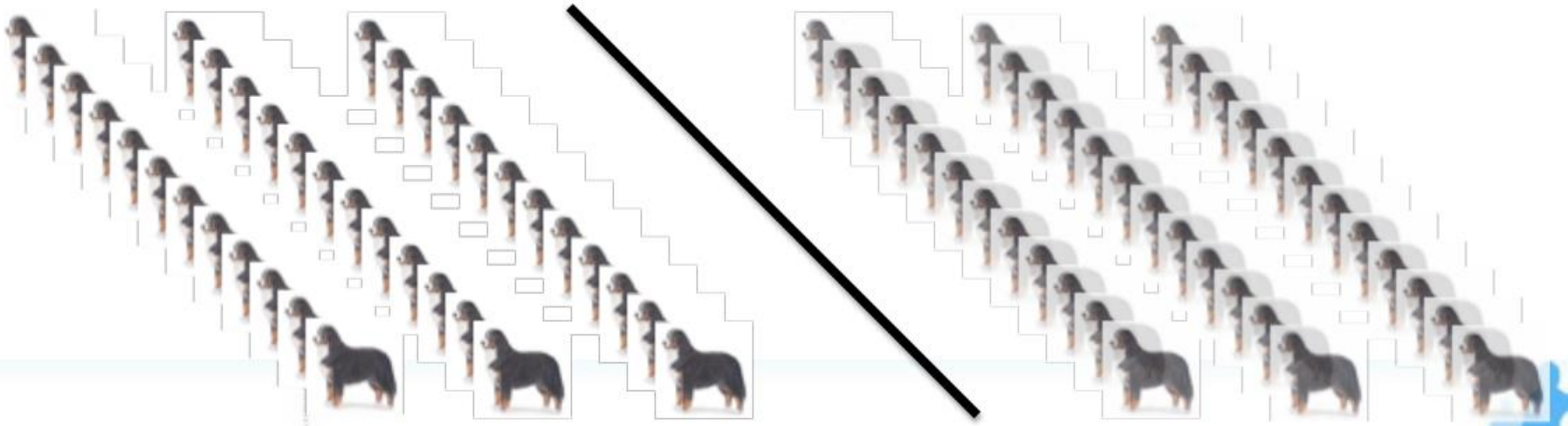
GWAS: 232 unaffected and 244 affected BMDs (American/European)



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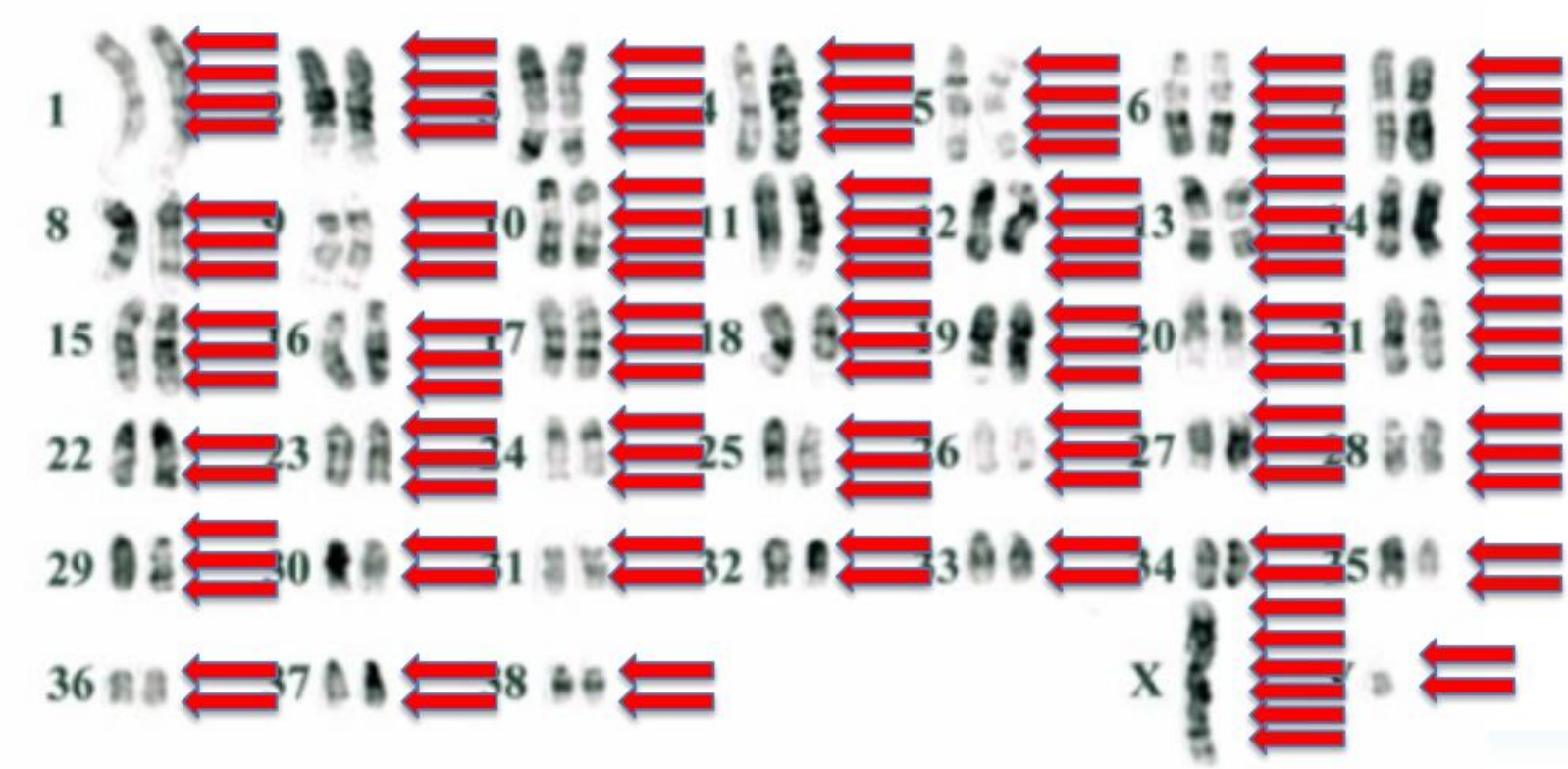

comparaison of 22 000 markers

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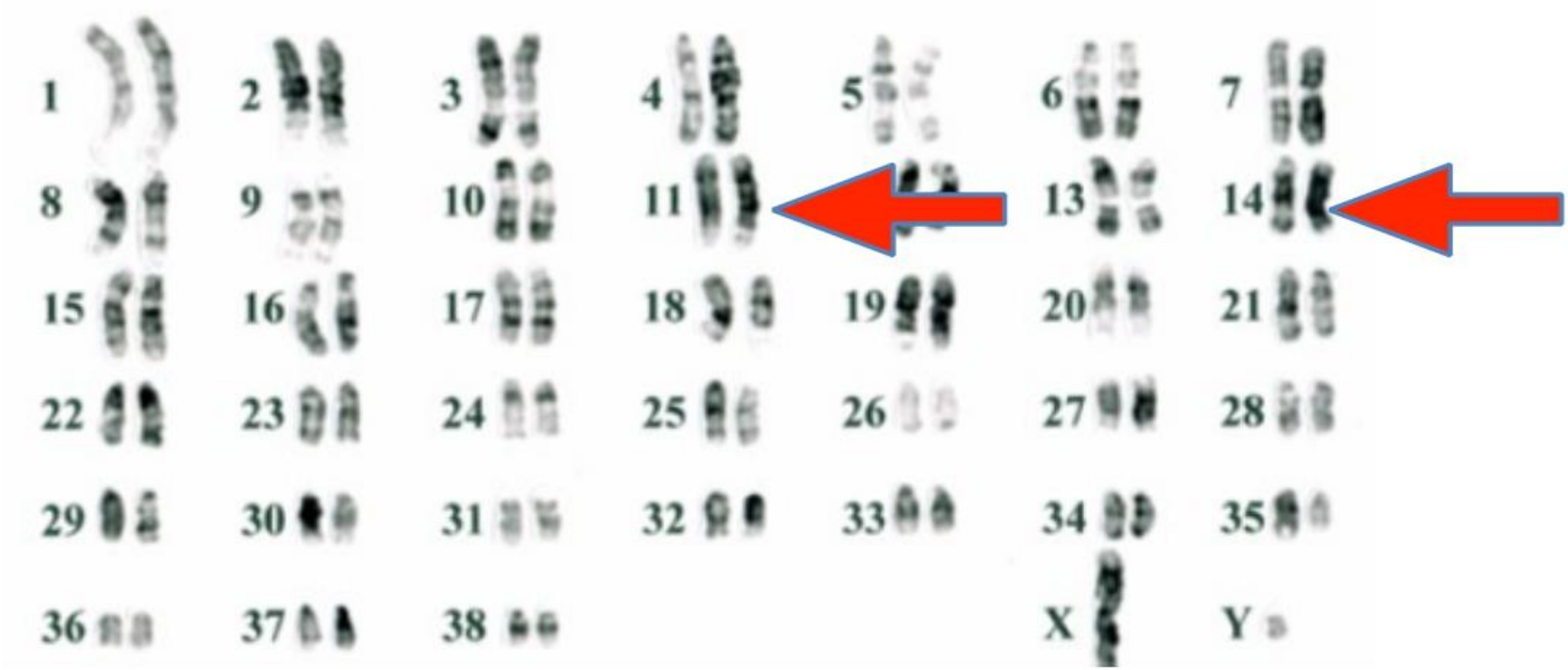

Markers differs between unaffected and affected BMDs

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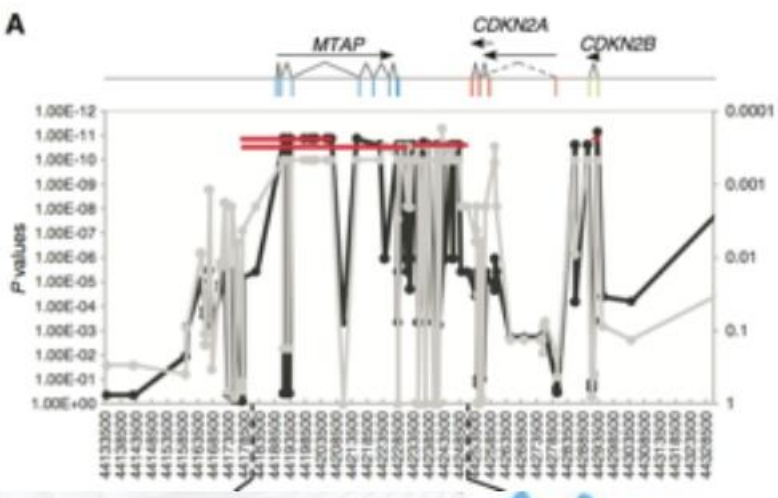
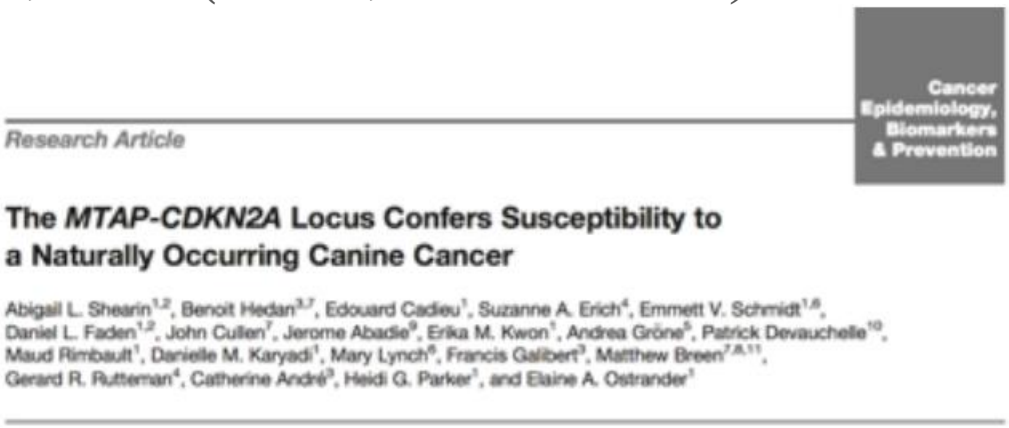
-> Identification of main loci : CFA11, CFA14 *(Shearin, Hedan et al. 2012)*





Research Article

Cancer Epidemiology, Biomarkers & Prevention

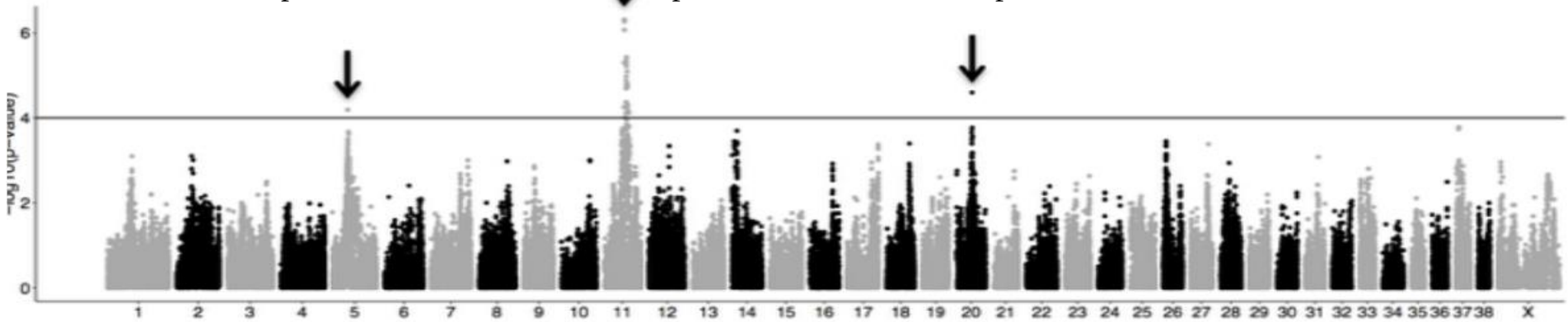
The *MTAP-CDKN2A* Locus Confers Susceptibility to a Naturally Occurring Canine Cancer

Abigail L. Shearin[1,2], Benoit Hedan[3,7], Edouard Cadieu[1], Suzanne A. Erich[4], Emmett V. Schmidt[1,6], Daniel L. Faden[1,2], John Cullen[7], Jerome Abadie[9], Erika M. Kwon[1], Andrea Gröne[5], Patrick Devauchelle[10], Maud Rimbault[1], Danielle M. Karyadi[1], Mary Lynch[8], Francis Galibert[3], Matthew Breen[7,8,11], Gerard R. Rutteman[4], Catherine André[3], Heidi G. Parker[1], and Elaine A. Ostrander[1]

Looking for predisposing genes: Research project

➢ New GWAS (Illumina SNP array 170K) 2011-2012: 154 unaffected and 172 affected BMDs

$p_{corrected}$: 6.4 x 10^{-5} $p_{corrected}$: 4.8 x 10^{-7} $p_{corrected}$: 2.5 x 10^{-5}



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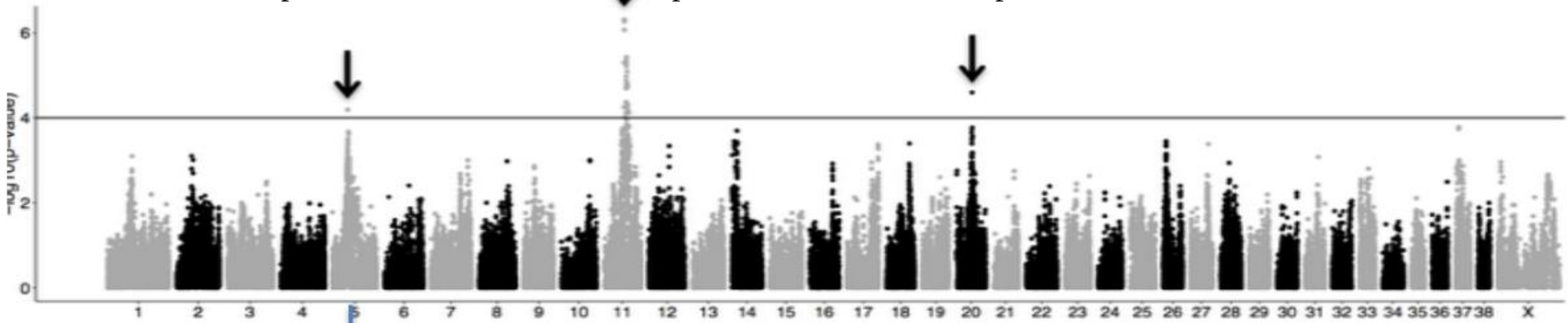

region involved in lymphoma and hemangiosarcoma predisposition (Tonomura et al. 2015)

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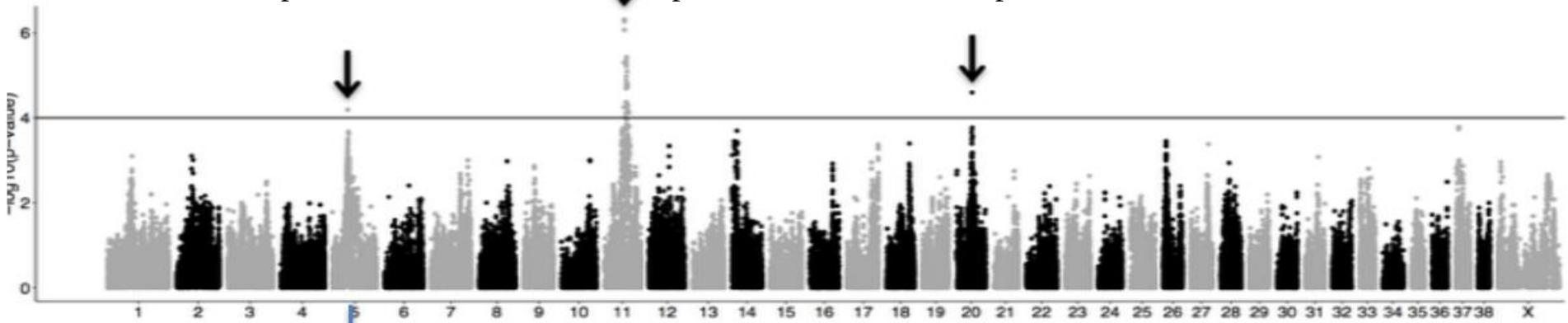

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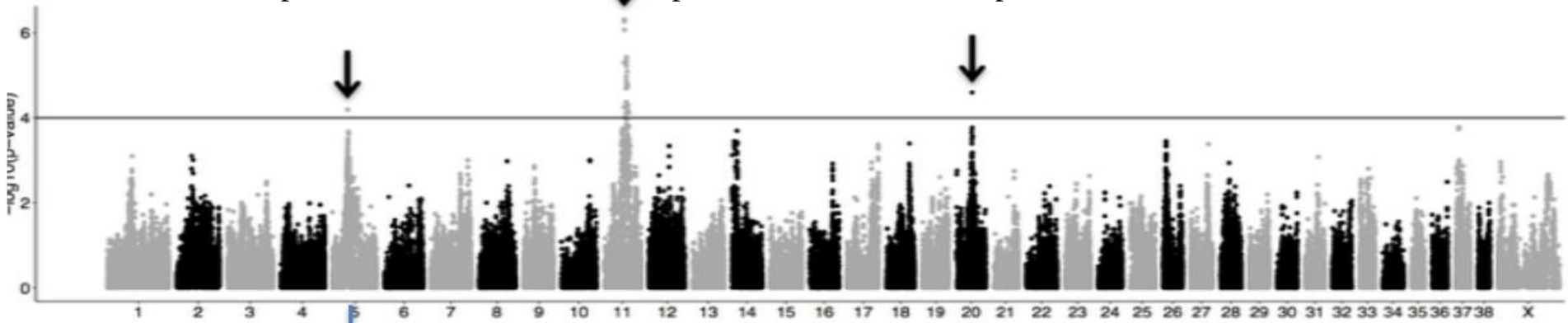

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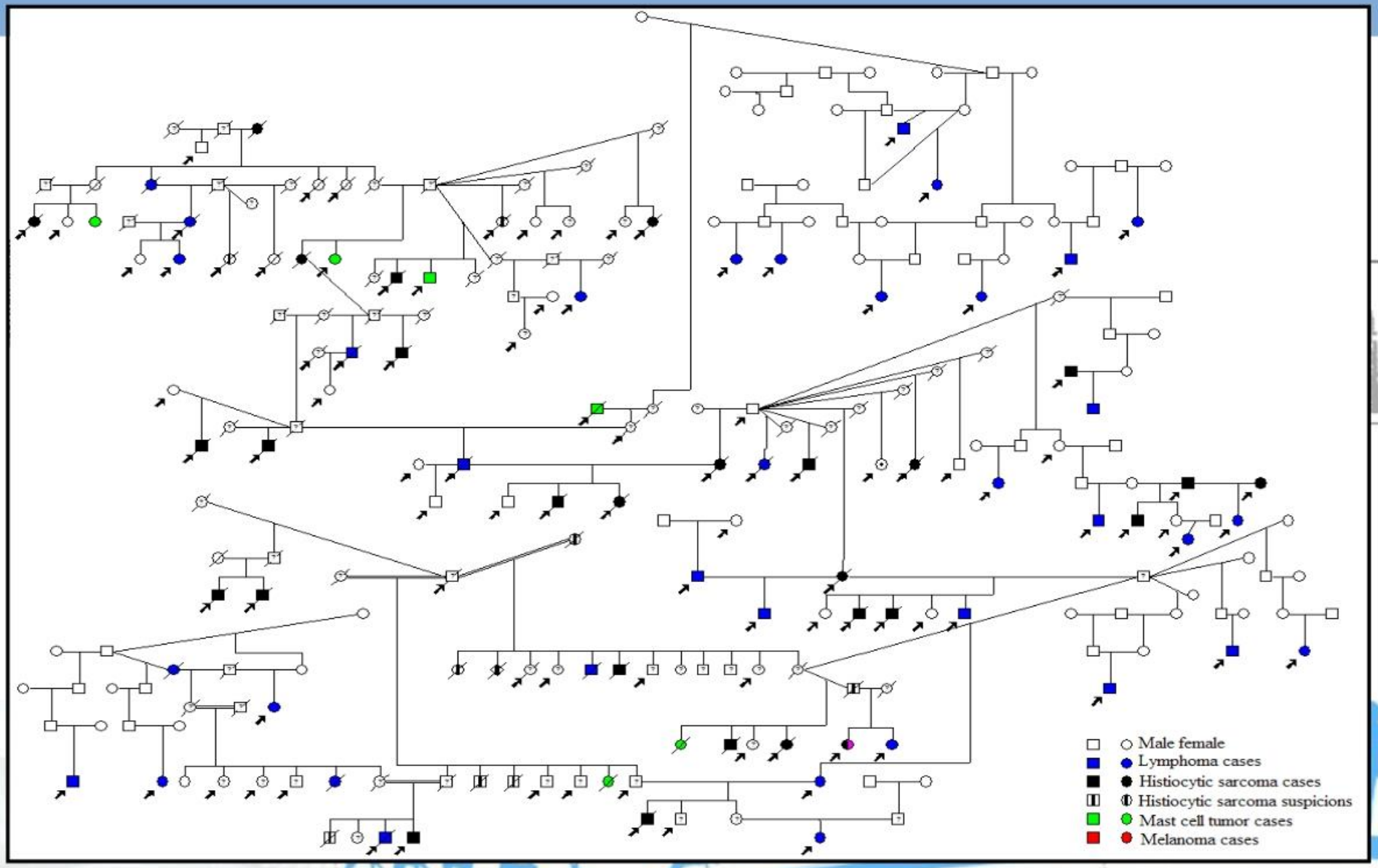

Benoit Hedan 2022

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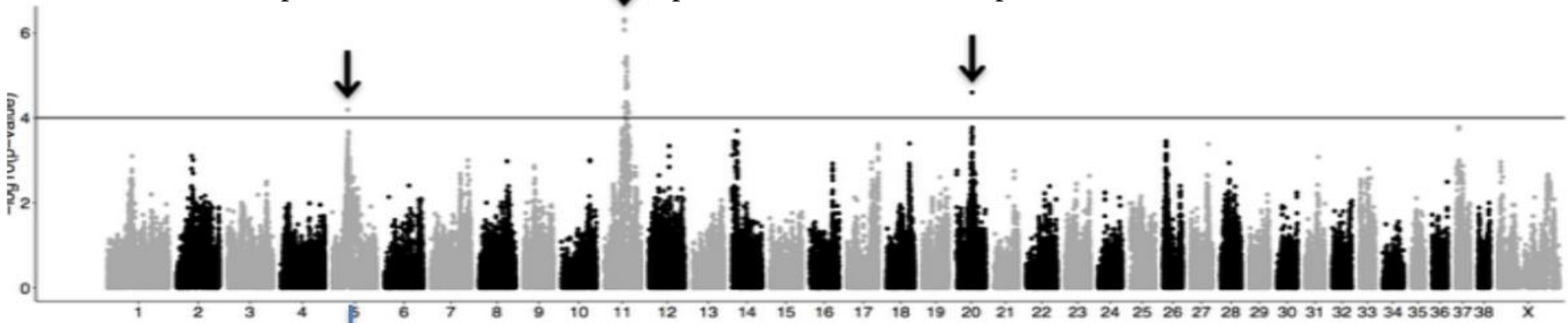

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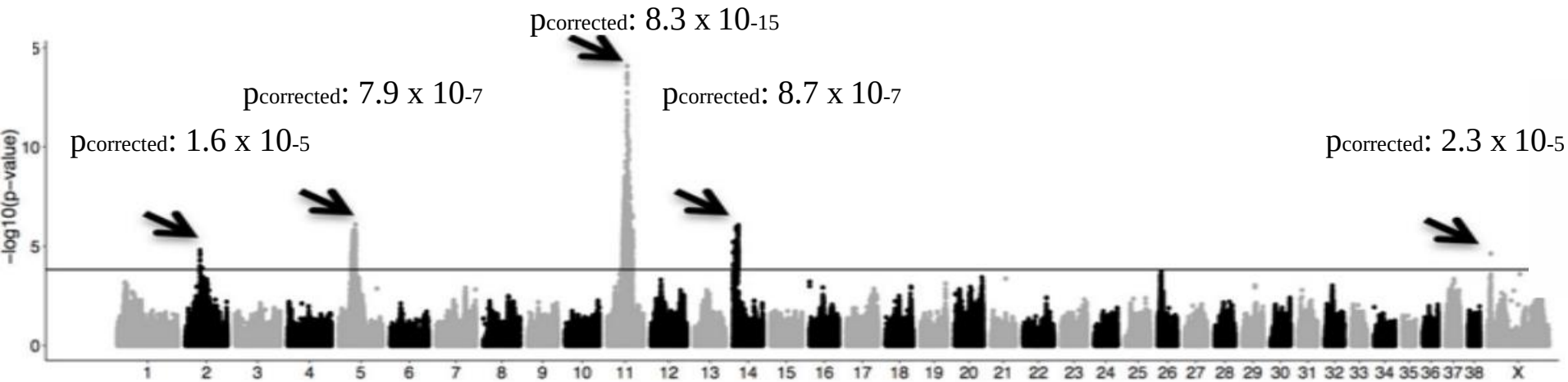
Looking for predisposing genes: Research project

➢ New GWAS on 3 predisposed breeds (BMD, Rottweiler, Flat coated retriever) 455 affected and 408 unaffected dogs.



3 main regions on CFA 5, 11 and 14 :

- the risk haplotype of CFA5 is shared between 3 breeds
- the risk haplotype of CFA11 is shared between BMDs and Rottweilers
- the protective haplotype of CFA14 is shared between 3 breeds

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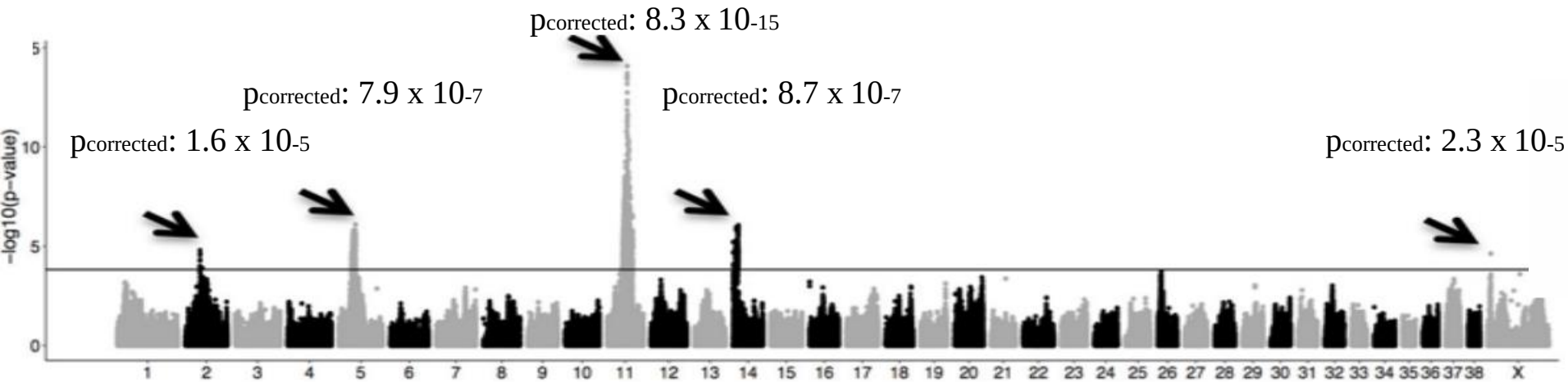

Risk of developing HS is linked to accumulation of risk alleles of these 3 main regions (CFA5, CFA11, CFA14)

frequencies of risk alleles in the 3 breeds		
number of risk alleles	affected	unaffected
≥5 risk alleles	0,7	0,27
4 risk alleles	0,23	0,34
≤3 risk alleles	0,07	0,29

Odds Ratio		
Odds Ratio	interval	pval
5.41	[4.04-7.24]	1.14x10-31
0.5	[0.37-0.67]	2.74x10-6
0.15	[0.1-0.23]	2.17x10-21

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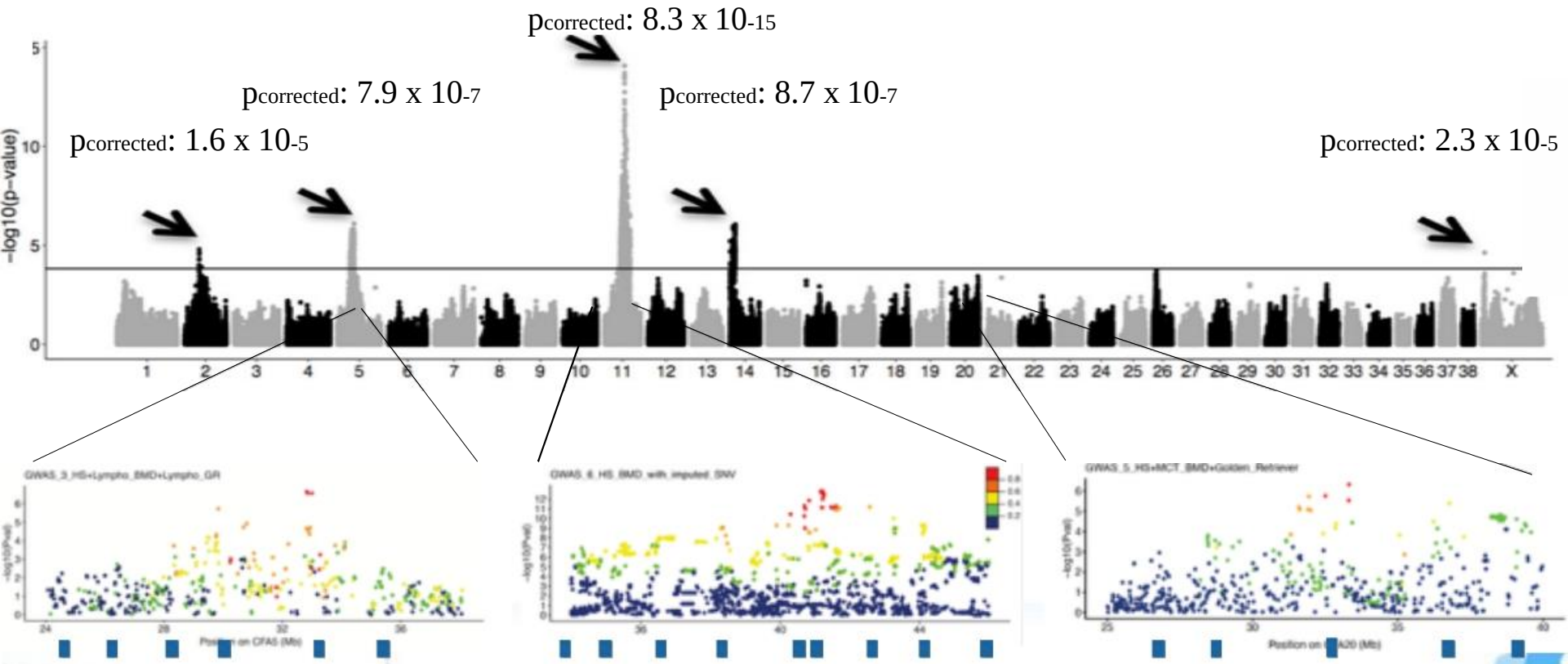

Predisposition -> accumulation of many regions and subregions

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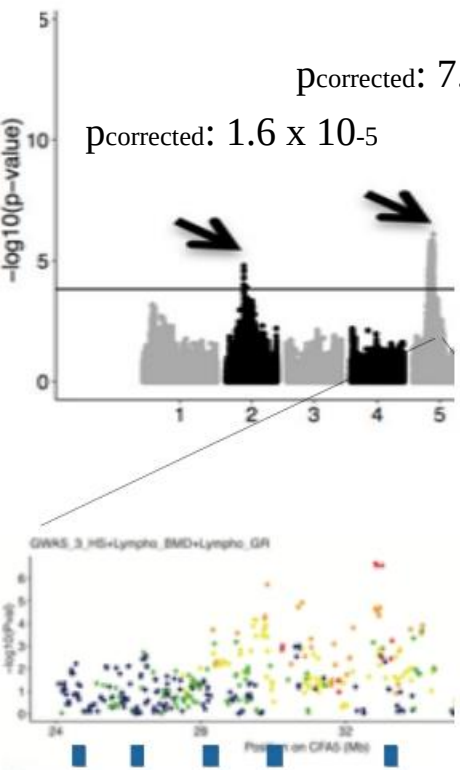

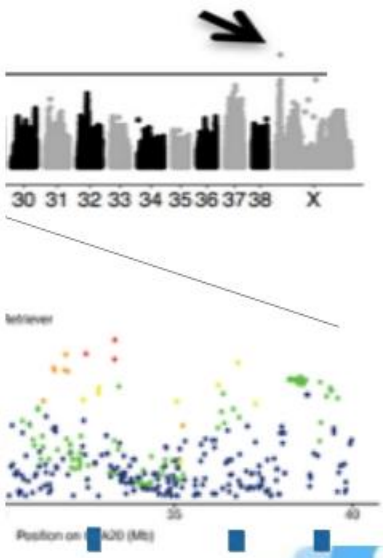

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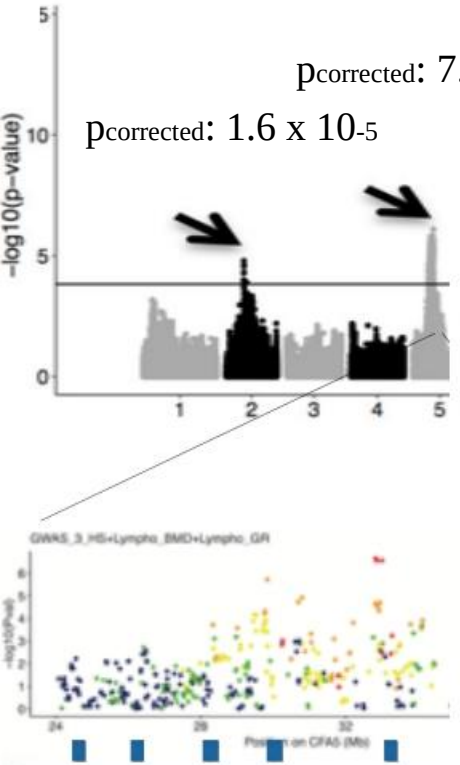

PLOS GENETICS

Multi-omics approach identifies germline regulatory variants associated with hematopoietic malignancies in retriever dog breeds

Jacquelyn M. Evans[1], Heidi G. Parker[1], Gerard R. Rutteman[2], Jocelyn Plassais[1], Guy C. M. Grinwis[3], Alexander C. Harris[1], Susan E. Lana[4], Elaine A. Ostrander[1]*

1 Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, United States of America, 2 Department of Clinical Sciences, division Internal Medicine of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands, 3 Department Biomedical Health Sciences, division Pathology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands, 4 College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, United States of America

*postrand@mail.nih.gov

OPEN ACCESS

Citation: Evans JM, Parker HG, Rutteman GR, Plassais J, Grinwis GCM, Harris AC, et al. (2021) Multi-omics approach identifies germline regulatory variants associated with hematopoietic malignancies in retriever dog breeds. PLoS Genet 17(5): e1009543. https://doi.org/10.1371/journal.pgen.1009543

Editor: William Hendricks, Translational Genomics Research Institute, UNITED STATES

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Data Availability Statement: SRA accession numbers for WGS are in S8 Table. RNA-seq and ChIP-seq data are in SRA (PRJNA685036). SNP chip data are in GEO (GSE163784). Remaining relevant data are within the manuscript and Supporting Information files.

Funding: This work was supported by the Intramural Program of the National Human Genome Research Institute at NIH (https://www.genome.gov/) with partial support from the NCI.

Abstract

Histiocytic sarcoma is an aggressive hematopoietic malignancy of mature tissue histiocytes with a poorly understood etiology in humans. A histologically and clinically similar counterpart affects flat-coated retrievers (FCRs) at unusually high frequency, with 20% developing the lethal disease. The similar clinical presentation combined with the closed population structure of dogs, leading to high genetic homogeneity, makes dogs an excellent model for genetic studies of cancer susceptibility. To determine the genetic risk factors underlying histiocytic sarcoma in FCRs, we conducted multiple genome-wide association studies (GWASs), identifying two loci that confer significant risk on canine chromosomes (CFA) 5 ($P_{meta2} = 4.83 \times 10^{-15}$) and 19 ($P_{meta2} = 2.25 \times 10^{-7}$). We subsequently undertook a multi-omics approach that has been largely unexplored in the canine model to interrogate these regions, generating whole genome, transcriptome, and chromatin immunoprecipitation sequencing. These data highlight the PI3K pathway gene *PIK3R6* on CFA5, and proximal candidate regulatory variants that are strongly associated with histiocytic sarcoma and predicted to impact transcription factor binding. The CFA5 association colocalizes with susceptibility loci for two hematopoietic malignancies, hemangiosarcoma and B-cell lymphoma, in the closely related golden retriever breed, revealing the risk contribution this single locus makes to multiple hematological cancers. By comparison, the CFA19 locus is unique to the FCR and harbors risk alleles associated with upregulation of *TNFAIP6*, which itself affects cell migration and metastasis. Together, these loci explain ~35% of disease risk, an exceptionally high value that demonstrates the advantages of domestic dogs for complex trait mapping and genetic studies of cancer susceptibility.

PLOS Genetics | https://doi.org/10.1371/journal.pgen.1009543 May 13, 2021 1 / 20

Predisposition -> accumulation of many regions and subregions

Looking for predisposing genes: Research project

➢ Work in progress to identify variants associated with HS predisposition

➢ Cancers impact BMD life span

➢ Cancer : polyfactorial disease -> involving genetic and environmental factors

➢ Many regions are involved in HS predisposition of which CFA11, CFA5, CFA14

> Needs of research to better understand predisposing mutations and interactions

BUT data produced by research could be useful for breeders to help selection!

How these data could be useful for breeders?

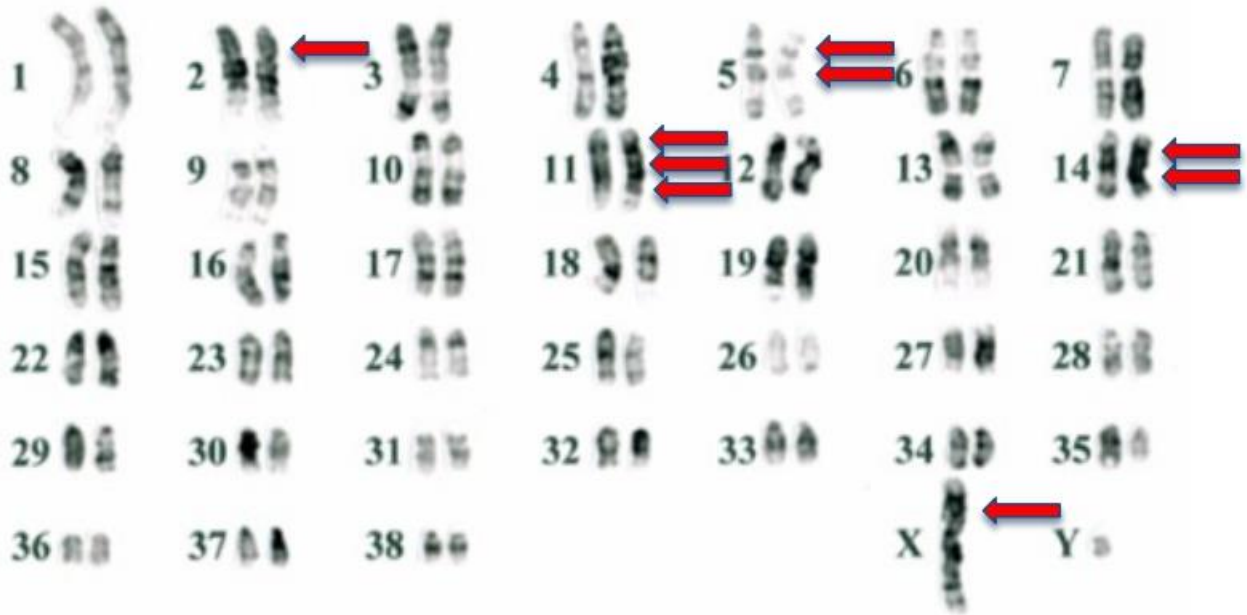
In 2012 , in collaboration with Antagene , we decided to develop a genetic risk test

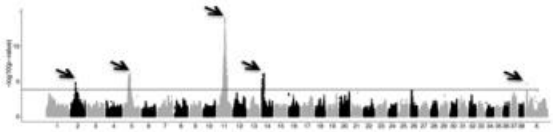
- Based on our first GWAS data, selection of 140 markers associated with HS

- Genotyping of 1081 French BMDs (256 affected dogs + 165 unaffected dogs)

  -> selection of the 9 markers more significantly associated with risk to develop HS

In 2012
How these data could be useful for breeders?





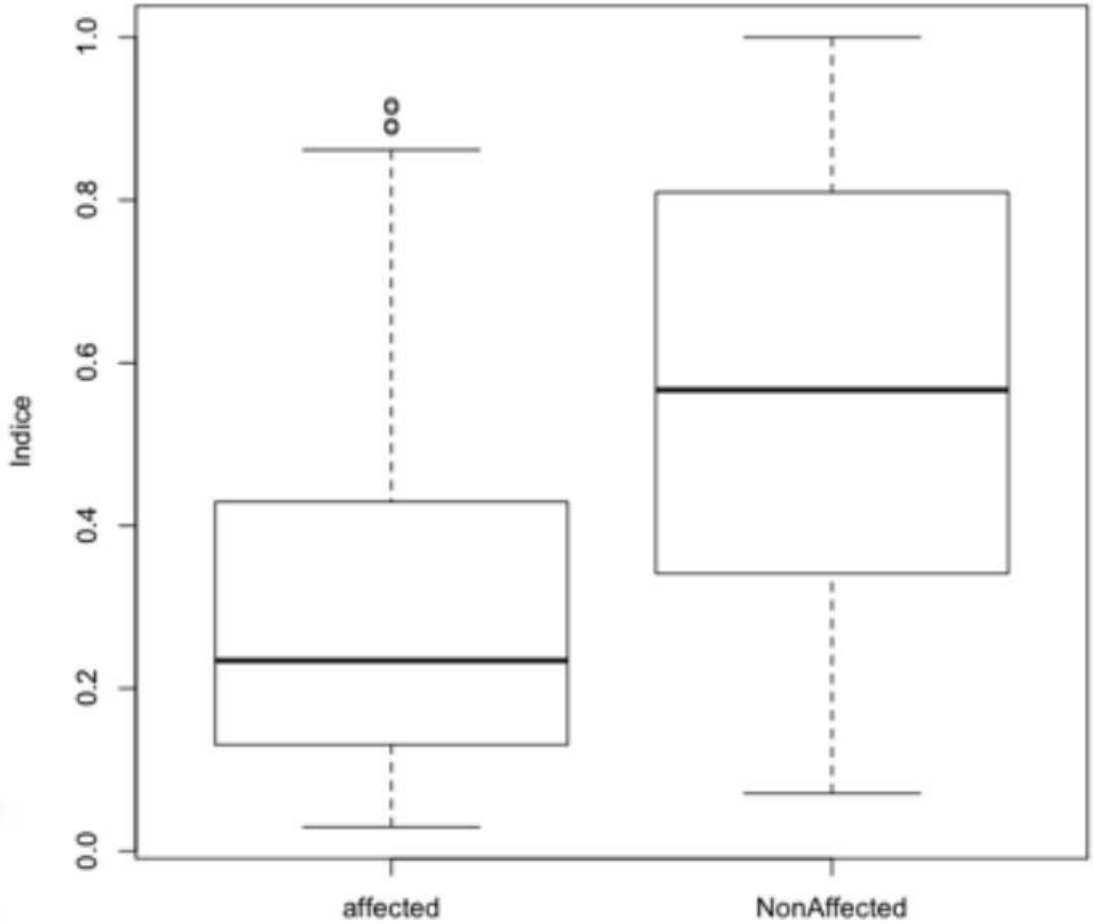
➢ Estimation of risk to be unaffected (GLM model) :

$$\text{Risk} = \frac{\exp\left(\sum_{1}^{n} (\alpha_i \, SNP_i)\right)}{1+\exp\left(\sum (\alpha_i \, SNP_i)\right)}$$

Index

Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

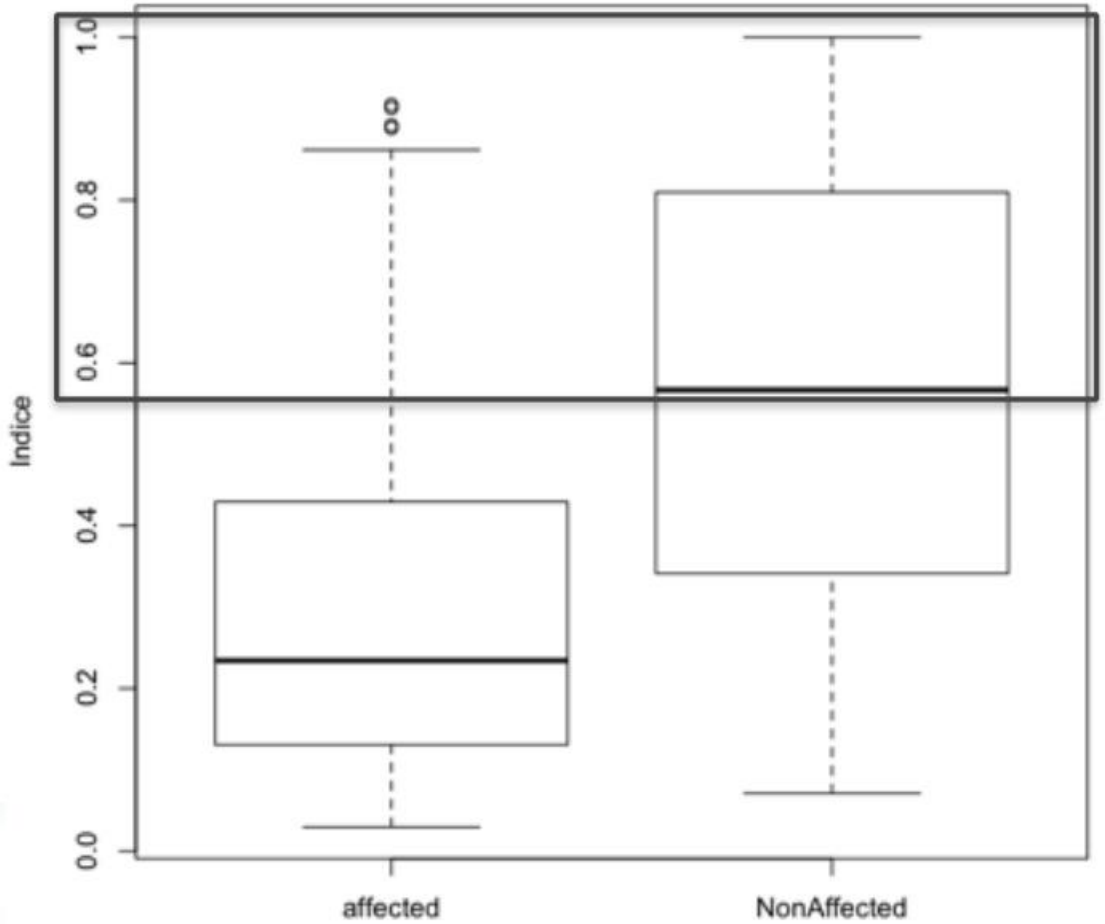


p-value : 1.754 10-16 (Mann Whitney test)

Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

Category A



47 % of healthy
10% of affected

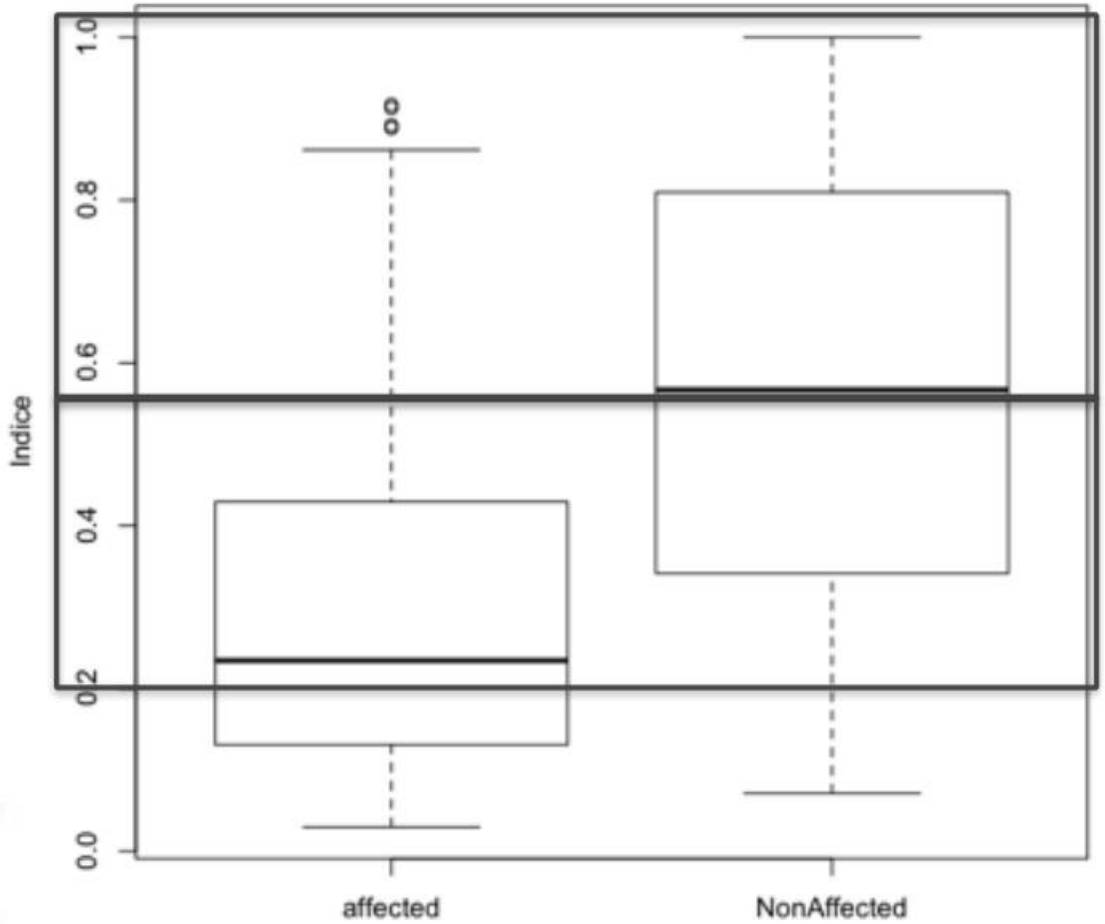
p-value : 1.754 10-16 (Mann Whitney test)

Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

Category A

Category B



47 % of healthy
10% of affected

43 % of healthy
50% of affected

p-value : 1.754 10-16 (Mann Whitney test)

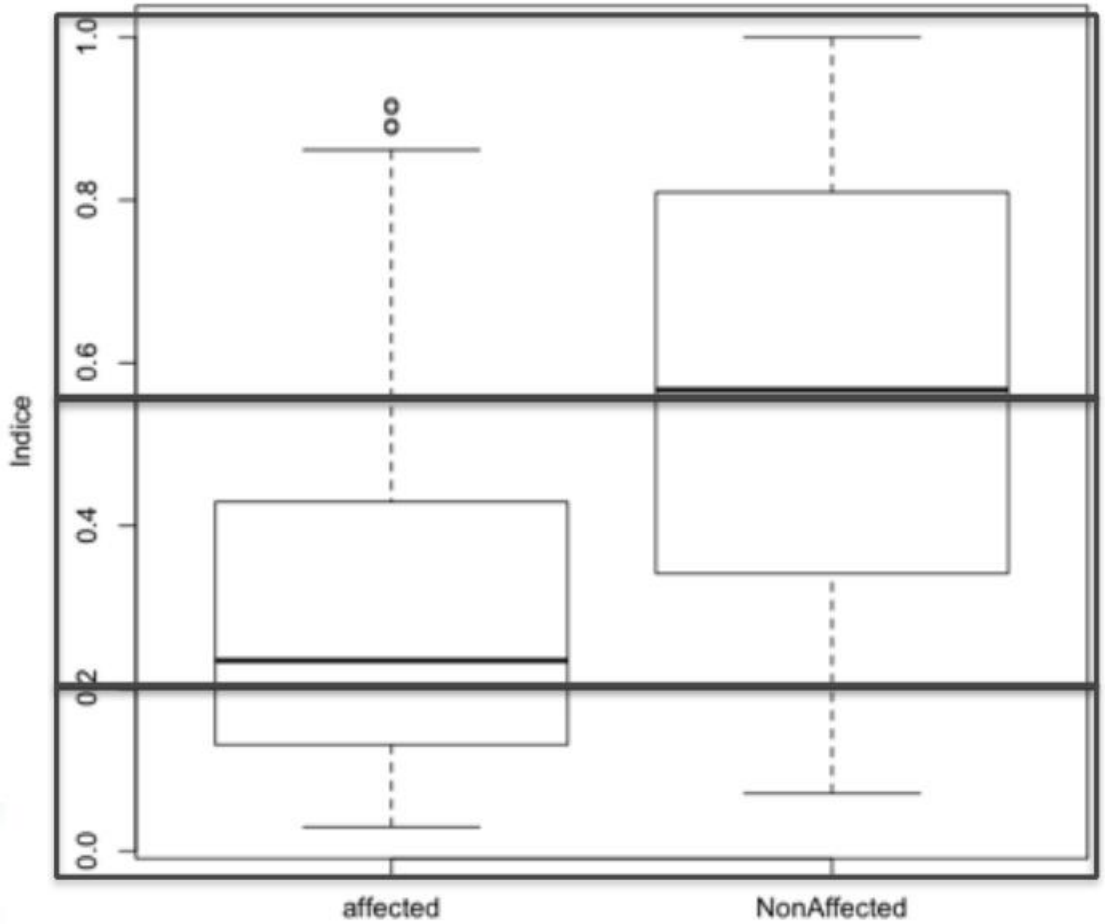
Index development

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

Category A

Category B

Category C



47 % of healthy
10% of affected

43 % of healthy
50% of affected

10 % of healthy
40% of affected

p-value : 1.754 10-16 (Mann Whitney test)

Index Validation

In collaboration with the European breeder clubs and the BernerGarde (Dr V Yuzbasiyan-Gurkan). In total, **718** dogs tested for development and validation of the pre-test

- 415 dogs with Histiocytic Sarcoma
- 303 unaffected dogs (> 10 years)

	A	B	C
Affected	***14%***	***43.1%***	***42.9%***
Healthy	***41.5%***	***46.5%***	***11.9%***

- **Statistics :** 88% p-value : 2.1 10-24 (Xi2 test)

Index A to detect healthy dogs :

Specificity ie **probability that an affected dog is not A** : **86%**

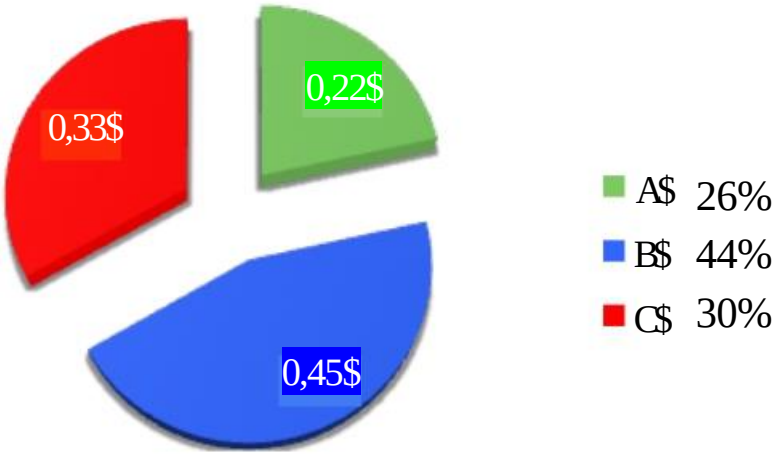
Index C to detect affected dogs :

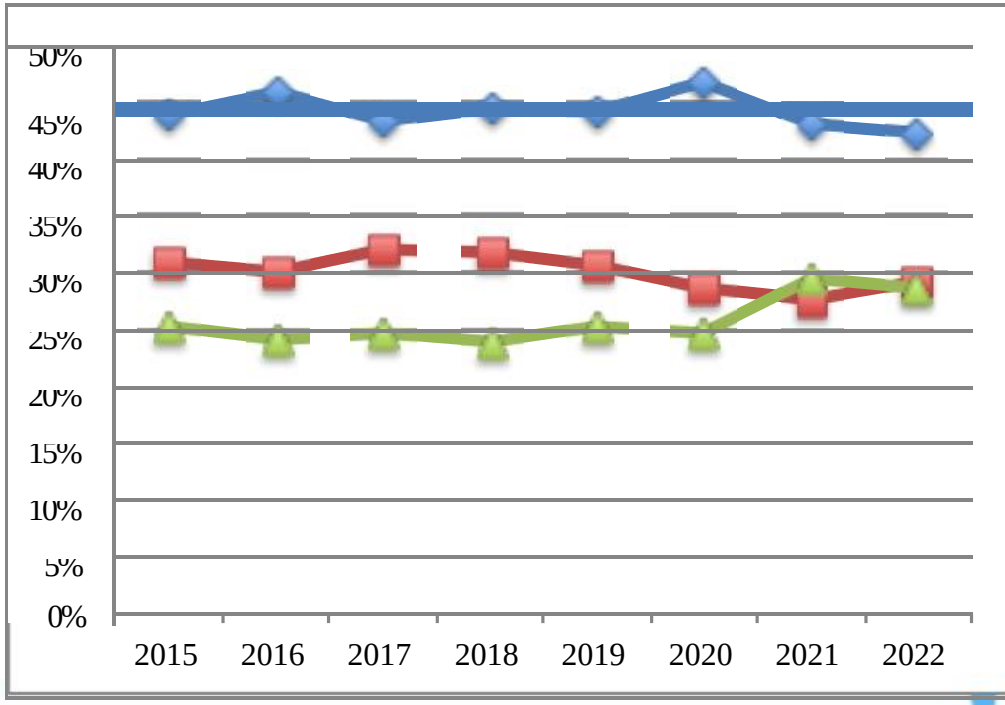
Specificity ie **probability that a healthy dog is not C** : **88%**

Summary of HS tests performed by Antagene

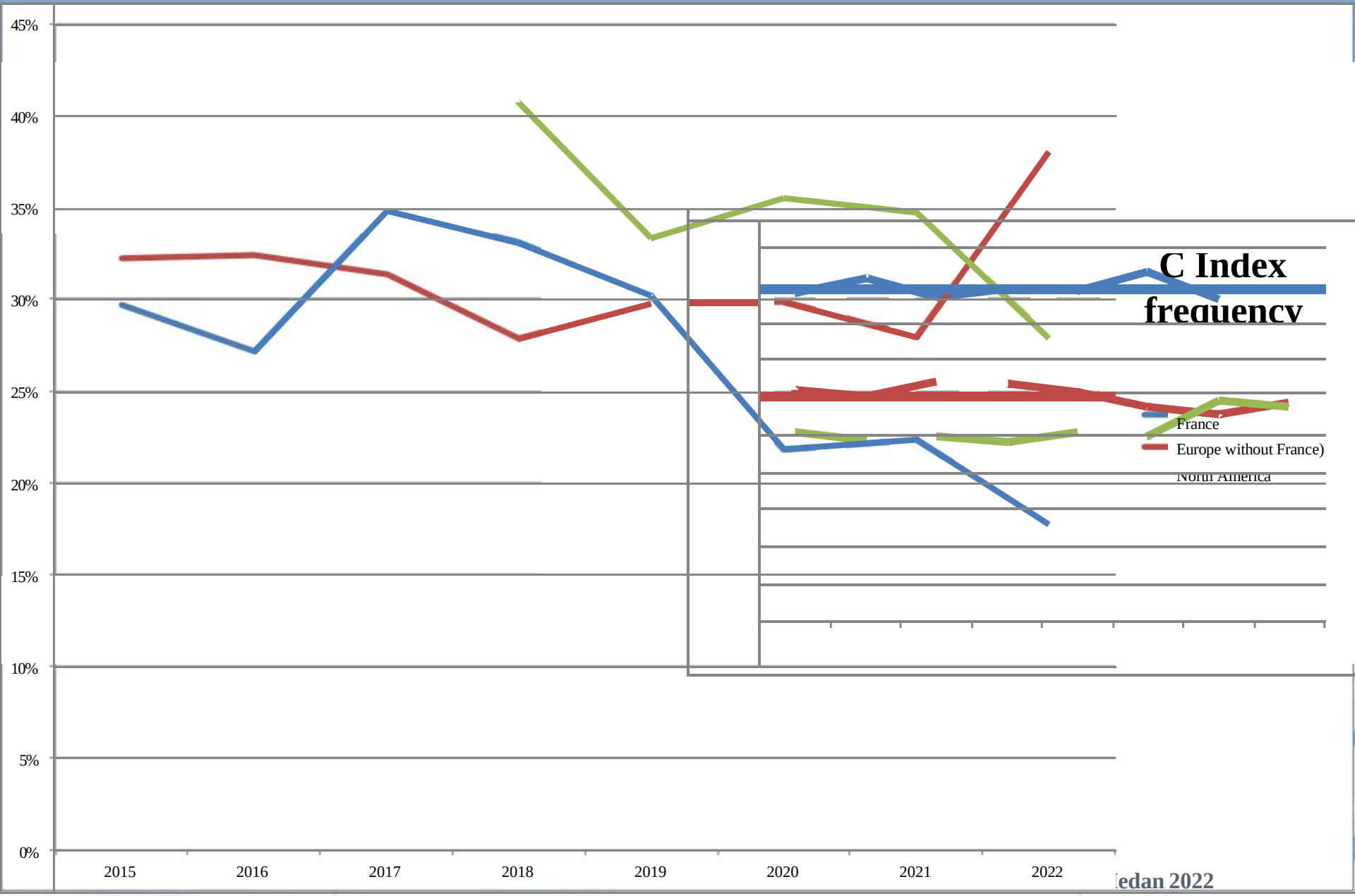
Since 2012 , Antagene has tested >6700 dogs (unknown phenotypes):

distribution of index in this population:





Summary of HS tests HS by Antagene



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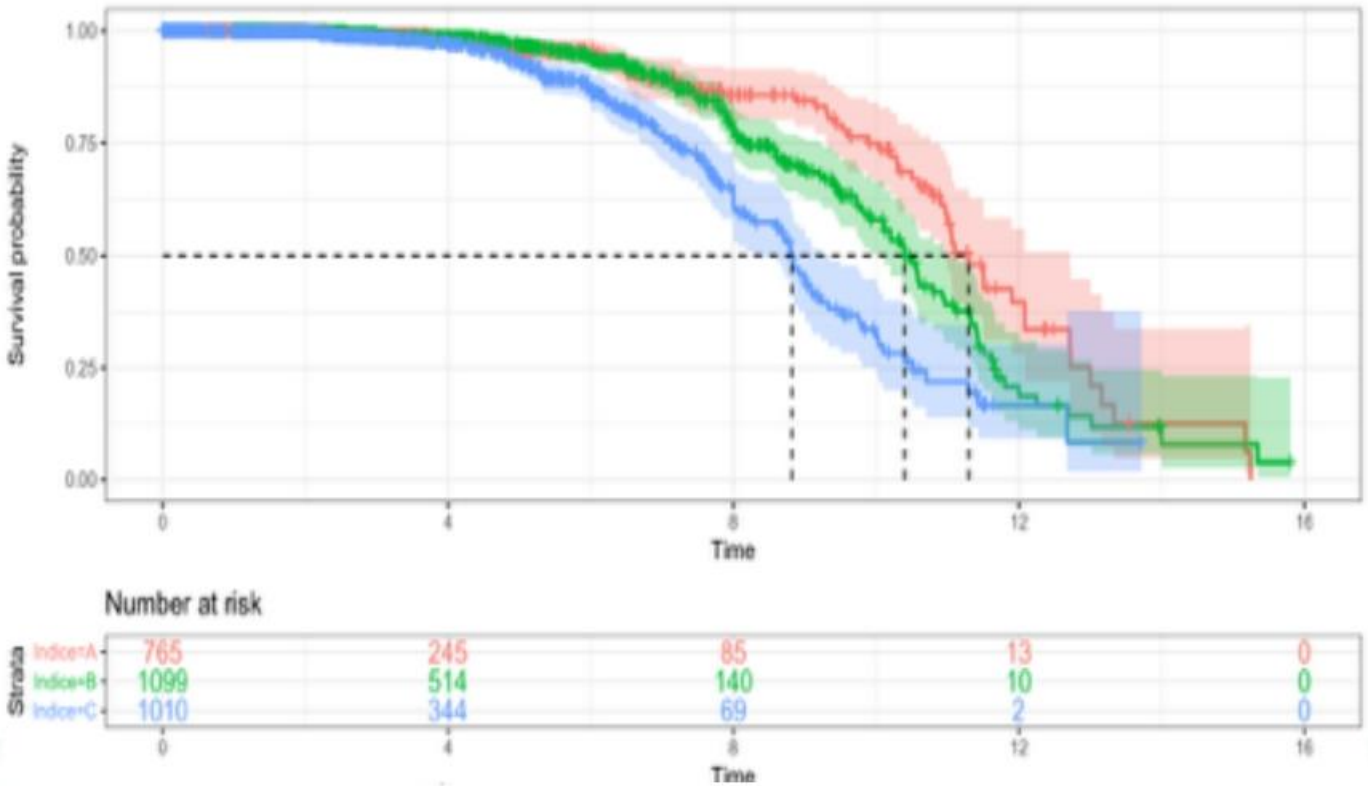

strata	mean	median
A	11.1	11.3
B	10.3	10.4
C	9	8.8

pvalue= 4x10-10

-> on the whole population, even with unknown cause of death, we observe significant differences of lifespan according to index

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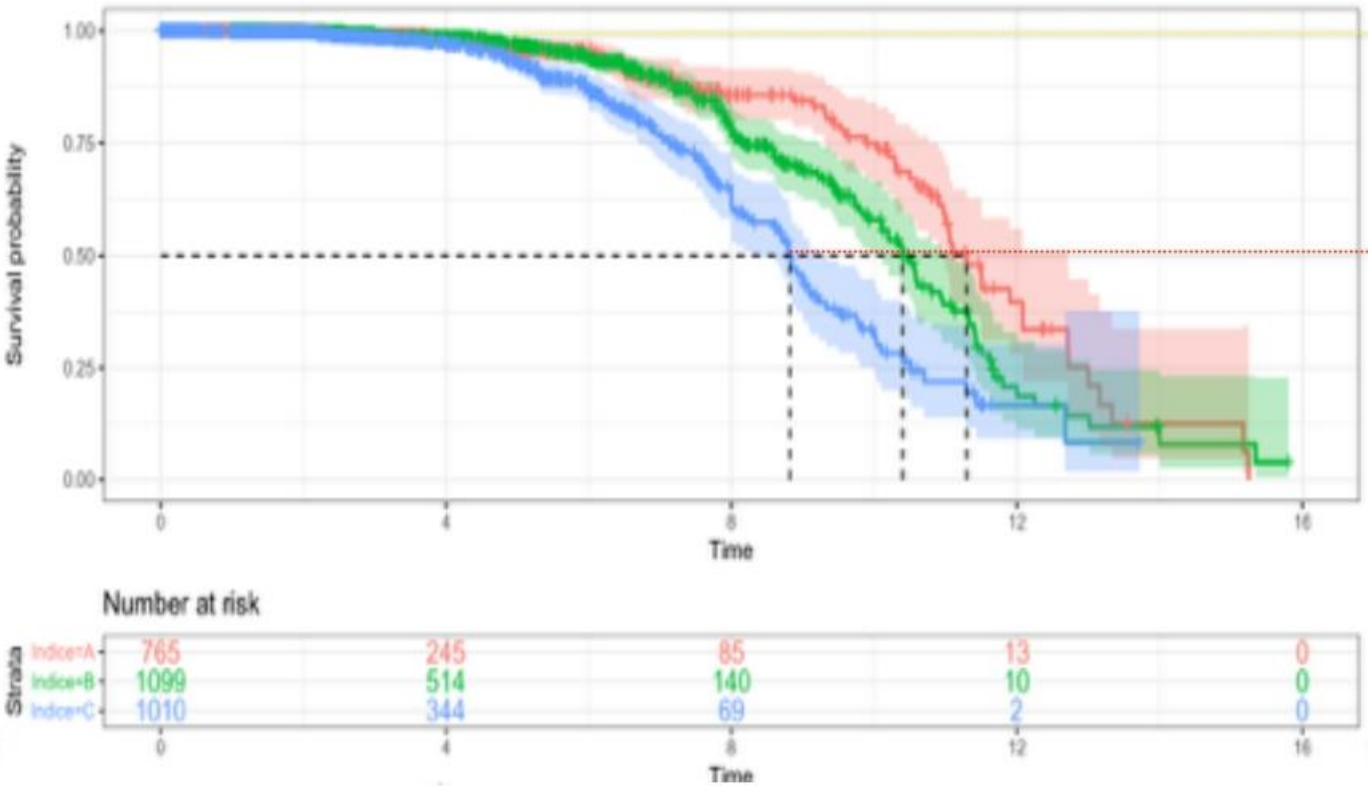


A : 10.6% of deaths
B : 36.1% of deaths
C: 53.2% of deaths

strata	mean	median
A	11.1	11.3
B	10.3	10.4
C	9	8.8

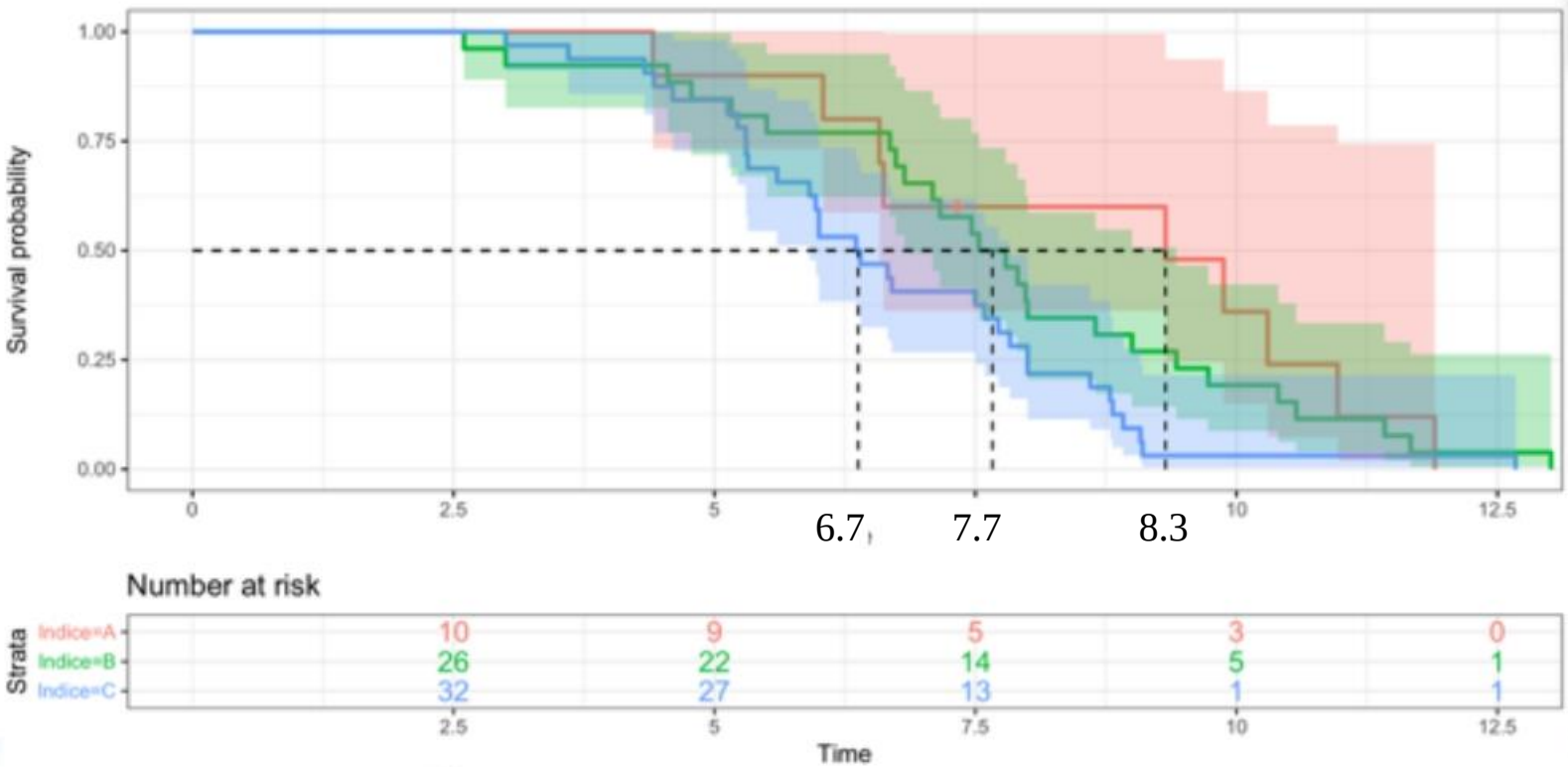
pvalue= 4x10-10

-> almost half of death between 0 and 8 years occurs on C dogs!

Summary of HS tests performed by Antagene

Since 2012 , Antagene has tested >6700 dogs (unknown phenotypes):

Focus on the 68 dogs with HS diagnosis (**Histological diagnosis**):



pvalue= 0.0.32

C Dogs are more likely to be affected younger (mean 6.7 years) while A dogs are more likely to be affected older (mean 8.3 years)

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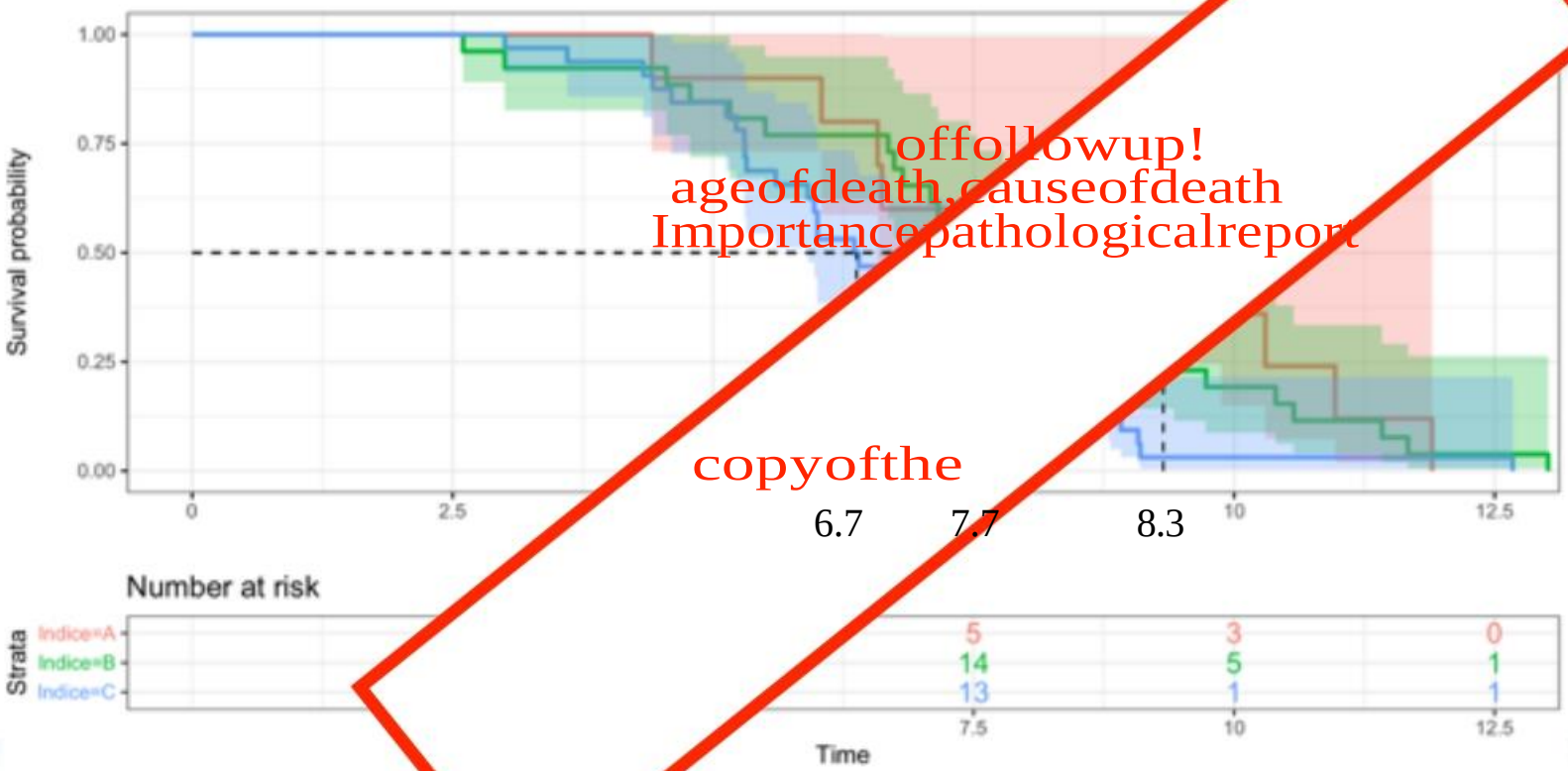

offollowup!
ageofdeath,causeofdeath
Importancepathologicalreport

copyofthe

6.7 7.7 8.3

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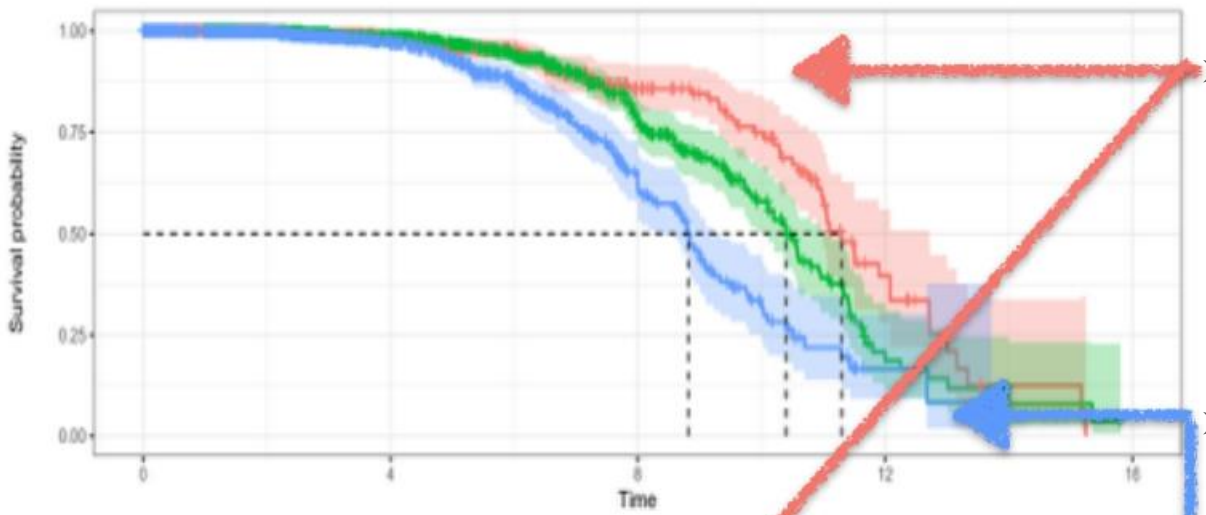

➢ **Why ~14 % of affected are A?**

➢ **Why ~12% of unaffected are C?**

	A	B	C
Affected	***14%***	***43.1%***	***42.9%***
Healthy	***41.5%***	***46.5%***	***11.9%***

Summary of Index Validation

What explains the differences? -> Histiocytic sarcoma = polyfactorial disease

- Other involved chromosomes

HS test based on 9 known markers but others regions involved
-> work on discordant dogs (GWAS/sequencing)

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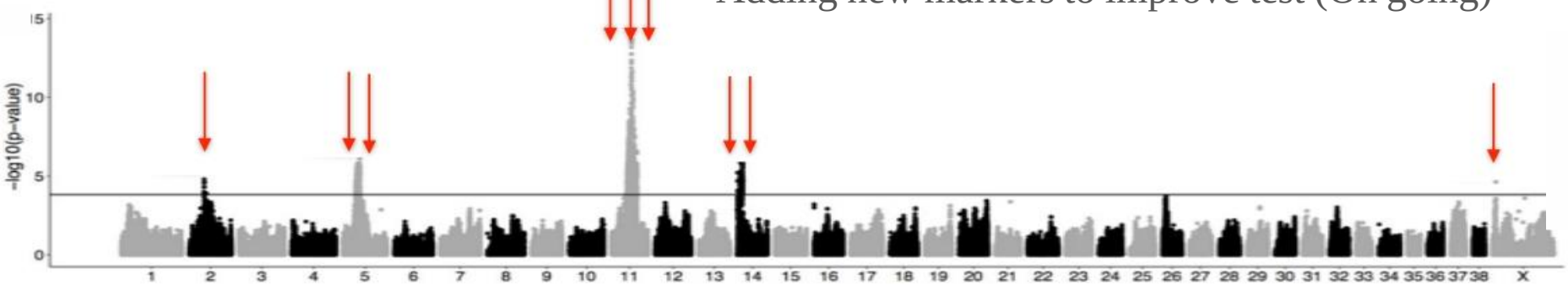

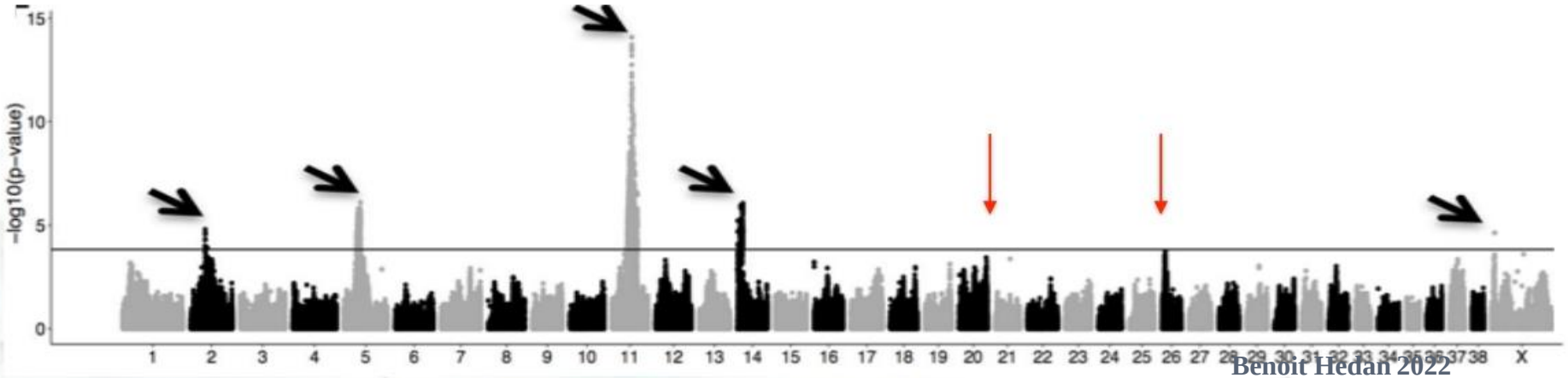

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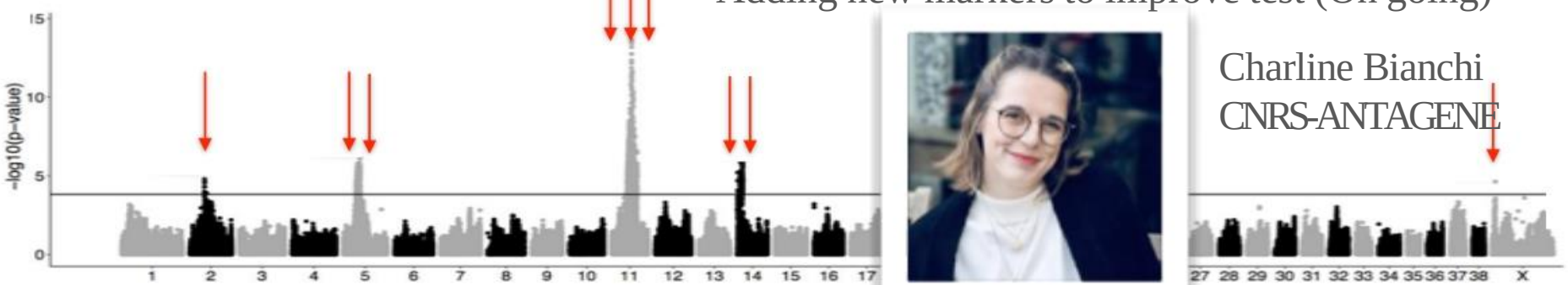
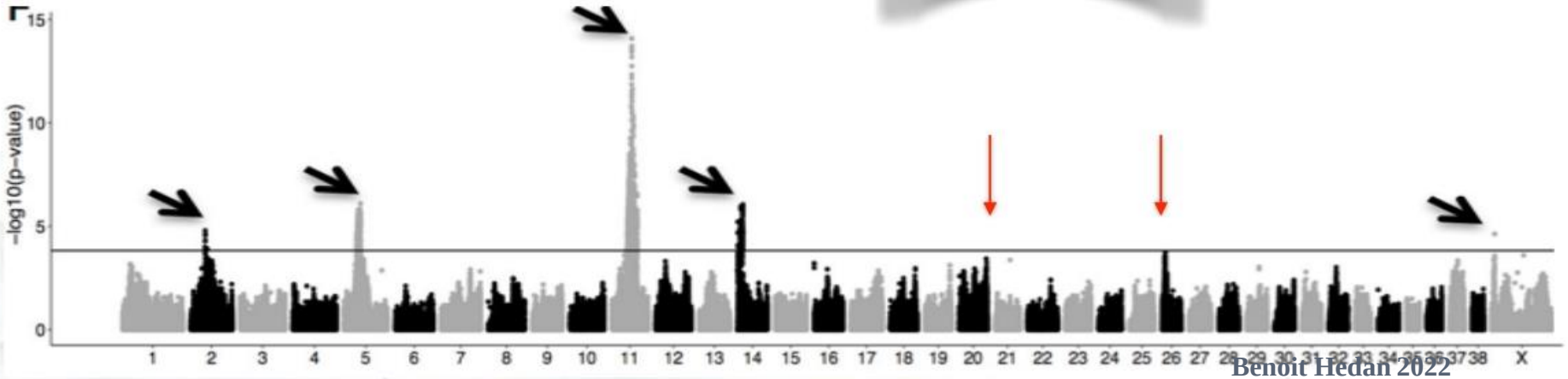


Charline Bianchi
CNRS-ANTAGENE



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➢ environnemental factors :

- sexual hormones

- inflammation : orthopaedic conditions…

(Manor et al. 2017, Kujik et al. 2013, Ruple et al 2016

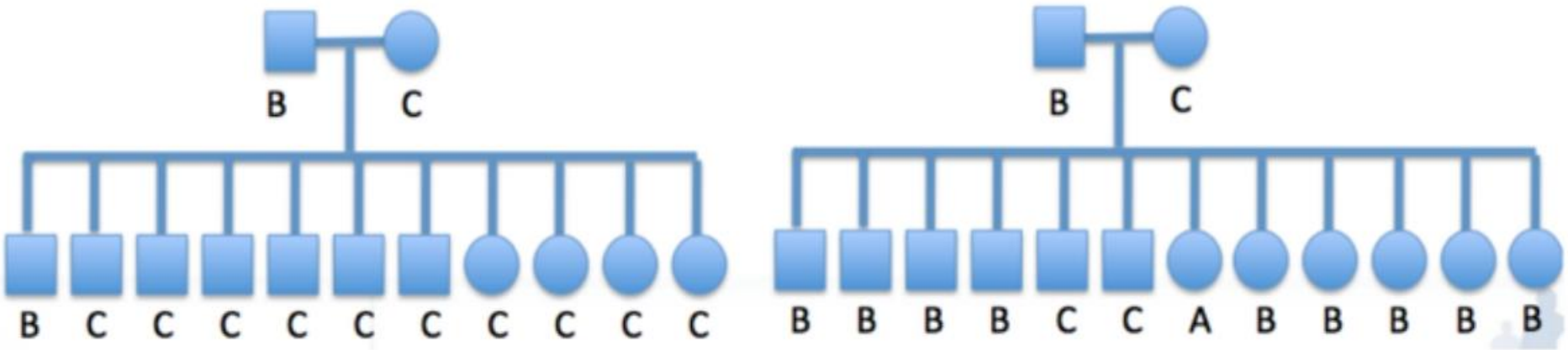
Inflammation may be a modifiable risk factor for the development of HS in BMD

Ruple et al 2016

- treatments : anti-inflammatory medications (Ruple et al. 2016)

Use of the test:
Test of litters in 2012

Test of 112 puppies from 18 litters



different compatibility of dogs

Histiocytic Sarcoma Index Mate Selection (HSIMS)

Development of Histiocytic Sarcoma Index Mate Selection (HSIMS) online webtool

Participation of numerous Bernese mountain clubs for this online development : **unique in veterinary medicine** !

French, Austrian, German, American, Swedish, Czech, Slovenian, Slovak and Swiss clubs

4123 sires and dams used for simulations

around 50 000 simulations per year

Histiocytic Sarcoma Index Mate Selection (HSIMS)

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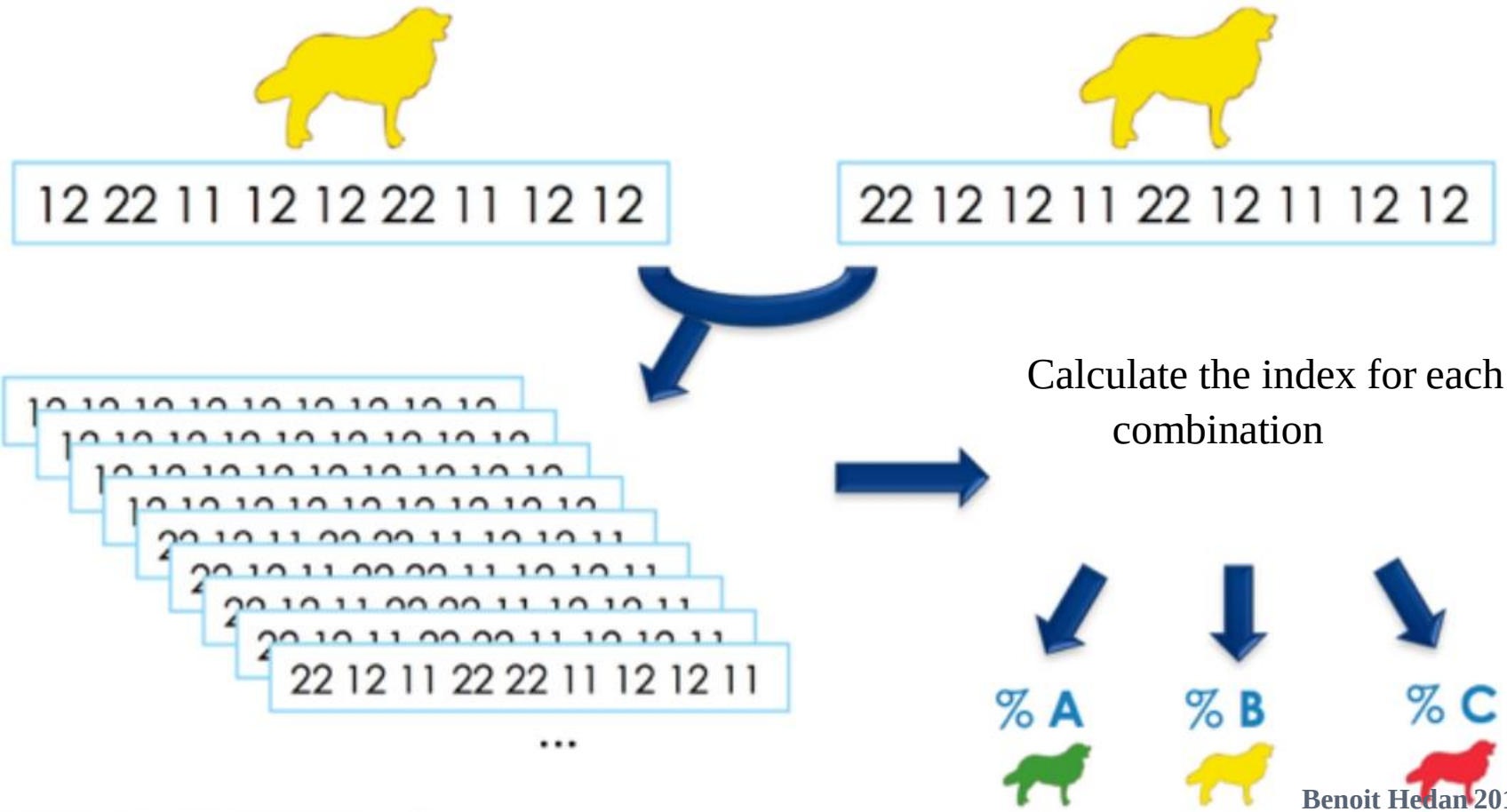

Benoit Hedan 2019

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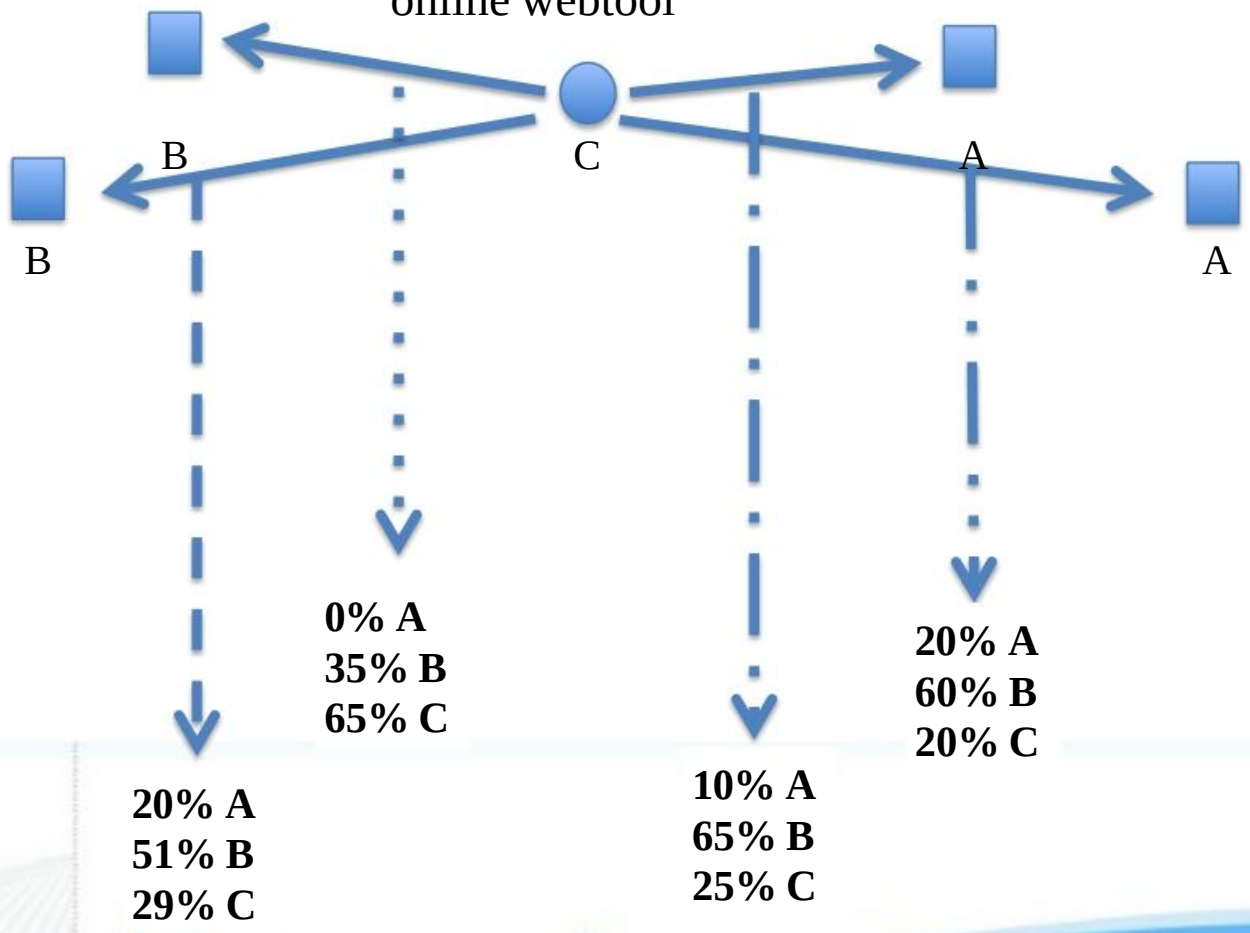

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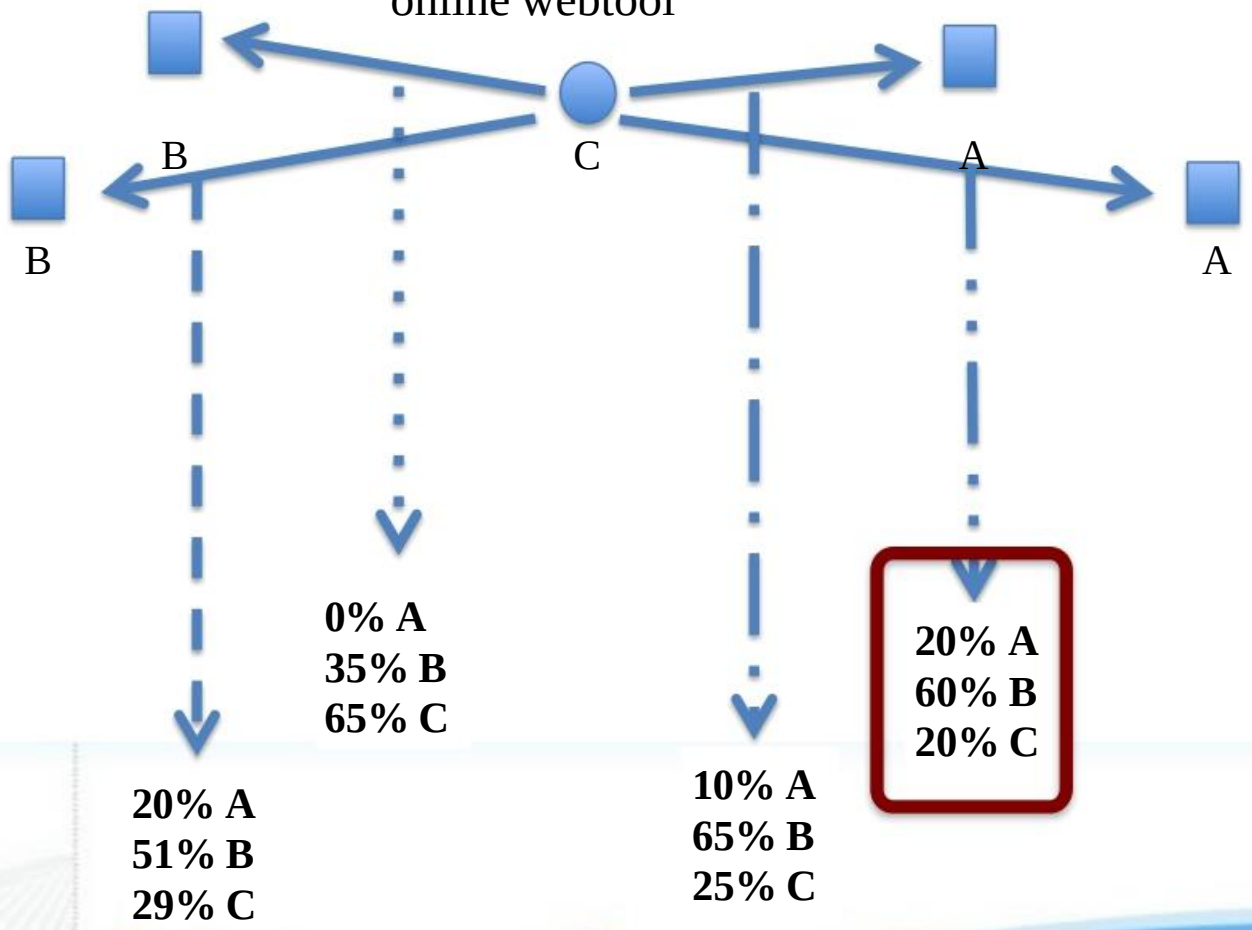

Histiocytic Sarcoma Index Mate Selection (HSIMS)

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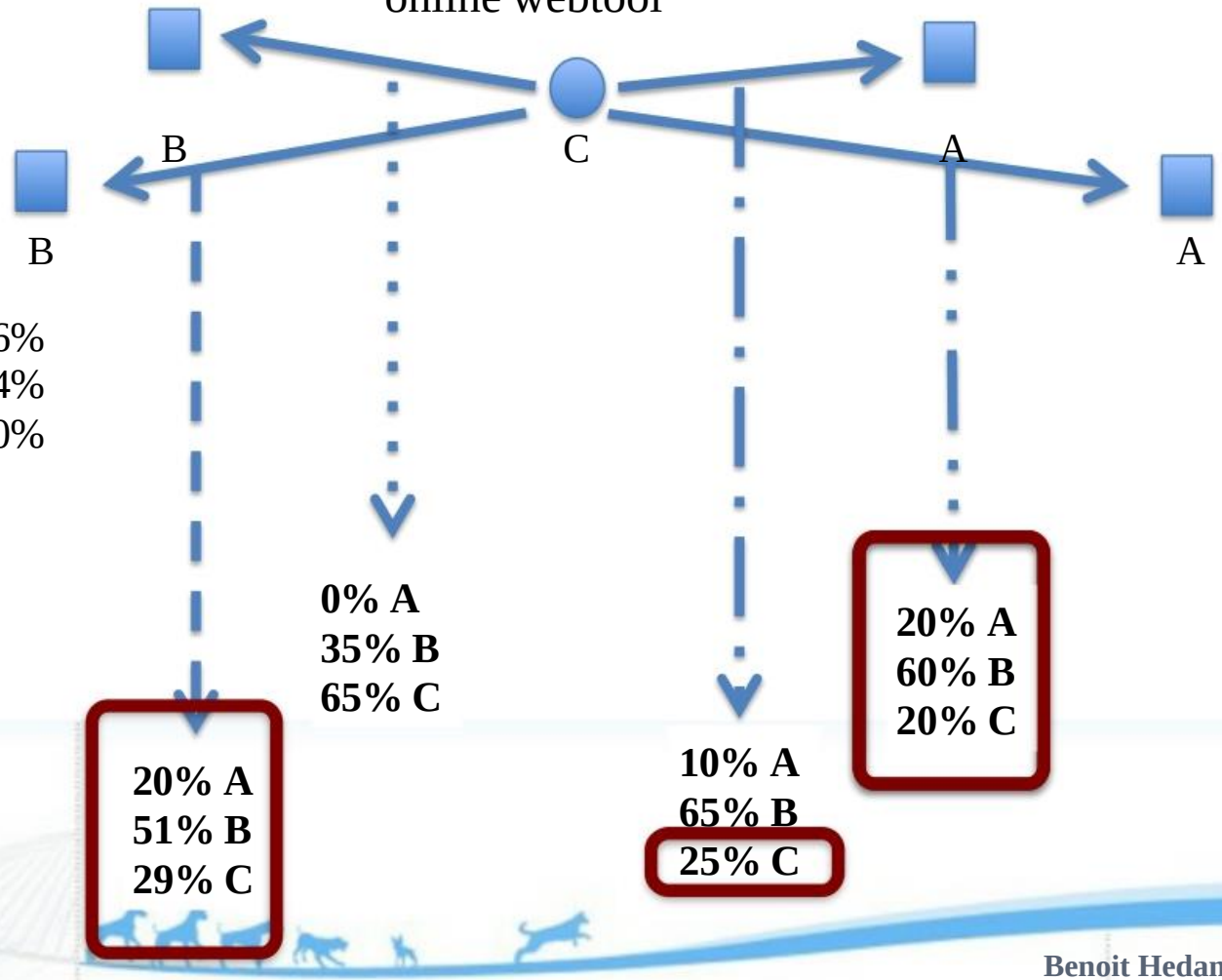
Development of Histiocytic Sarcoma Index Mate Selection (HSIMS) online webtool

26%
44%
30%



Summary of the HS test

How to use this test ?

➢ help for selection and mating based on **actual knowledge**
other importants unknown genetic factors

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C

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compatible sire (A,B,C) C

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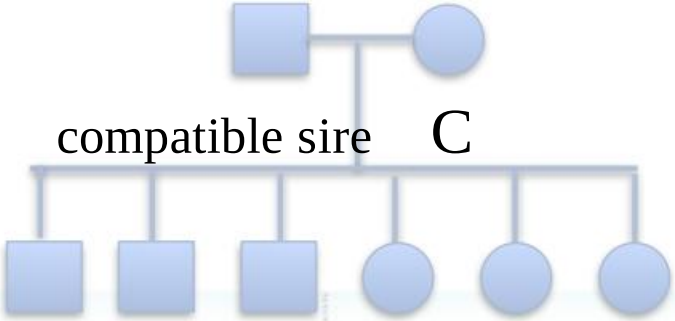

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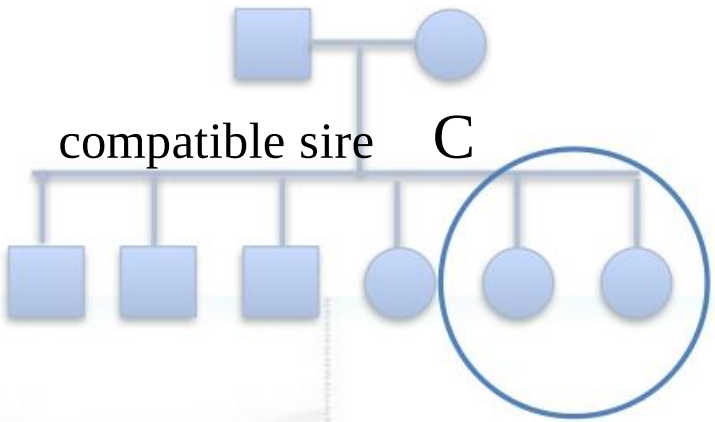

Selection of your best candidate futur dams

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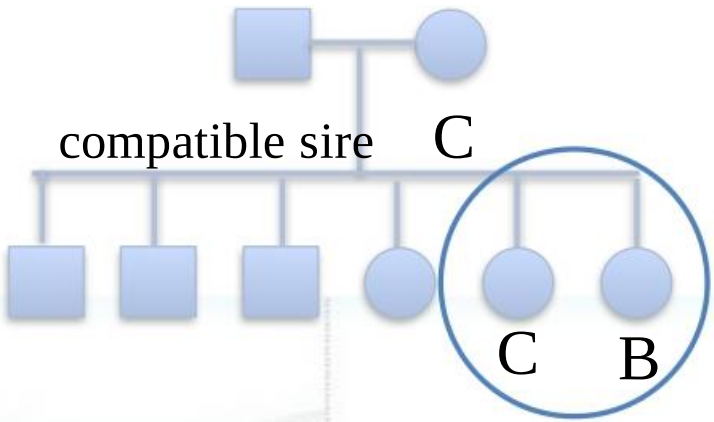

Selection of your best candidate futur dams
TEST SH

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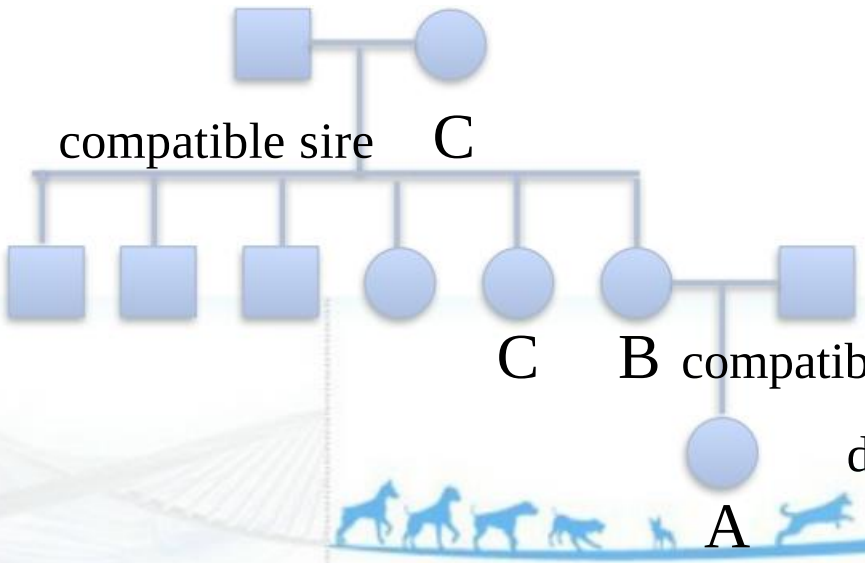

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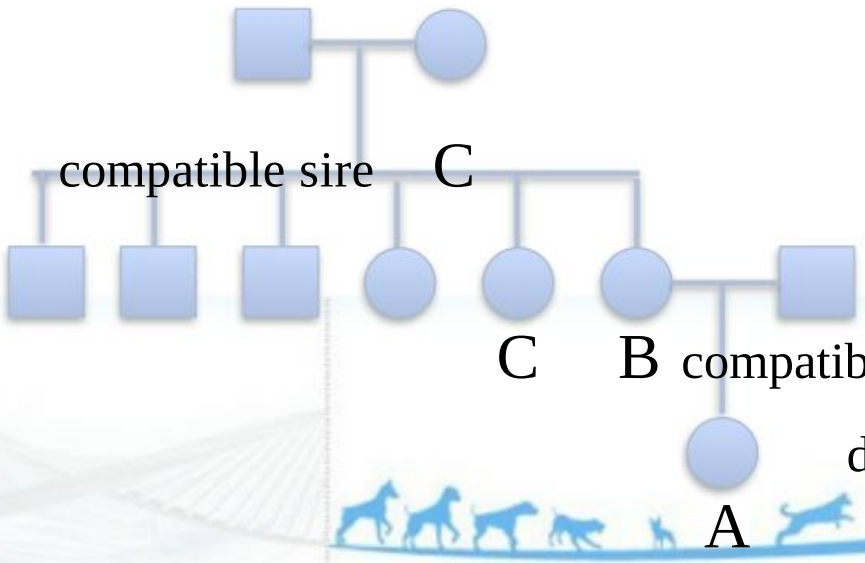
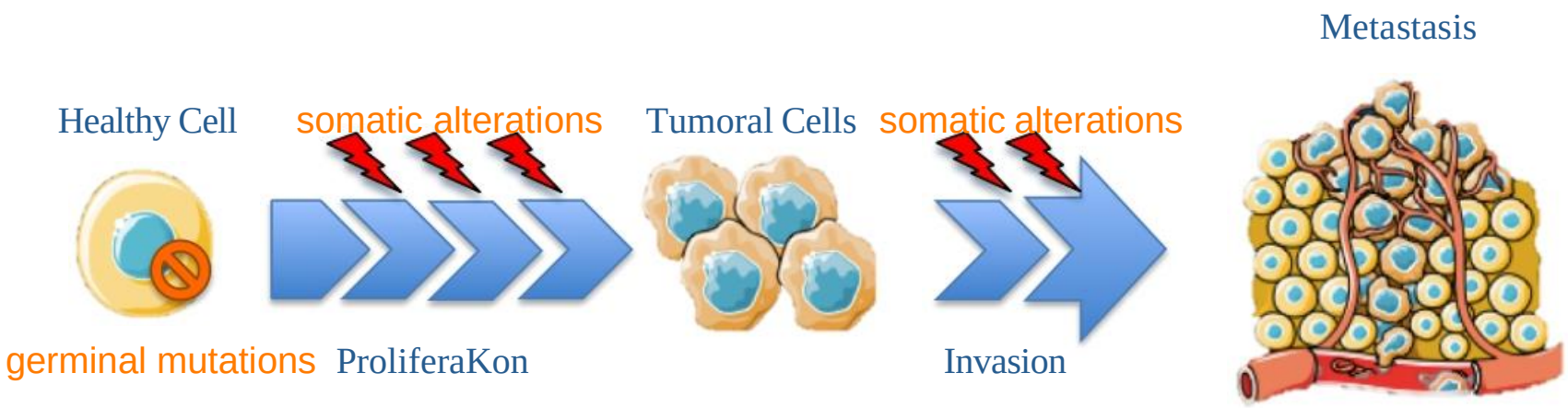


➢ **keep diversity and select slowly**

A long term goal :
Identify genetic mechanisms involved in HS

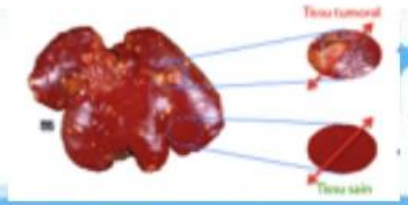


- Identify predisposing genes and risk alleles

-> Blood collection

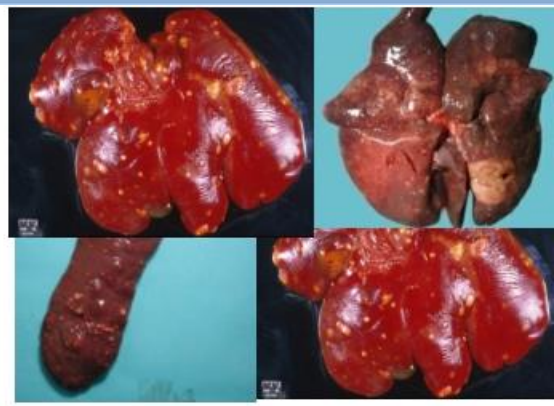
- Identify recurrent somatic alterations associated with tumor progression

-> Tissue collection



Canine histiocytic sarcoma: Somatic mutations

Recurrent somatic mutations identify by RNAseq of 4 tumors in the genes *TP53* and *PTPN11 (*MAPKinase pathway*)* :



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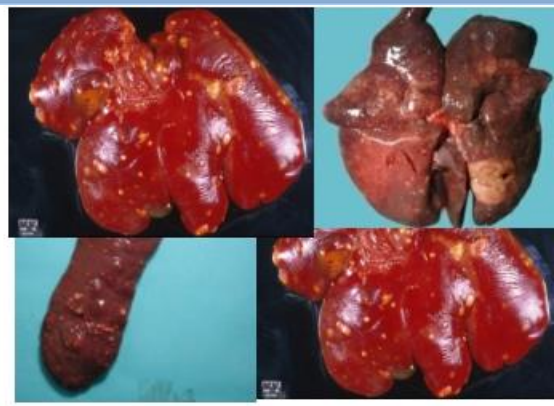

Gene mutated in human myelomonocytic leukemia

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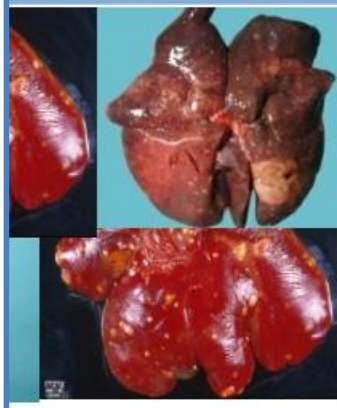

Proliferation survival differentiation….

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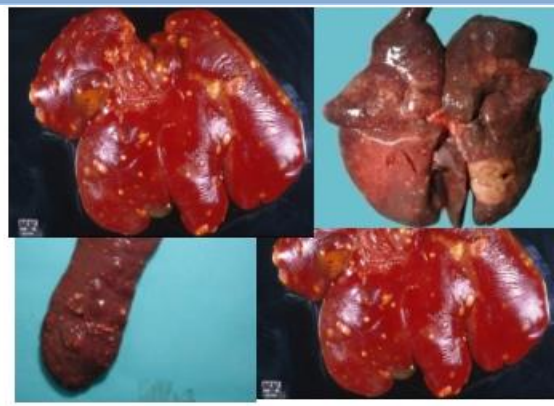

Gene mutated in human myelomonocytic leukemia

Sequencing of *PTPN11* in >100 HS - different breeds, with different clinical presentations

-> *PTPN11* mutated in 56,75% (63/111) of cases

-> major event for HS development

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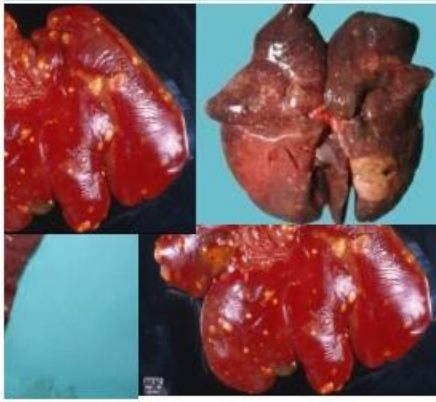
-> *PTPN11* mutated in 56,75% (63/111) of cases

-> major event for HS development



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ORIGINAL ARTICLE

Gain-of-function mutation in *PTPN11* in histiocytic sarcomas of Bernese Mountain Dogs

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common in certain breeds of dogs. High prevalence of fatal, disseminated HS has been described in Bernese Mountain Dogs (BMDs). Support for genetic predisposition to develop HS has been presented in several studies, but to date, causative genetic events have not been reported. In addition, no driver mutations have been identified in tumours. Recently, E76K gain-of-function mutation in SHP2 encoded by the *PTPN11* gene has been described in human histiocytic malignancies. In our study, we identified the PTPN11^{E76K} in HS of BMDs. Amplification of exon 3 of the *PTPN11* gene followed by Sanger sequencing was used to detect the ... and 10 other ... compared with 8.69% in other breeds. No mutation was identified in normal tissues from 10 BMDs with HS that carried the mutation and 12 control dogs with no neoplastic disease, including 6 BMDs. Increased immunoreactivity for AKT, phosphorylated ERK1/2 and phosphorylated AKT in a small subset of BMDs with PTPN11^{E76K} suggests that a gain-of-function might be mediated by the ERK and AKT pathways. These data suggest PTPN11^{E76K} as an important driver mutation of HS in BMDs. This information may not only aid in unravelling the tumourigenic events associated with HS in BMDs, but also help in identifying more promising therapeutic strategies.

Bernese Mountain Dog, gain-of-function mutation, histiocytic sarcoma, *PTPN11* gene

1 | INTRODUCTION

Histiocytic sarcoma (HS) is the most aggressive entity in the spectrum of histiocytic diseases in dogs.[1] HS was first recognized in Bernese Mountain Dogs (BMDs),[2] as a neoplasm derived from cells with the phenotypic profile of interstitial dendritic cells.[3] While HS has been sporadically reported in retriever dogs and other dog breeds, BMDs are considered to be a predisposed breed for HS with a polygenic mode of inheritance.[4–6] The disseminated form of HS has a strong negative prognosis and occurs most frequently in BMDs. Moreover, the median age of diagnosis of HS is earlier in BMDs than in Golden Retrievers or other dog breeds.[7] Therefore, it has been suggested that the potential breed-specific genetic background may be responsible for the tumourigenesis of HS in BMDs.[5–7]

The genomic loci involved in HS in BMDs and Flat-Coated Retrievers have been studied extensively,[8,9] but not definitively. ... (CDKN2A/B, RB1 and PTEN) were detected in HS in both breeds, suggesting that these genetic alterations are more likely associated with a high risk to develop HS than with specific breeds.[8] HS-associated loci in BMDs were investigated, and BMDs in North America and Europe shared the same HS-associated locus, which features a single haplotype spanning *MTAP* and part of *CDKN2A* ... *CDKN2A* and *CDKN2B* genes with *INK4A/ARF/INK4B* dysregulation is considered a primary contributor to HS susceptibility in BMDs, neither a causative genetic event nor driver mutation of HS in BMD has yet been identified.

In humans, HS is a rare neoplasm that tends to have an aggressive course and responds poorly to treatment.[10] The most frequent mutations in human HS cases were those identified in the *BRAF* gene,

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Canine histiocytic sarcoma: Somatic mutations

Recurrent somatic mutations identify by RNAseq of 4 tumors in the genes *TP53* and *PTPN11 (*MAPKinase pathway*)* :

Gene mutated in human myelomonocytic leukemia

Sequencing of *PTPN11* in >100 HS - different breeds, with different clinical presentations -> *PTPN11* mutated in 56,75% (63/111) of cases

PTPN11 Mutations linked to internal tumors:

	Mutated	WT
BMD	48	36
other breeds	6	12

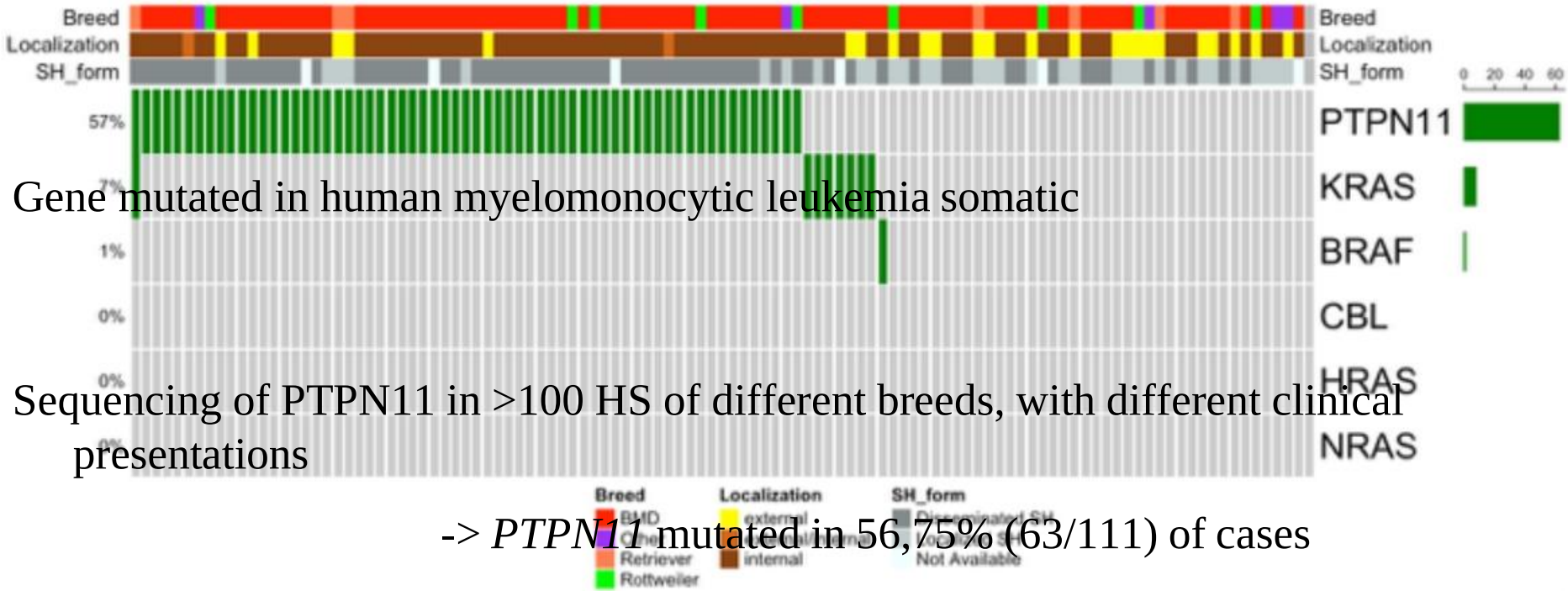
p-value = 0.07 (Exact Fisher test)

	Mutated	WT
internal	42	25
external	5	20

p-value = 0.00035 (Exact Fisher test)

Canine histiocytic sarcoma: Somatic mutations

Mutations in oncogenes of the MAPKinase pathway



Gene mutated in human myelomonocytic leukemia somatic

Sequencing of PTPN11 in >100 HS of different breeds, with different clinical presentations

-> *PTPN11* mutated in 56,75% (63/111) of cases

-> Alteration of this pathway in >65% of HS- a key event in HS development

Canine histiocytic sarcoma: Somatic mutations

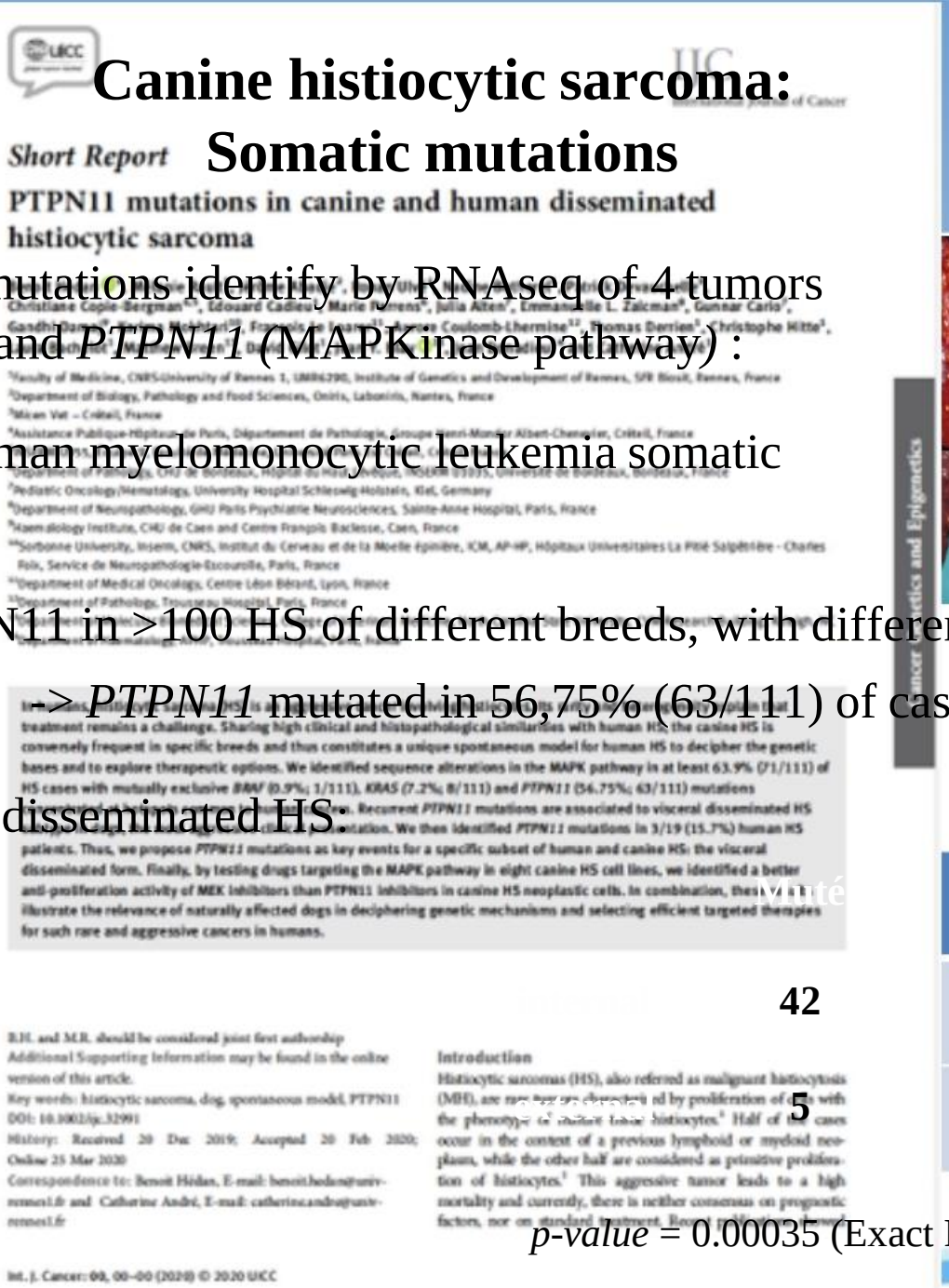
Recurrent somatic mutations identify by RNAseq of 4 tumors in the genes *TP53* and *PTPN11* (MAPKinase pathway) :

Gene mutated in human myelomonocytic leukemia somatic

Sequencing of PTPN11 in >100 HS of different breeds, with different clinical presentations -> *PTPN11* mutated in 56,75% (63/111) of cases

Mutations linked to disseminated HS:

(J Donnadieu)



Short Report

PTPN11 mutations in canine and human disseminated histiocytic sarcoma

Christiane Copie-Bergman[4,5], Edouard Cadieu[1], Marie Hoeppner[6], Julia Alten[7], Emmanuelle L. Zalcman[8], Gunnar Carlo[9], Gaëlle Bonsergent[10], Florence Le Loarer[11], Aurore Coulomb-Lhermine[12], Thomas Derieux[1], Christophe Hitte[1], [illegible]

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[2]Department of Biology, Pathology and Food Sciences, Oniris, Laboniris, Nantes, France
[3]Alcan Vet – Créteil, France
[4]Assistance Publique-Hôpitaux de Paris, Département de Pathologie, Groupe Henri-Mondor Albert-Chenevier, Créteil, France
[5]Department of Pathology, CHU de Bordeaux, Hôpital du Haut-Lévêque, INSERM U1035, Université de Bordeaux, Bordeaux, France
[7]Pediatric Oncology/Hematology, University Hospital Schleswig-Holstein, Kiel, Germany
[8]Department of Neuropathology, GHU Paris Psychiatrie Neurosciences, Sainte-Anne Hospital, Paris, France
[9]Haemobiology Institute, CHU de Caen and Centre François Baclesse, Caen, France
[10]Sorbonne University, Inserm, CNRS, Institut du Cerveau et de la Moelle épinière, ICM, AP-HP, Hôpitaux Universitaires La Pitié Salpêtrière - Charles Foix, Service de Neuropathologie-Escourolle, Paris, France
[11]Department of Medical Oncology, Centre Léon Bérard, Lyon, France
[12]Department of Pathology, Tenon Hospital, Paris, France

Cancer Genetics and Epigenetics

treatment remains a challenge. Sharing high clinical and histopathological similarities with human HS, the canine HS is conversely frequent in specific breeds and thus constitutes a unique spontaneous model for human HS to decipher the genetic bases and to explore therapeutic options. We identified sequence alterations in the MAPK pathway in at least 63.9% (71/111) of HS cases with mutually exclusive *BRAF* (0.9%; 1/111), *KRAS* (7.2%; 8/111) and *PTPN11* (56.75%; 63/111) mutations [illegible]. Recurrent *PTPN11* mutations are associated to visceral disseminated HS [illegible] mutation. We then identified *PTPN11* mutations in 3/19 (15.7%) human HS patients. Thus, we propose *PTPN11* mutations as key events for a specific subset of human and canine HS: the visceral disseminated form. Finally, by testing drugs targeting the MAPK pathway in eight canine HS cell lines, we identified a better anti-proliferation activity of MEK inhibitors than PTPN11 inhibitors in canine HS neoplastic cells. In combination, these [illegible] illustrate the relevance of naturally affected dogs in deciphering genetic mechanisms and selecting efficient targeted therapies for such rare and aggressive cancers in humans.

B.H. and M.R. should be considered joint first authorship
Additional Supporting Information may be found in the online version of this article.
Key words: histiocytic sarcoma, dog, spontaneous model, PTPN11
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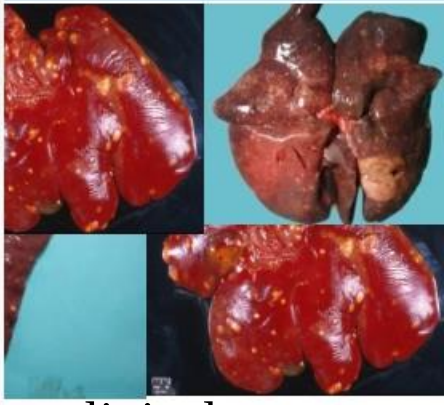
History: Received 20 Dec 2019; Accepted 20 Feb 2020; Online 25 Mar 2020
Correspondence to: Benoit Hédan, E-mail: benoit.hedan@univ-rennes1.fr and Catherine André, E-mail: catherine.andre@univ-rennes1.fr

Introduction
Histiocytic sarcomas (HS), also referred as malignant histiocytosis (MH), are rare [illegible] characterized by proliferation of cells with the phenotype of mature tissue histiocytes.[1] Half of the cases occur in the context of a previous lymphoid or myeloid neoplasm, while the other half are considered as primitive proliferation of histiocytes.[1] This aggressive tumor leads to a high mortality and currently, there is neither consensus on prognostic factors, nor on standard treatment. Recent [illegible]

Int. J. Cancer: 00, 00–00 (2020) © 2020 UICC



	WT
42	25
5	20

p-value = 0.00035 (Exact Fisher test)

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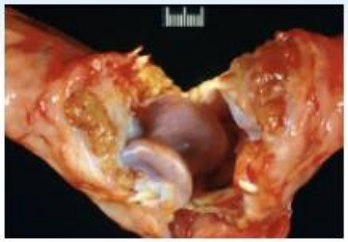
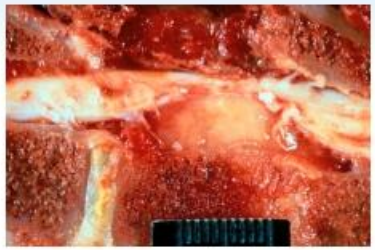
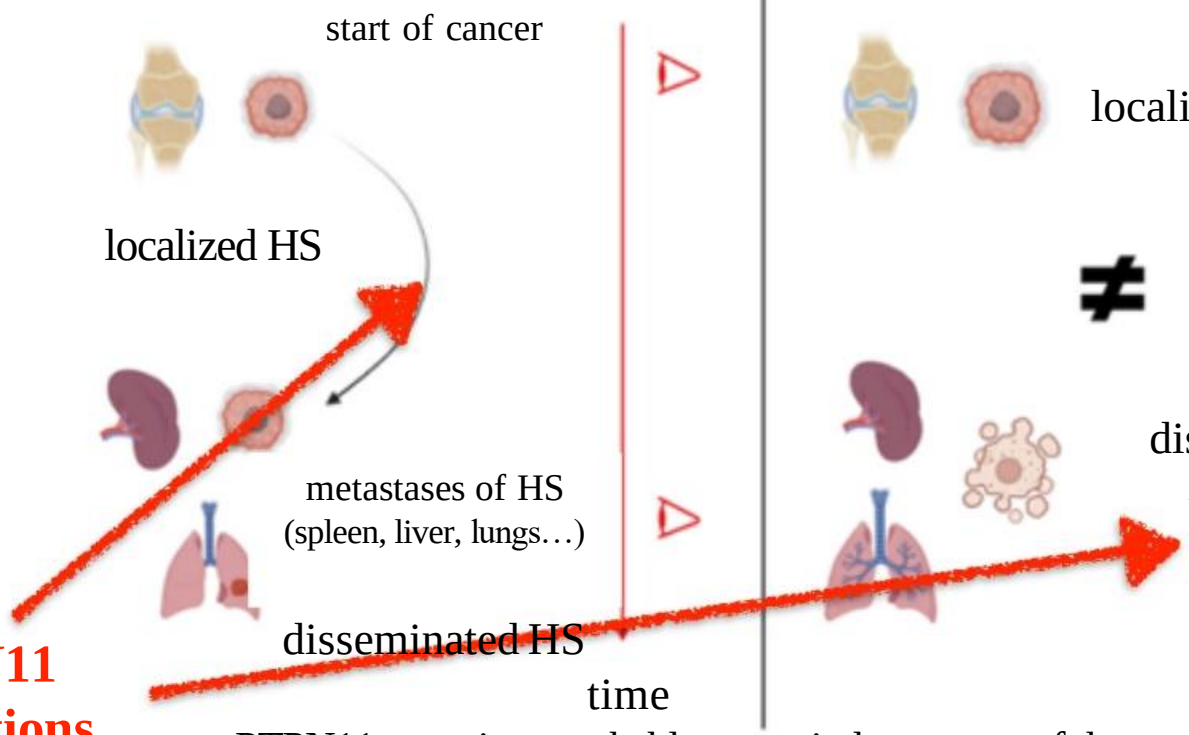
Clinical presentations





Hypothesis 1: same cancers observed at different time

Hypothesis 2: different subtypes of Histiocytic cells



PTPN11 mutations probably occur in late stages of the tumor

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Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

Interest for the diagnosis:

- specific of HS : there are no *PTPN11* nor *KRAS* mutations in any of the lymphoma samples (Takada et *al.* 2019 Genes)

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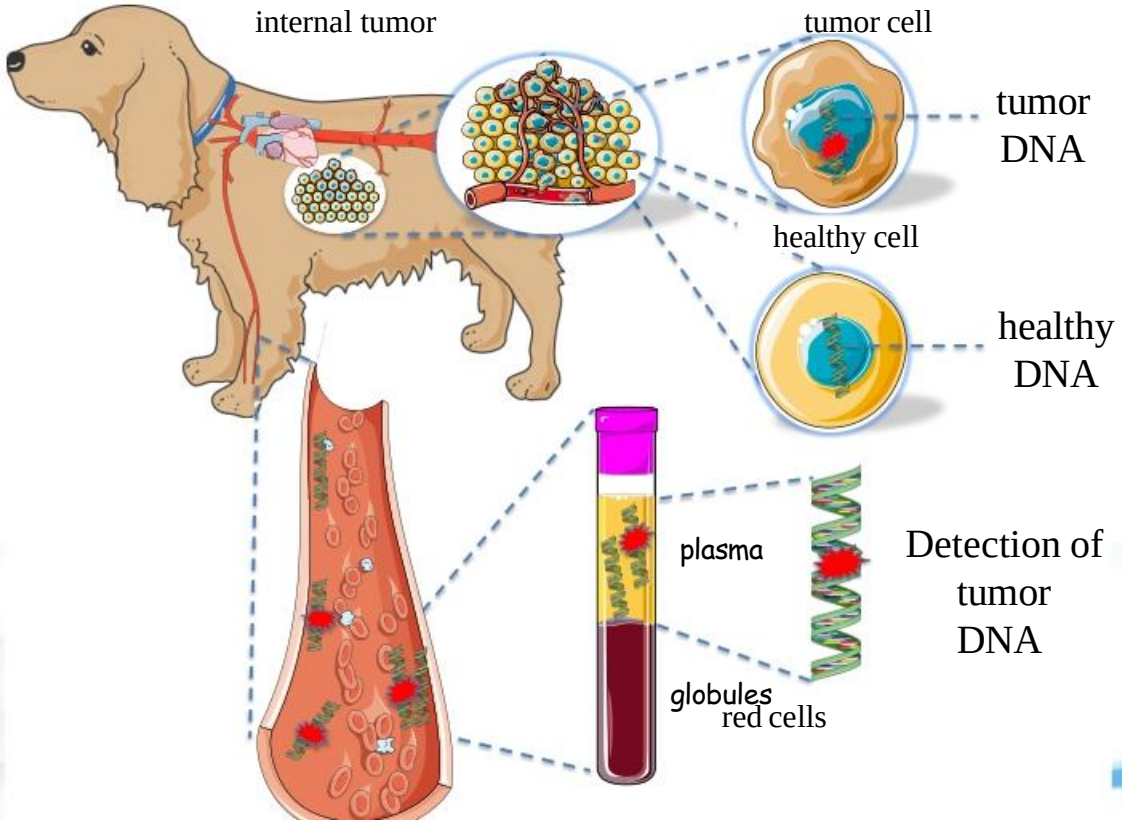

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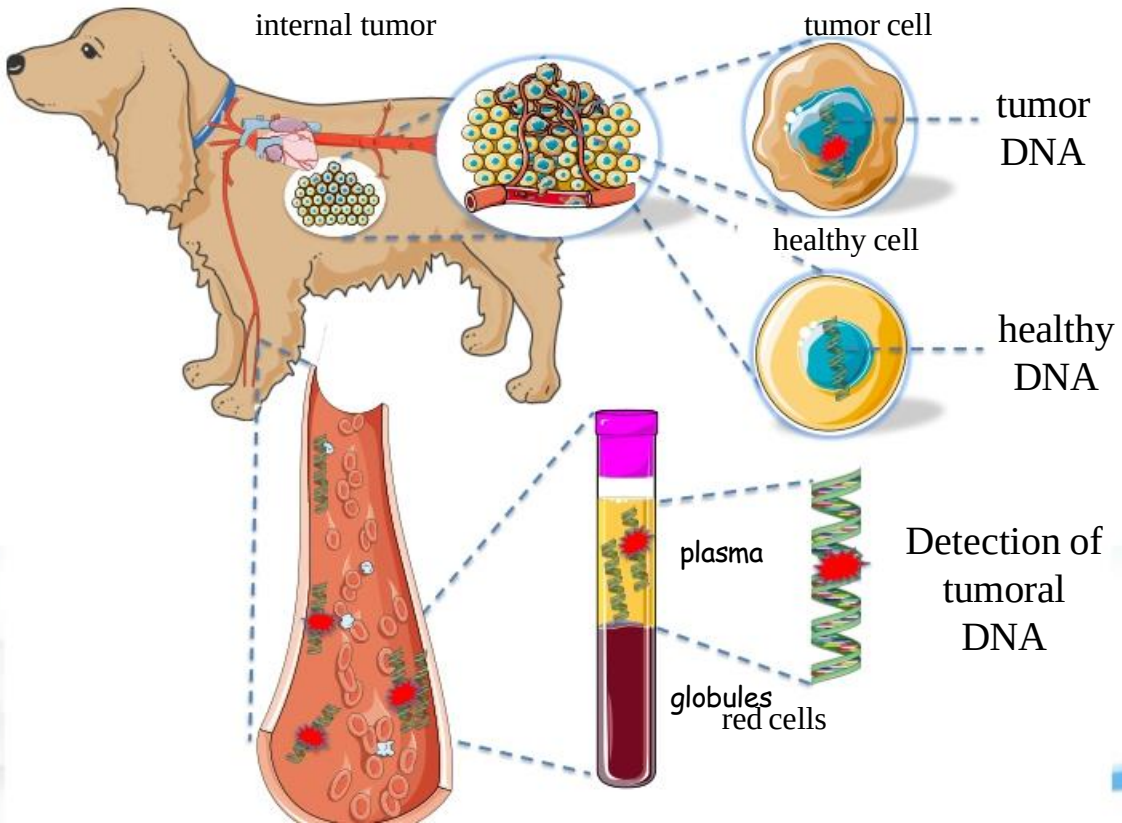

45 histiocytic sarcomas

23 mutated

22 WT

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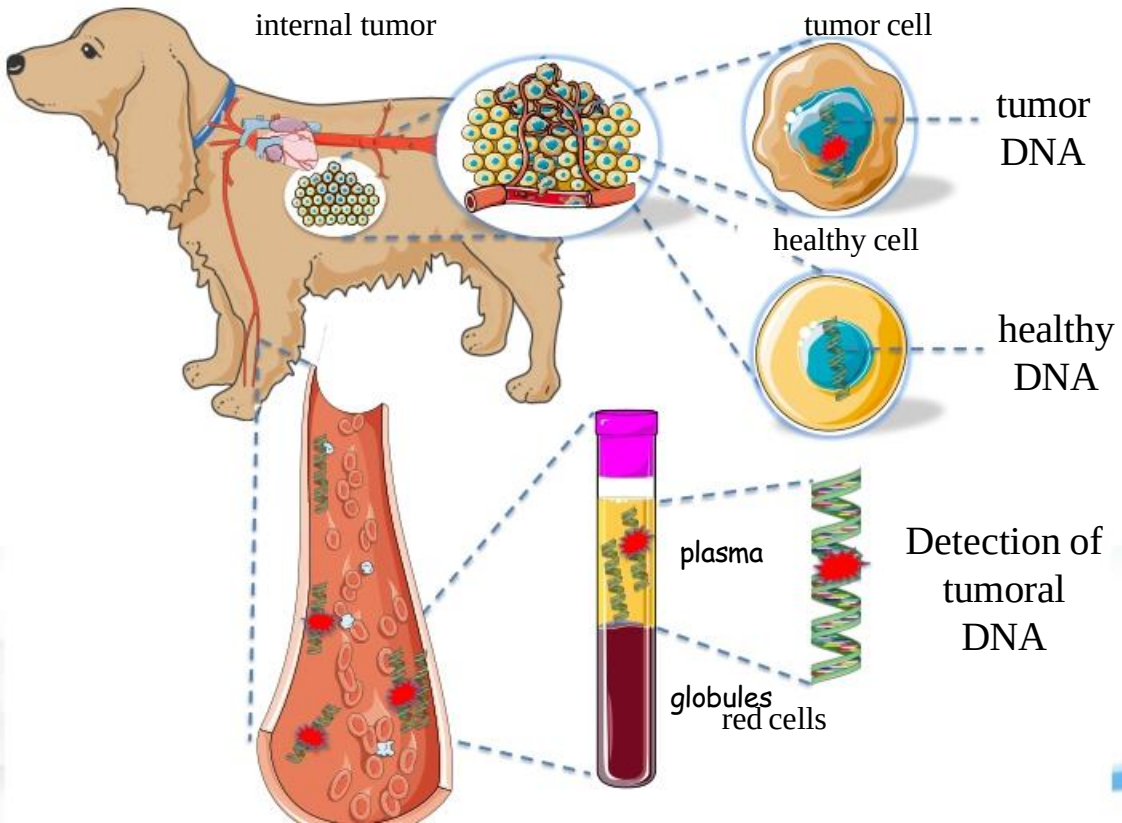

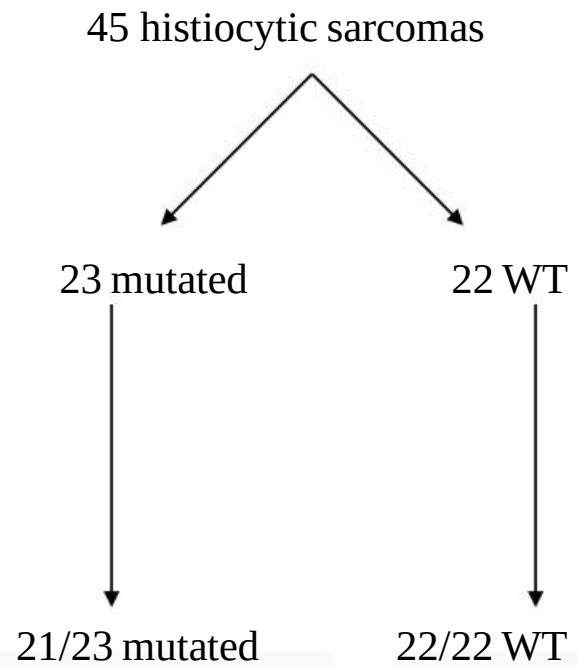

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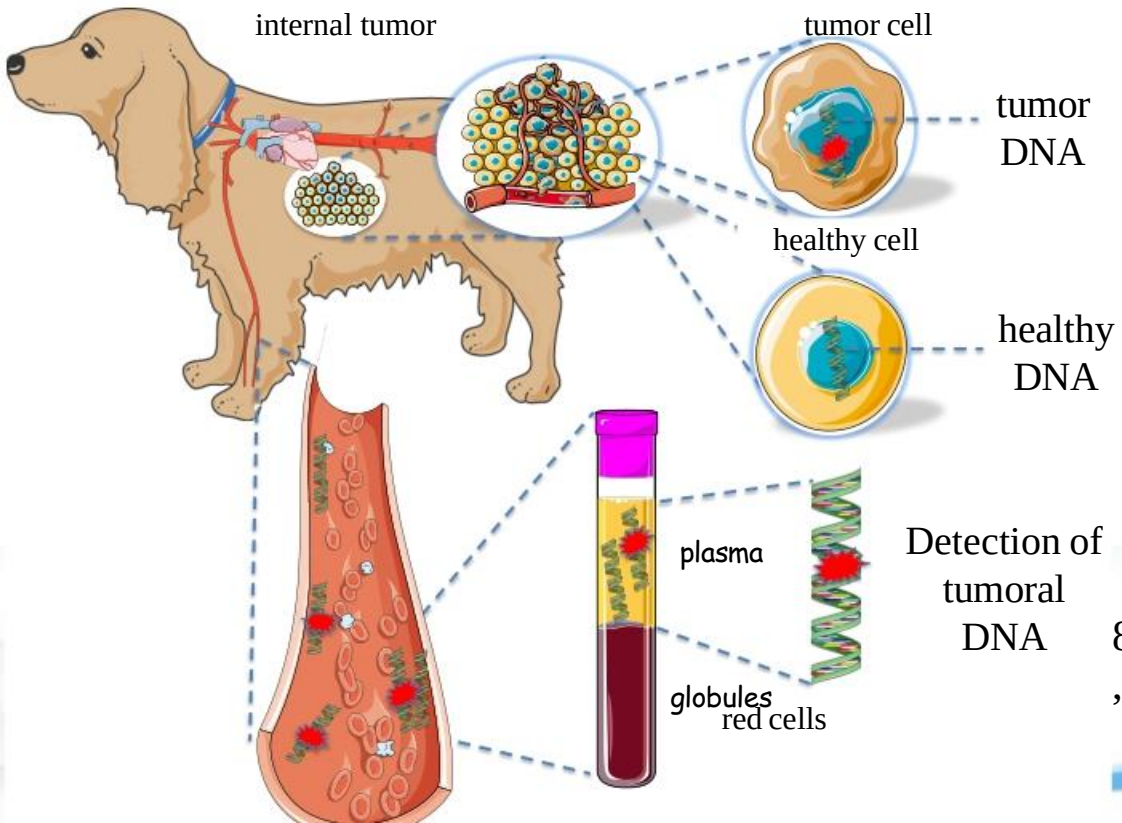

45 histiocytic sarcomas

23 mutated → 21/23 mutated

22 WT → 22/22 WT

Detection of tumoral DNA

84 control dogs (lymphoma, melanoma , mast cell tumor, healthy):
-> 1/84 false positive

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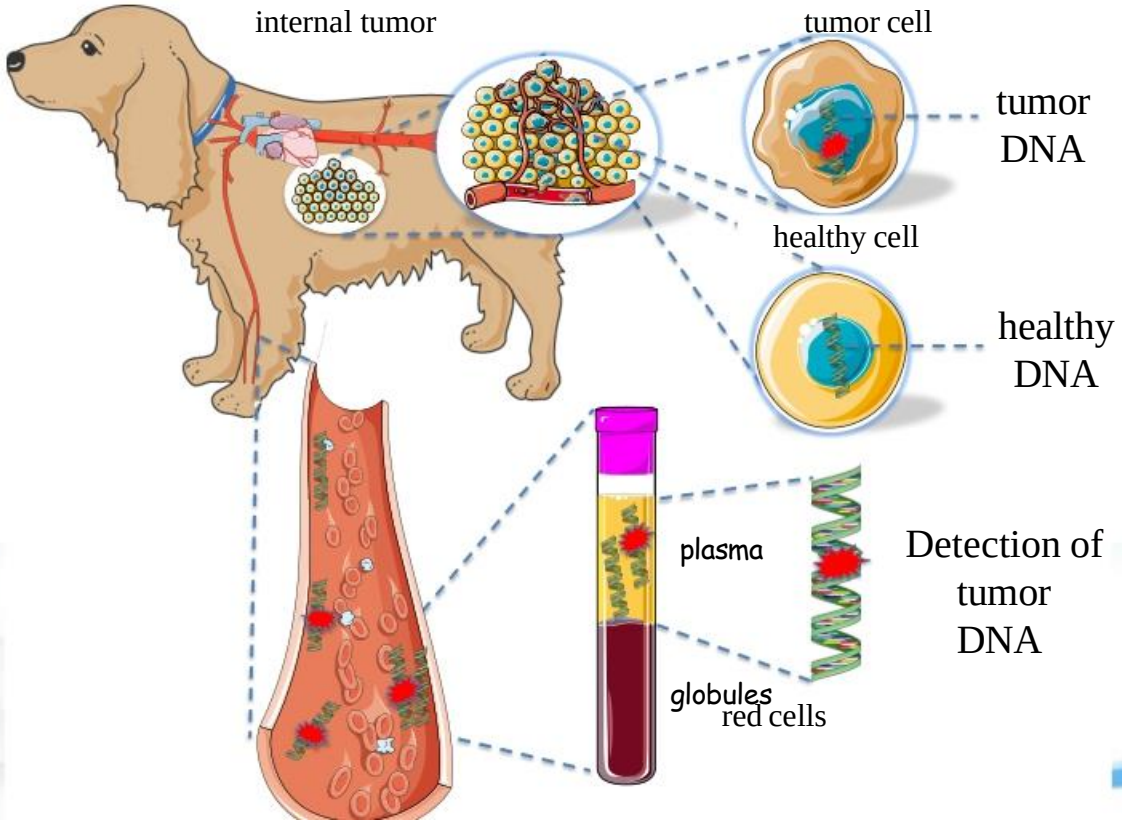
sensitivity 42.8 %
(up to 77% for lung localization)

specificity 98.8 %

Prouteau et al. 2021



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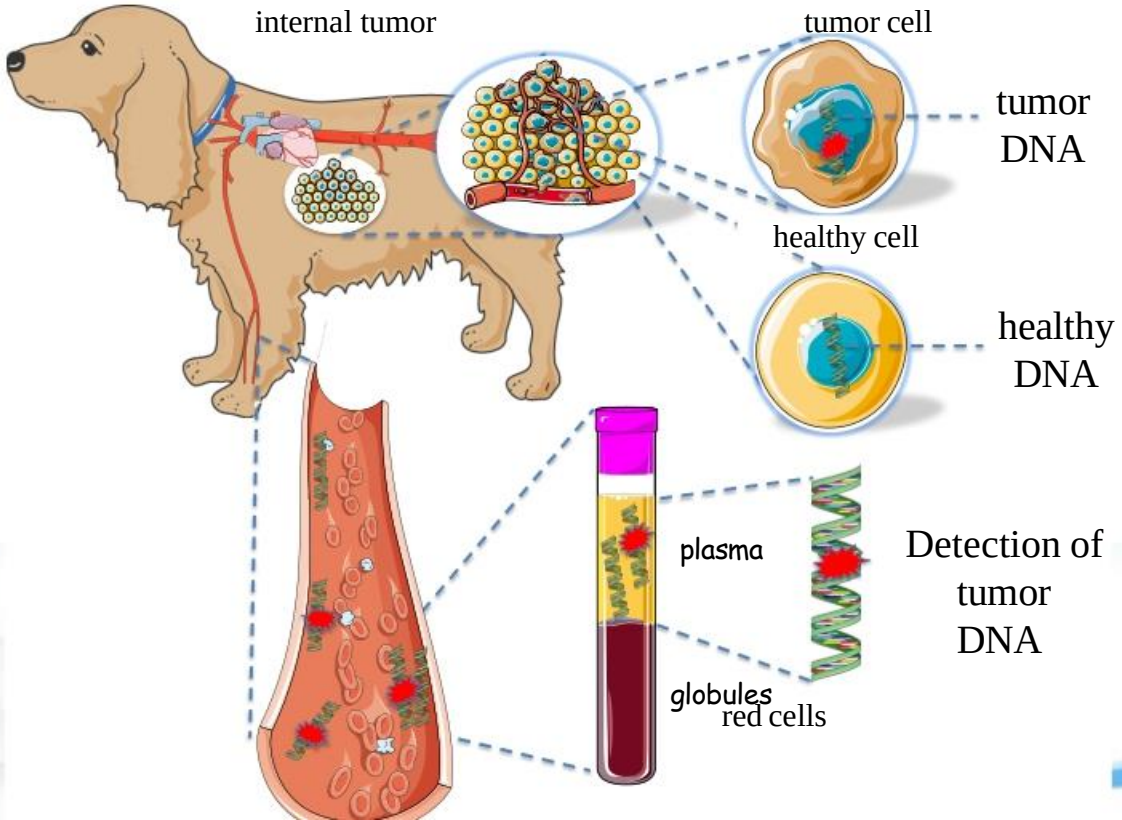


sensitivity 42.8 %
(up to 77% for lung localization)

specificity 98.8 %

Prouteau et al. 2021

-> a tool for diagnosis of none biopsable masses

-> a prognostic tool?

-> to follow treatment efficacy?

-> useful for earlier diagnosis?

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

Prognosis marker ?

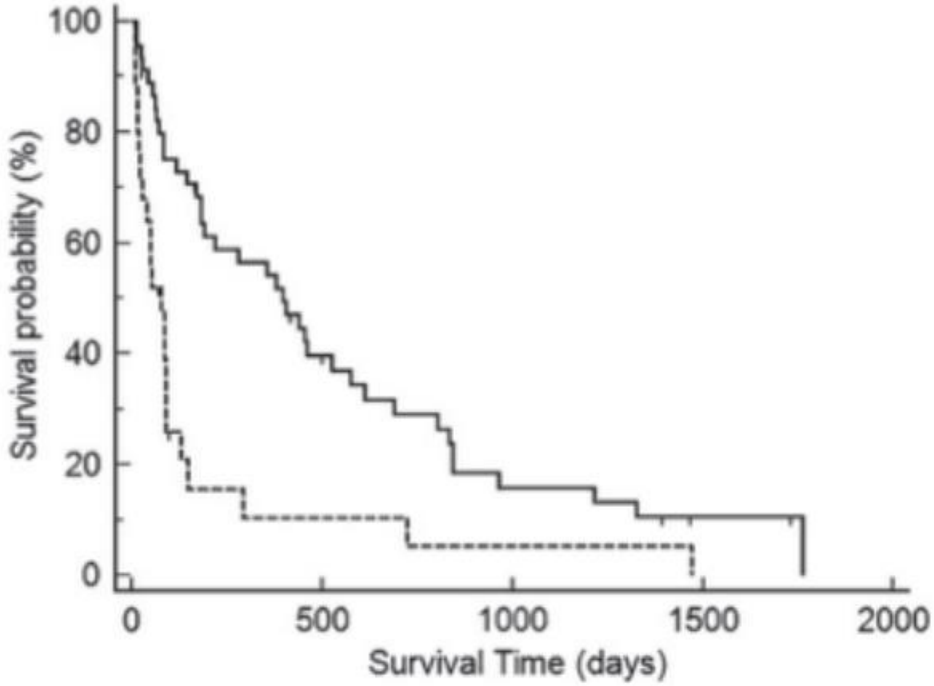


Figure 3. Kaplan–Meier survival curve estimating median OS for dogs diagnosed with LHS (solid line; $n = 46$) compared to dogs with DHS (dashed line; $n = 25$). Dogs diagnosed with LHS had significantly ($P = 0.0002$) longer median OS (398 days) than dogs diagnosed with DHS (78 days). Vertical tick marks represent animals that were censored (LHS group $n = 8$; DHS group $n = 2$).

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

Prognosis marker ? -> Exploration of HS cases from different french histological labs

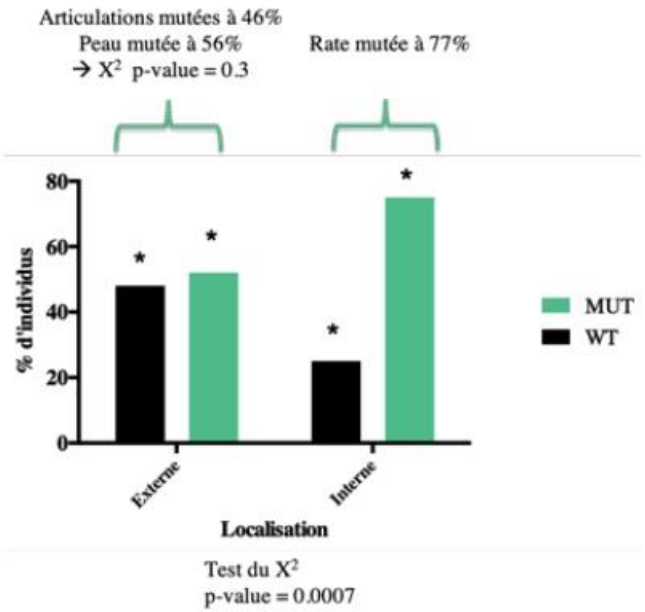
Collection of 305 blocs FFPE (Oniris, IHP, LAPVSO, Amboise) with epidemiological data (Annabelle Garand, Julien Ascencio, Charline Bianchi)

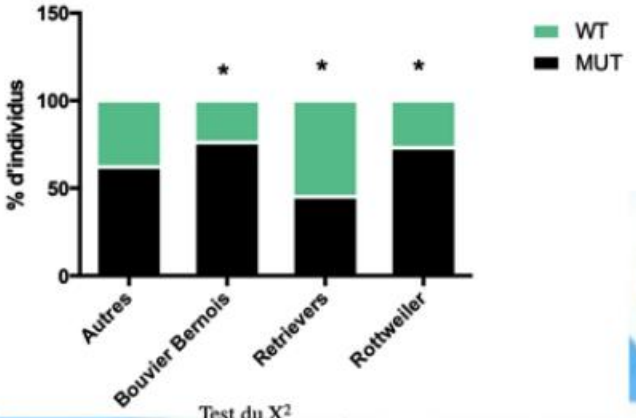
breed	Internal masses	External masses	both	Total
Akita Inu	0	1	0	1
American Stafford Terrier	1	3	0	4
Australian Shepherd	2	4	0	6
Beagle	1	1	0	2
Beauceron	1	3	0	4
Belgian Shepherd	3	1	0	4
Bernese mountain dog	42	50	1	93
Bleu de Gascogne	1	0	0	1
Border	1	3	0	4
Bulldog	11	7	0	18
Boxer	2	4	0	6
Canaan dog	1	0	0	1
Chihuahua	1	1	0	2
Chow Chow	0	1	0	1
Cocker	5	1	0	6
Coton de Tuléar	1	0	0	1
cross breed	5	4	0	9
Dachshund	0	1	0	1
Doberman	2	3	0	5
Flat Coat Retriever	1	5	0	6
Fox	3	0	0	3
German Shepherd	4	2	0	6
Golden Retriever	7	11	0	18
Husky	0	2	0	2
Irish woljound	1	0	0	1
Jack Russel	4	2	0	6
King Charles Spaniel	1	3	0	4
Labrador	16	10	0	26
Maltese dog	1	1	0	2
Munster spaniel	0	1	0	1
other	0	2	0	2
Pinsher	0	1	0	1
Poodle	2	2	0	4
Pyrenean Sheepdog	0	1	0	1
Rolweiler	21	15	1	37
Scomsh Terrier	0	1	0	1
Shar Peï	1	0	0	1
Shepherd	3	0	0	3
Silky Terrier	1	0	0	1
Welsh Corgi	1	0	0	1
Yorkshire Terrier	6	3	0	9
		Total		305

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

Prognosis marker ? -> Selection of 180 HS cases

		localisaKon	
	total	externe	interne
American Bully	1	1 muté	
American Stafford Terrier	4	2 WTs	2 mutés
Berger Allemand	6	1 muté/1 WT	2 muté/2 WT
Berger Australien	5	3 mutéS	2 mutés
Berger Belge Malinois	4	1 muté/1 WT	2 mutés
Border	4	3 WTs	1 mutés
Bouledogue	20	5 muté/4 WT	8 muté/3 WT
Bouvier Bernois	41	12 muté/7 WT	18 muté/4 WT
Boxer	3	2 mutés	1 mutés
Caniche	4	2 mutés	1 muté/1WT
Cavalier King Charles	4	1 muté/2 WT	1 mutés
Cocker	6	1 WT	5 mutés
Dobermann	5	1 muté/2 WT	2 mutés
Flat Coat Retriever	11	5 mutés/4 WT	1 muté/1WT
Golden Retriever	11	2 mutés/3 WT	3 muté/3WT
Jack Russel Terrier	6	2 mutés	1 muté/3WT
Labrador	9	3 WTs	3 muté/3WT
Labrador x epagneul	1		1 mutés
Rolweiler	30	10 mutés/5 WT	12 mutés/3 WT
Yorkshire Terrier	8	1 mutés/2 WT	3 mutés/2 WT
Yorkshire TerrierX	1		1 mutés



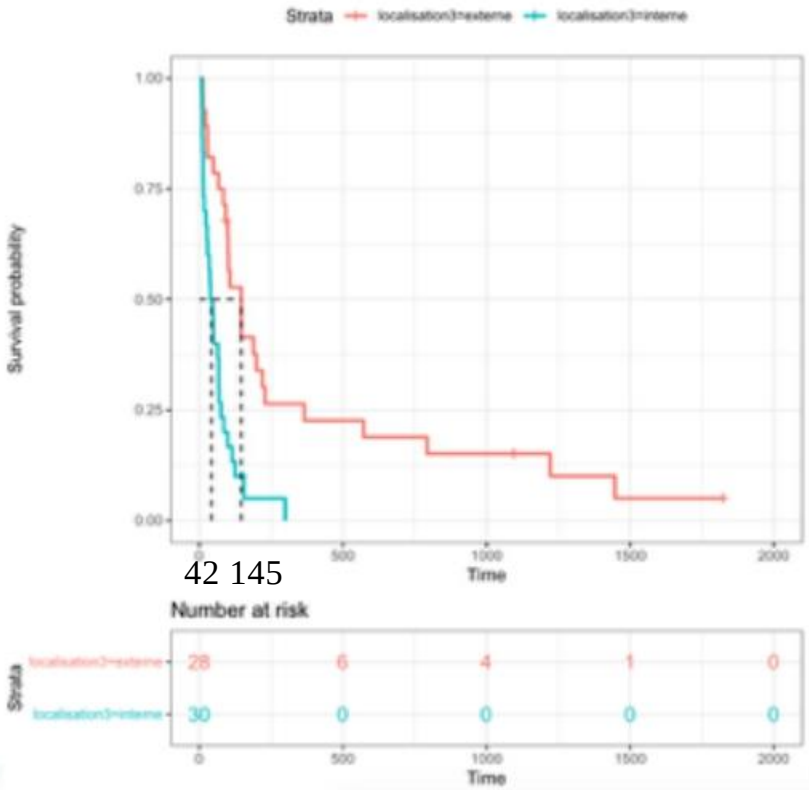


Test du X²
p-value = 0.03

Mutations linked to internal localisation and enriched in the breeds :
BMDs, Rottweiler, French Bulldog..

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

Prognosis marker ? -> Selection of 180 HS cases



pvalue = 1 x10-5

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

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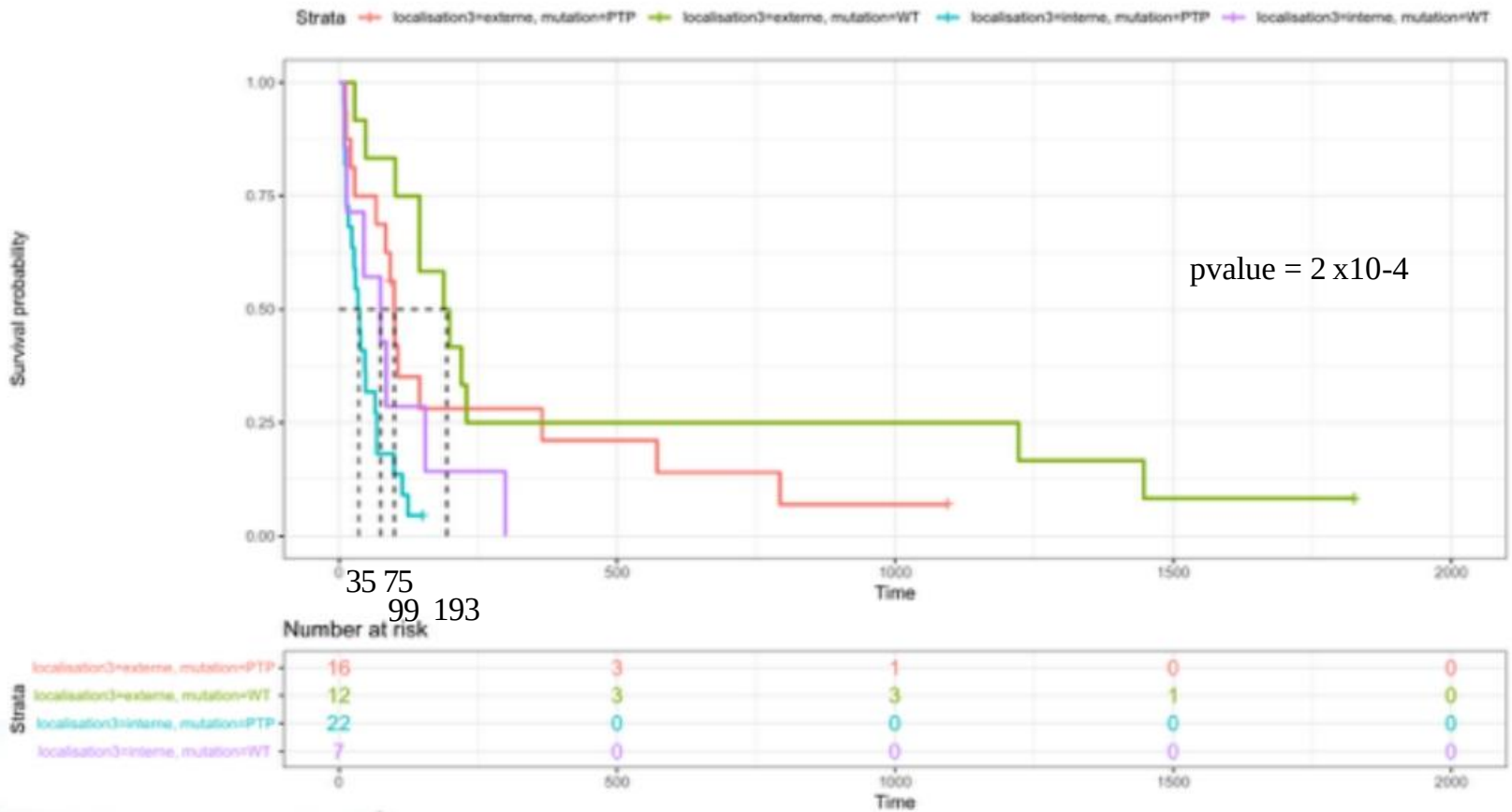
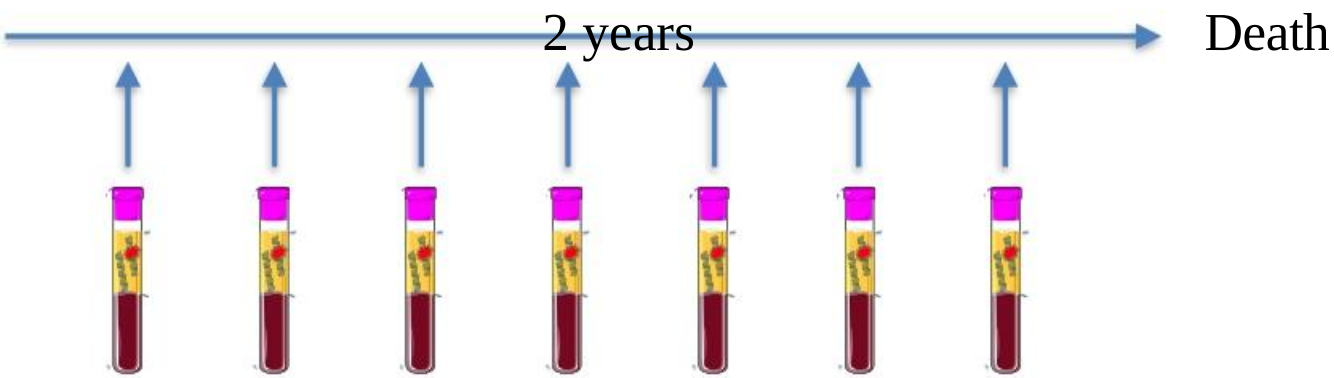


PTPN11 mutation is a prognosis marker

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

Follow up 30 dogs from two breeders : at risk (Age/ HS-test)



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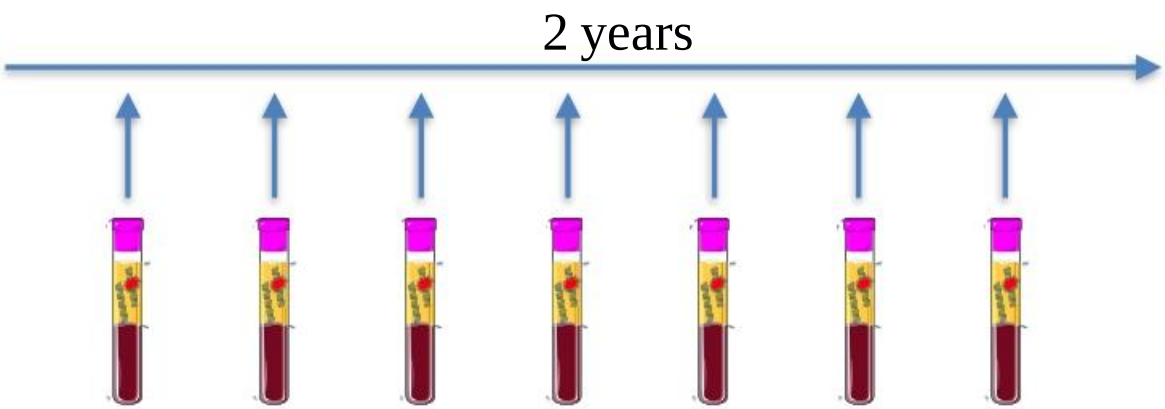
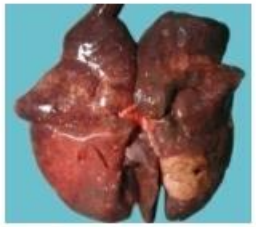


deaths -> 4 HS
with PTPN11 mutation



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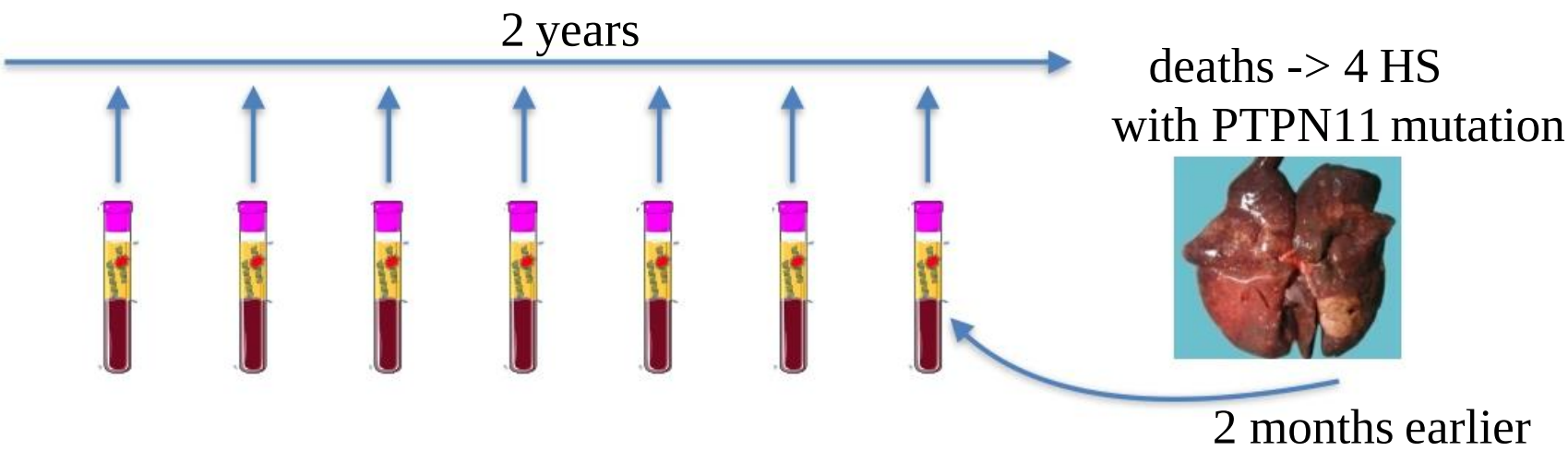

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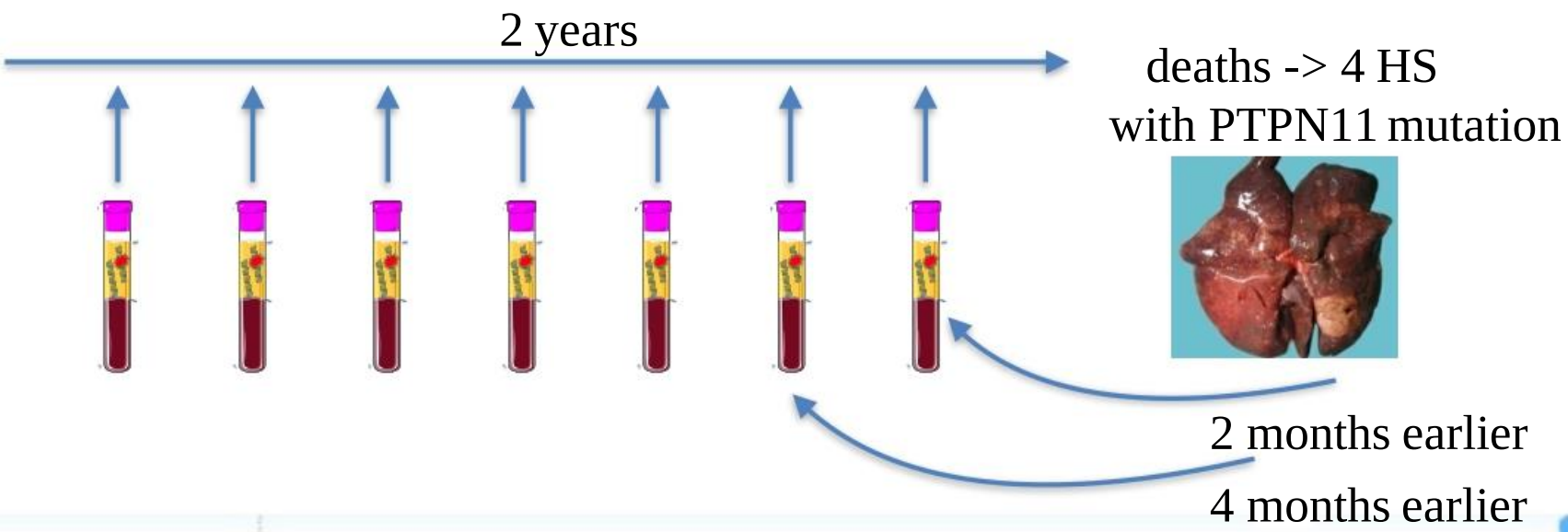
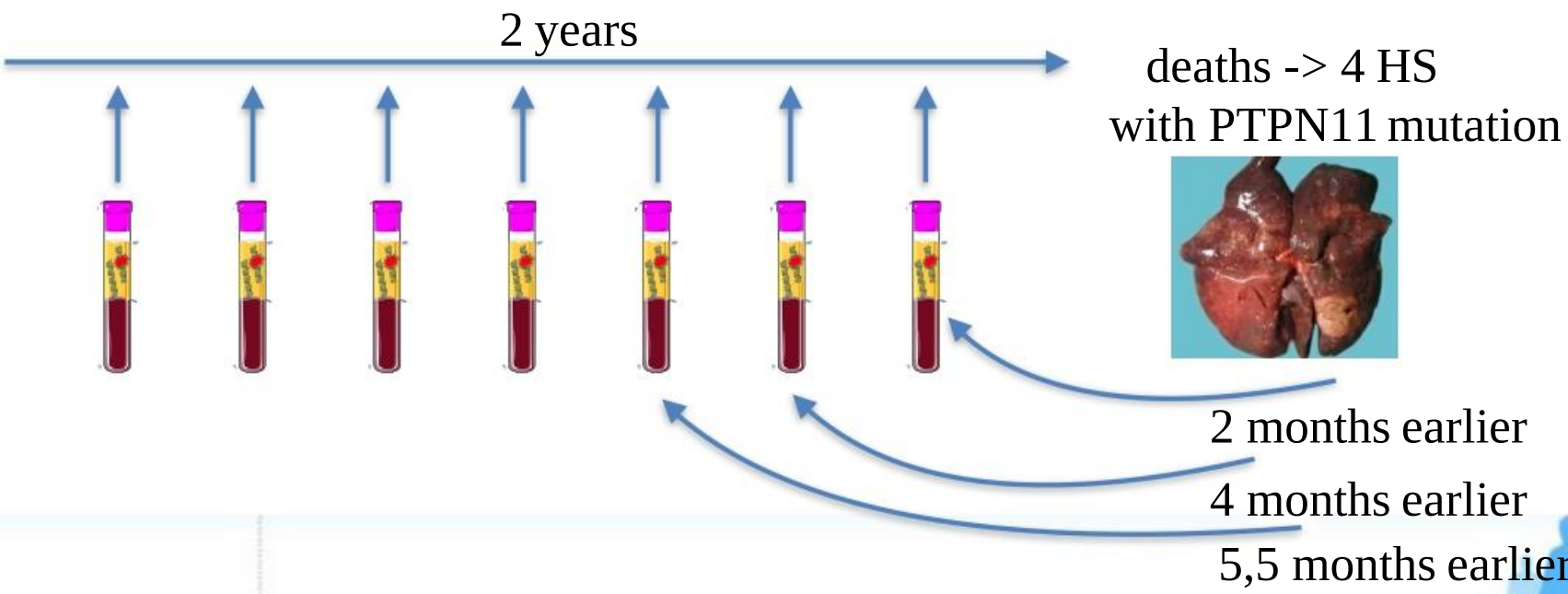
Follow up 30 dogs from two breeders : at risk (Age/ HS-test)



Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

Follow up 30 dogs from two breeders : at risk (Age/ HS-test)



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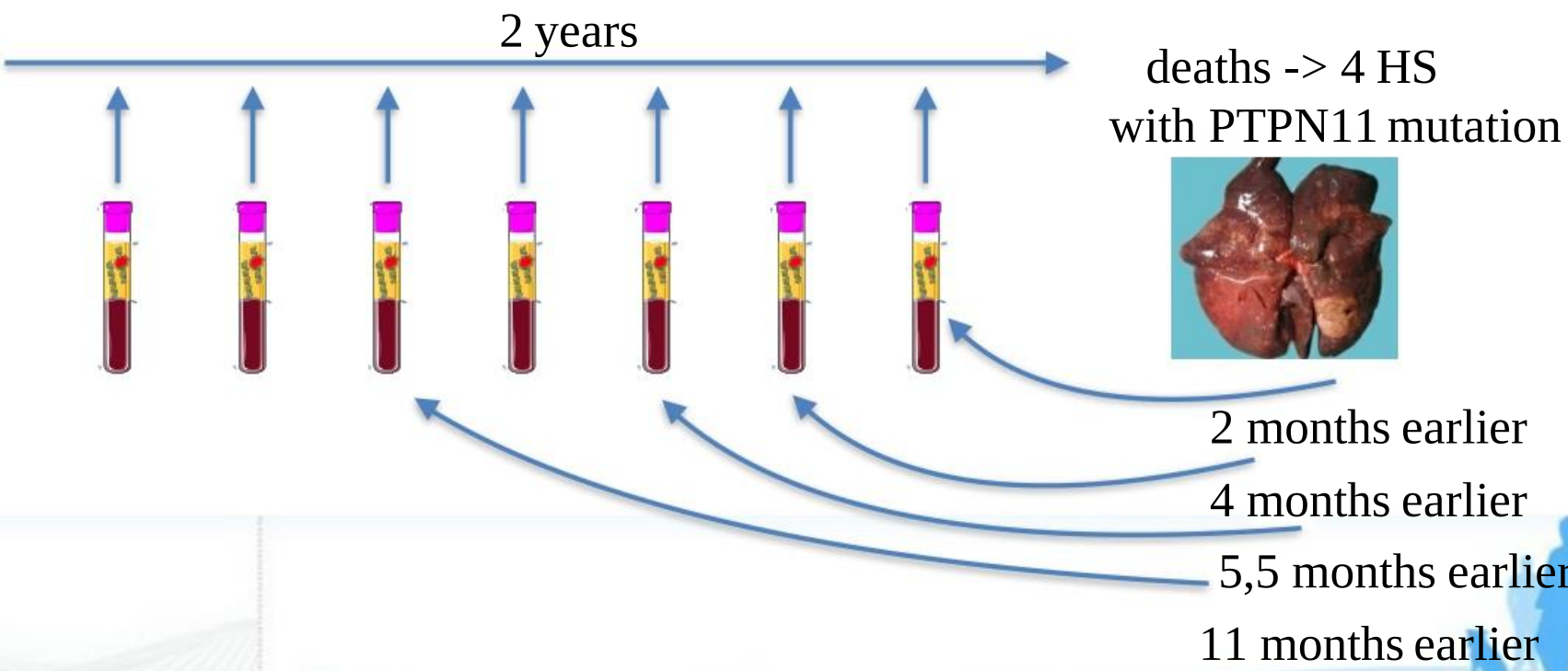

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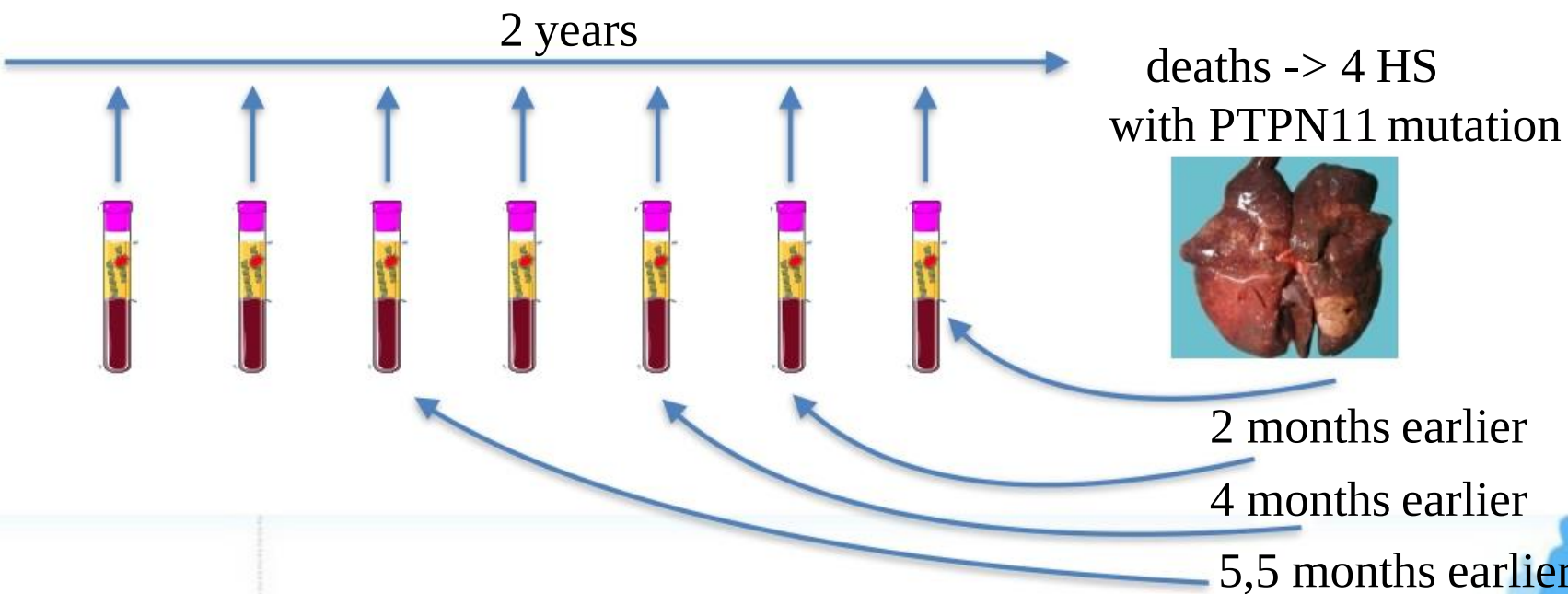


These markers could be used for an earlier diagnosis!

5,5 months earlier

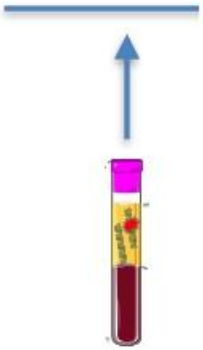
Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

Follow up 30 dogs ~~from to~~ breeder at risk (Age/HS-test)

2 years

deaths -> 4 HS with PTPN11 mutation



2 months earlier

4 months earlier

5,5 months earlier

11 months earlier

These markers could be used for an earlier diagnosis!

Detection of cancer 5 months earlier

PLOS ONE

RESEARCH ARTICLE

Clinical validation of a next-generation sequencing-based multi-cancer early detection "liquid biopsy" blood test in over 1,000 dogs using an independent testing set: The CANcer Detection in Dogs (CANDiD) study

Andi Flory[1,2,3], Kristina M. Kruglyak[1], John A. Tynan[1], Lisa M. McLennan[1], Jill M. Rafalko[1]*, Patrick Christian Fiaux[1], Gilberto E. Hernandez[1], Francesco Marass[1], Prachi Nakashe[1], Carlos A. Ruiz-Perez[1], Donna M. Faris[1], Thuy Lenhart[1], Rita Montalti-Pepio[1], Kate Wotrang[1], Angela L. McCleary-Wheeler[14], Susan Lana[5], Brenda Phillips[2], Brian K. Flesner[4,6], Nicole F. Leibman[7], Tracy LaDue[8], Chelsea D. Tripp[9], Brenda L. Coomber[10], J. Paul Woods[11], Mairin Miller[3], Sean W. Aiken[2], Amber Wolf-Ringwall[12], Antonella Borgatti[12], Kathleen Kraska[2], Christopher B. Thomson[3], Alane Kosanovich Cahalane[13], Rebecca L. Murray[9], William C. Kisseberth[14], Maria A. Camps-Palau[7], Franck Floch[15,16], Claire Beaudu-Lange[17], Aure´ lia Klajer-Peres[18], Olivier Keravel[18], Luc-Andre´ Fribourg-Blanc[19], Pascale Chigot-Marcotte[20], Angelo Marco[21], Molly B. McLeod[22], Erin Portillo[23], Terry S. Clark[24], Scott Judge[25], C. Kirk Feinberg[21], Marie Benitez[21], Candace Runyan[26], Lindsey Hackett[27], Scott Lafey[28], Danielle Richardson[11], Sarah Vineyard[29], Mary Tefend Campbell[30], Nilesh Dharajiya[31,33], Taylor J. Jensen[32,33], Dirk van den Boom[33], Luis A. Diaz, Jr.[33,34], Daniel S. Grosu[1], Arthur Polk[1], Kalle Marsal[1], Susan Cho Hicks[1], Katherine M. Lytle[1], Lauren Holtvoigt[1], Jason Chibuk[1], Ilya Chorny[1], Dana W. Y. Tsui[1]

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Benoit Hedan 2022

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

Follow up 30 dogs from to breeder : at risk (Age/ HS-test) -> interest for an earlier diagnosis

Confirm these results on more dogs

Addition of new mutations to be more sensitive

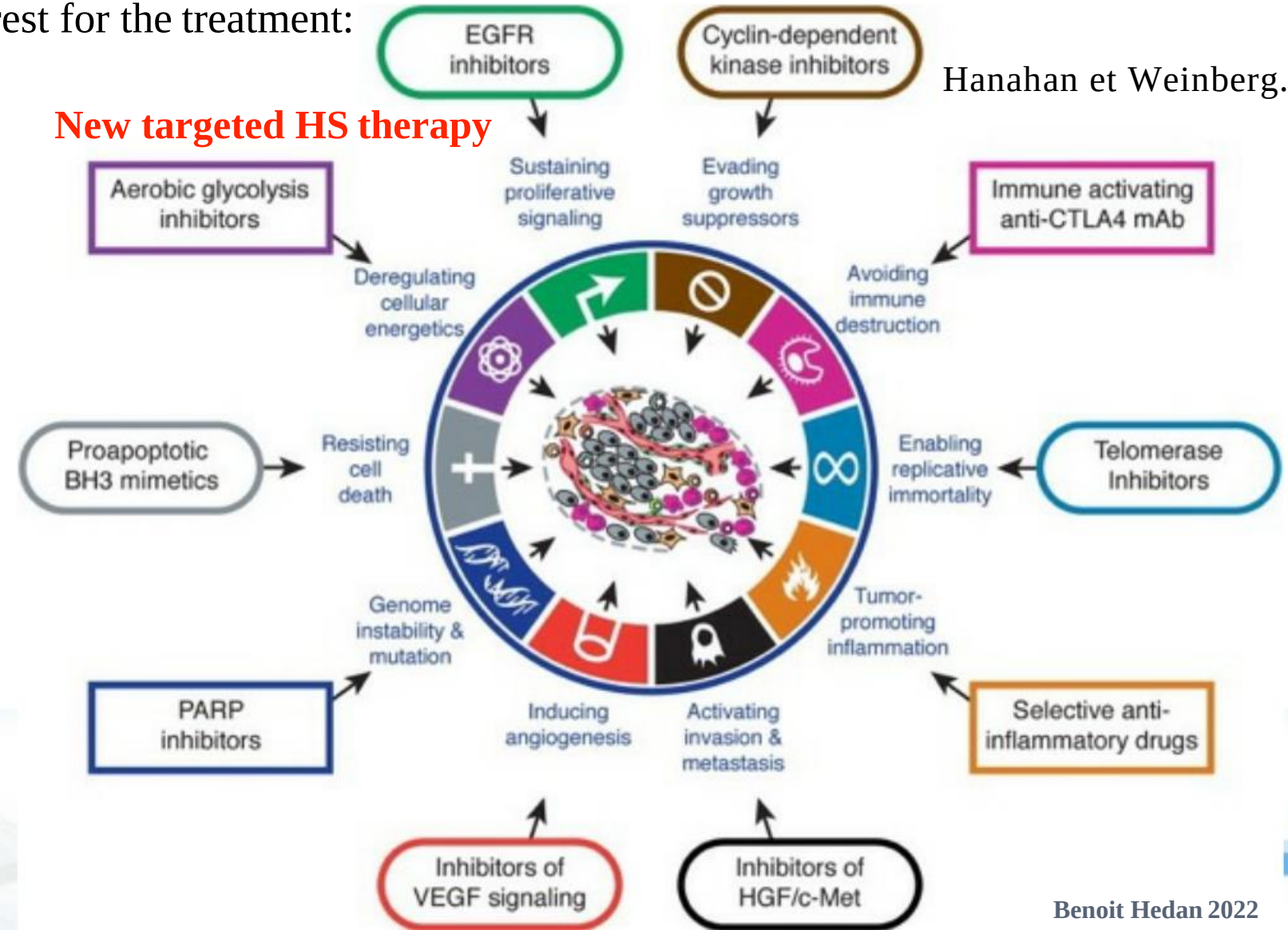
If we are able to detect earlier HS, do we are able to improve treatment of HS?

Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

Interest for the treatment:

Hanahan et Weinberg. Cell. 2

New targeted HS therapy



Benoit Hedan 2022

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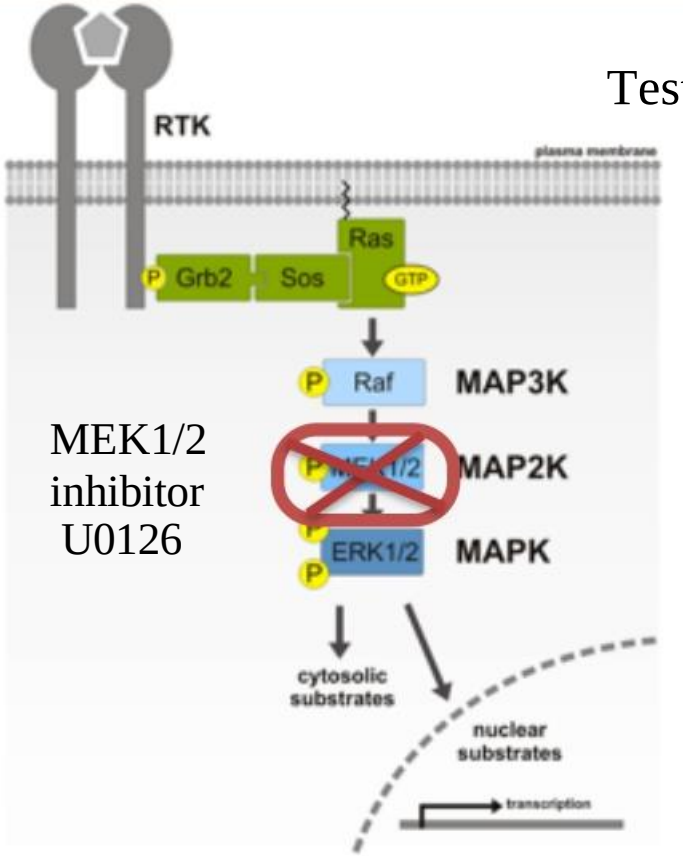
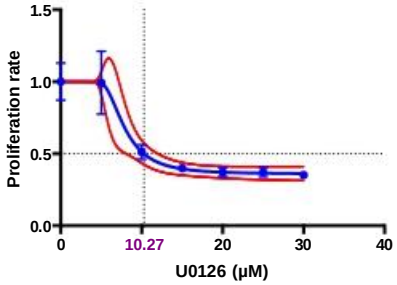


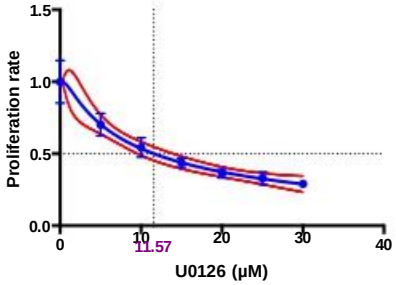
MEK1/2 inhibitor U0126

Tests of proliferation rate (72h) with MEK inhibitor

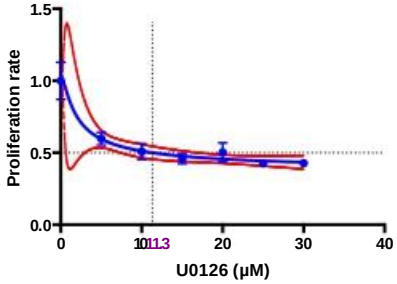
cell line 1



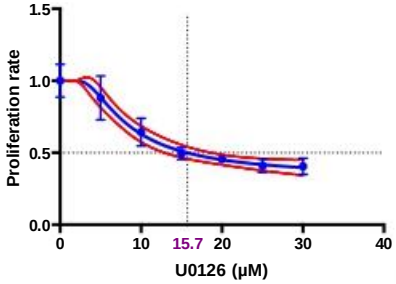
cell line 2



cell line 3

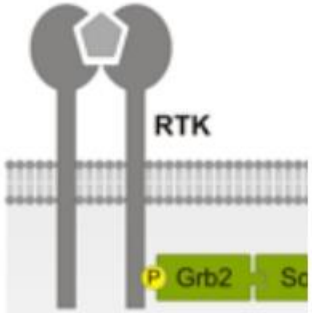


cell line 4



New therapy opportunities?

Mutations somatiques = opportunité pour développer des traitements ciblés



MEK1/2 inhibitor U0126

Cancer Biology and Translational Studies

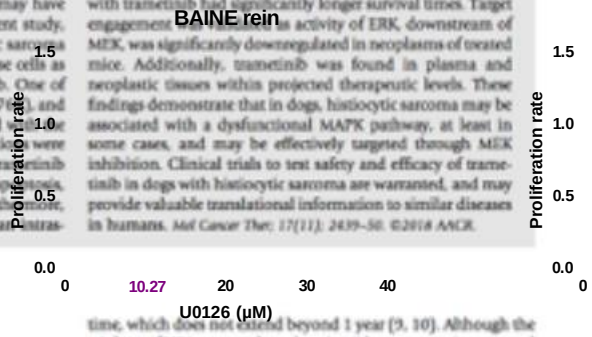
Molecular Cancer Therapeutics

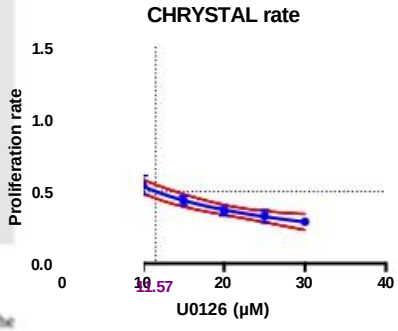
Targeting MEK in a Translational Model of Histiocytic Sarcoma

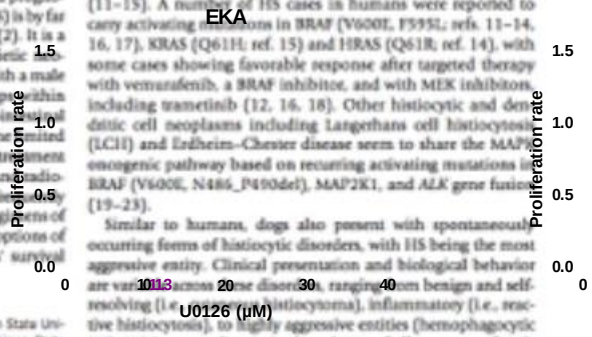
Marilia Takada[1], Jeremy M.L. Hix[2], Sarah Corner[1], Peter Z. Schall[1], Matti Kiupel[3], and Vilma Yuzbasiyan-Gurkan[1]

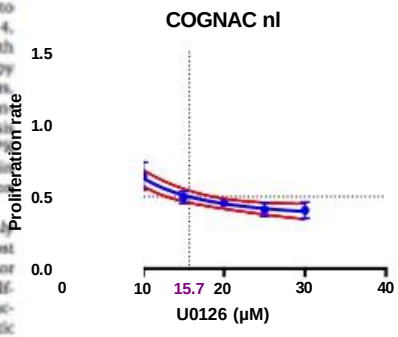
Abstract

Histiocytic sarcoma in humans is an aggressive orphan disease with a poor prognosis as treatment options are limited. Dogs are the only species that spontaneously develops histiocytic sarcoma with an appreciable frequency, and may have value as a translational model system. In the current study, high-throughput drug screening utilizing histiocytic sarcoma cells isolated from canine neoplasms identified these cells as particularly sensitive to a MEK inhibitor, trametinib. One of the canine cell lines carries a mutation in PTPN11 (E76K), and another one in KRAS (Q61H), which are associated with the activation of oncogenic MAPK signaling. Both mutations were previously reported in human histiocytic sarcoma. Trametinib inhibited sensitive cell lines by promoting cell apoptosis, indicated by a significant increase in caspase 3/7. Furthermore, *in vitro* findings were successfully recapitulated in an orthotopic-splenic orthotopic xenograft mouse model, which represents a disseminated aggressive form of histiocytic sarcoma. Mice with histiocytic sarcoma xenograft neoplasms that were treated with trametinib had significantly longer survival times. Target engagement and the level of activity of ERK, downstream of MEK, was significantly downregulated in neoplasms of treated mice. Additionally, trametinib was found in plasma and neoplastic tissues within projected therapeutic levels. These findings demonstrate that in dogs, histiocytic sarcoma may be associated with a dysfunctional MAPK pathway, at least in some cases, and may be effectively targeted through MEK inhibition. Clinical trials to test safety and efficacy of trametinib in dogs with histiocytic sarcoma are warranted, and may provide valuable translational information to similar diseases in humans. *Mol Cancer Ther; 17(11); 2439–50. ©2018 AACR.*









Introduction

Histiocytic and dendritic cell neoplasms encompass a group of proliferative entities with variable clinical behaviors and prognosis in humans (1). Among them, histiocytic sarcoma (HS) is by far the most aggressive disorder with the worst prognosis (2). It is a rare hematologic malignancy (<1% of all hematopoietic neoplasms) (3, 4) that affects all ages, but mostly adults, with a male predominance (5). Existing data show that HS develops within lymph nodes and at extranodal sites including the gastrointestinal tract, spleen, lungs, and head and neck (2, 5, 6). Given the limited occurrence, there is no consensus on standard-of-care treatment for HS in human patients. Although surgical resection and radiotherapy are attempted for local control, metastases frequently occur, and in these cases, systemic chemotherapy with regimens of drugs used for lymphoma is often utilized (6–8). More options of medical intervention are needed to improve patients' survival time, which does not extend beyond 1 year (9, 10). Although the etiology of HS is not yet clear, there is evidence suggesting a central role of the oncogenic RAS–RAF–MEK–ERK signaling pathway (11–15). A number of HS cases in humans were reported to carry activating mutations in BRAF (V600E, F595L; refs. 11–14, 16, 17), KRAS (Q61H; ref. 15) and HRAS (Q61R; ref. 14), with some cases showing favorable response after targeted therapy with vemurafenib, a BRAF inhibitor, and with MEK inhibitors, including trametinib (12, 16, 18). Other histiocytic and dendritic cell neoplasms including Langerhans cell histiocytosis (LCH) and Erdheim–Chester disease seem to share the MAPK oncogenic pathway based on recurring activating mutations in BRAF (V600E, N486_P490del), MAP2K1, and *ALK* gene fusions (19–23).

Similar to humans, dogs also present with spontaneously occurring forms of histiocytic disorders, with HS being the most aggressive entity. Clinical presentation and biological behavior are variable across these disorders, ranging from benign and self-resolving (i.e., reactive histiocytoma), inflammatory (i.e., reactive histiocytosis), to highly aggressive entities (hemophagocytic HS). HS is a rare disease in dogs (<1% of all cancers; ref. 24); however, it is overrepresented in certain breeds, especially in Bernese mountain dogs (BMD; incidence of about ~25% in the population; ref. [illegible]). Other breeds that also present with HS include flat-coated [illegible]. Males and females are equally affected, and dogs are most often in adult age (8–10 years; refs. 26, 27). In dogs, HS presents most commonly in the skin, bone/joint, spleen, lymph node, lungs, and liver (27–29). Due to a high incidence of metastasis, reported as 70% to 90% (27, 28, 30–32), systemic chemotherapy is the

[1]Comparative Medicine and Integrative Biology Program, Michigan State University, East Lansing, Michigan. [2]Department of Radiology, Michigan State University, East Lansing, Michigan. [3]Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, Michigan.

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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Nouvelles thérapies ?

Mutations somatiques = opportunité pour développer des traitements ciblés

Cancer Biology and Translational Studies

Molecular Cancer Therapeutics

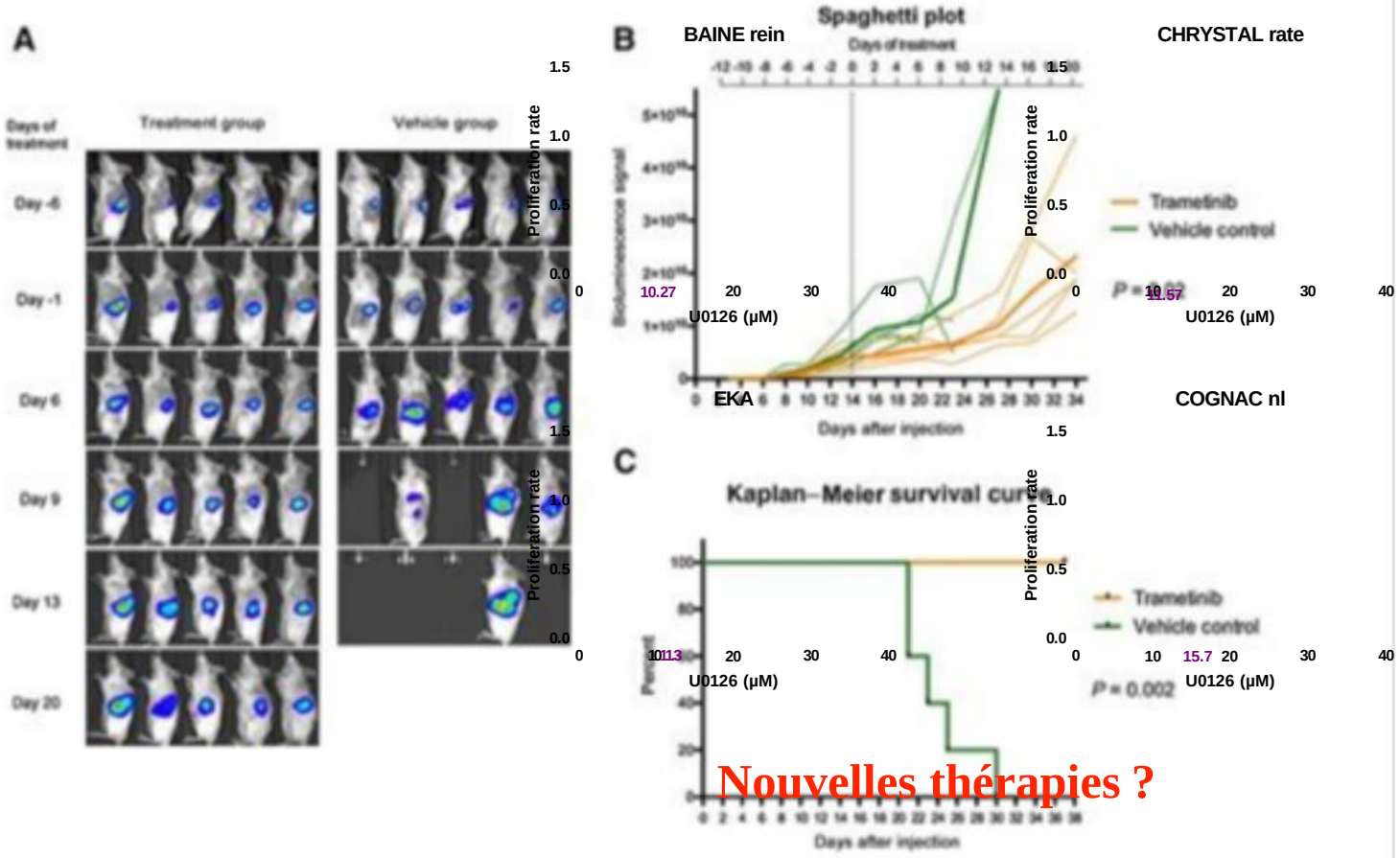
Targeting MEK in a Translational Model of Histiocytic Sarcoma

Marilia Takada[1], Jeremy M.L. Hix[2], Sarah Corner[1], Peter Z. Schall[2], Matti Kiupel[3], and Vilma Yuzbasiyan-Gurkan[1]

Tests de prolifération (72h) avec des inhibiteurs de MEK

Figure 3.

MEK1/2 inhibitor U0126



Nouvelles thérapies ?

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Cancer Biology and Translational Studies

Targeting MEK in a Translational Model of Histiocytic Sarcoma

Molecular Cancer Therapeutics

Tufts CUMMINGS VETERINARY MEDICAL CENTER

Clinical Trials Office

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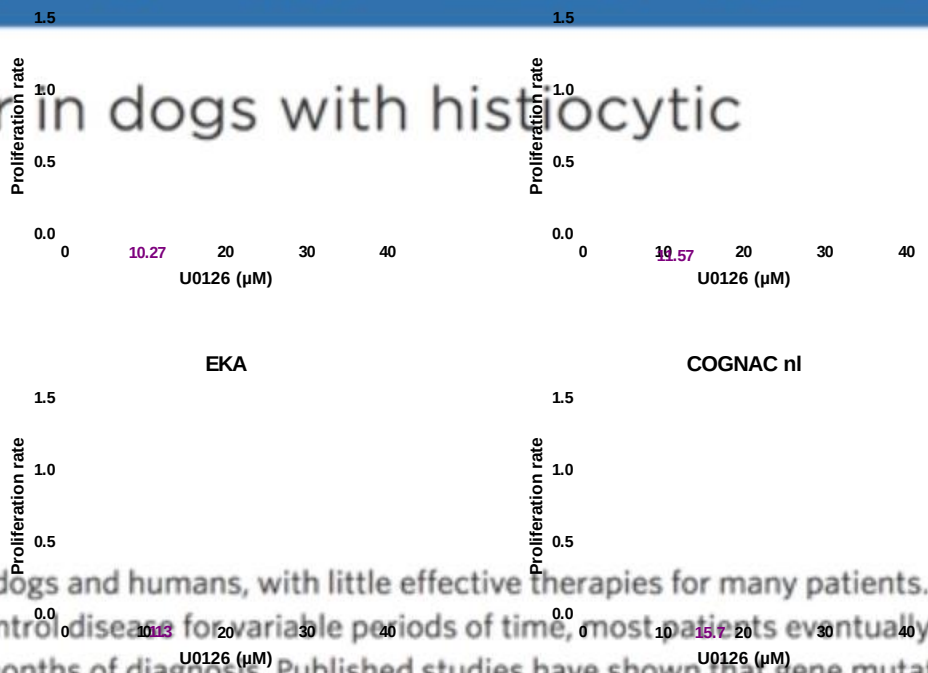
Tests de prolifération (72h) avec des inhibiteurs de MEK



Pilot study of a MEK inhibitor in dogs with histiocytic sarcoma

MEK1/2 inhibitor U0126

Status: Recruiting

Species: Dog

Veterinary Speciality: Oncology

Description

Histiocytic sarcoma represents a devastating disease in both dogs and humans, with little effective therapies for many patients. While surgery, radiation and chemotherapy can be used to control disease for variable periods of time, most patients eventually fail treatment and die of tumor spread (metastasis) within 6-12 months of diagnosis. Published studies have shown that gene mutations (mostly the PTPN11 gene) in canine histiocytic sarcoma drive activation of a specific cell signaling pathway called the MAPK pathway. One of the components of this pathway is a protein called MEK. Studies of canine histiocytic tumor cells in the laboratory and in mouse models of disease show that an inhibitor of MEK called trametinib can slow or stop growth of this tumor. The purpose of this clinical trial is to evaluate the effectiveness, safety and blood levels of the MEK inhibitor trametinib in dogs with histiocytic sarcoma. While trametinib was approved by the FDA in 2013 to treat people with melanoma (an aggressive skin cancer) and has now been used to treat people with several other kinds of cancers, it has not been studied formally in dogs (i.e., there have been no

Nouvelles thérapies ?

Conclusion

Test HS : first genetic risk test for dogs

-> validated for all BMD origins

-> follow up of dogs confirms the interest of this test for selection

-> research is still needed to improve our knowledge on genetic bases of HS predisposition and hematopoietic cancers!

Unique test for a canine cancer

Somatic mutations in HS tumors

- > Useful markers for an earlier diagnosis
for treatment ?

Double benefit for vet and human medicine

Looking for samples (suspicion/cases, old dogs…) to pursue research!

Looking for the follow up of dogs tested with the HS test!

Contact us : benoit.hedan@univ-rennes1.fr

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Matthew Breen

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Annemarie Thuri Kristensen

Antagene
Caroline Dufaure de Citres
Guillaume Queney
Lyon France

French Vet Schools
Jerome Abadie
Patrick Devauchelle

University of Uppsala
Kerstin Lindblad-Toh

Owners, breeders,

All clubs, Berner Garde,... who sent samples

Fundings CNRS, AKC, INCa,
BMD clubs :

Diagnostic of HS

- Gold standard histology:
  large pleomorphic cells, whorls of spindle cells, or a mixture of the two

  initial differential diagnoses include synovial cell sarcoma , lymphoma and plasmacytoma , mast cell tumor, and amelanotic melanoma

- Immunostaining is necessary in many cases to confirm histiocytic origin of tumoral cells: IBA1 (Macrophage/dendritic cells) (Pierezan et al. 2014), CD204 (Macrophage cells) (Kato et al. 2014). Our cases are confirmed with CD204 by Jerome Abadie (ONIRIS)

Diagnostic of HS
use of genetic tools

Genome-wide DNA copy number analysis and targeted transcriptional analysis of canine histiocytic malignancies identifies diagnostic signatures and highlights disruption of spindle assembly complex

Check for updates

Katherine Kennedy · Rachael Thomas ·
Jessica Durrant · Tao Jiang · Alison Motsinger-R
Matthew Breen

The copy number of CFA 16 and 31 are diagnostic signatures for HM. When comparing HM to
diseases that are frequently included in the differential process (HEM, MEL, LSA, MCT, CYT, and PL), the overall specificity is 94.7%.

Received: 11 January 2019 / Revised: 18 February 2019 / Acc
© Springer Nature B.V. 2019

Abstract Canine histiocytic malignancies (HM) are rare across the general dog population, but overrepresented in certain breeds, such as Bernese mountain dog and flat-coated retriever. Accurate diagnosis relies on immunohistochemical staining to rule out histologically similar cancers with different prognoses and treatment strategies (e.g., lymphoma and hemangiosarcoma). HM are generally treatment refractory with overall survival of less than 6 months. A lack of understanding regarding the mechanisms of disease development and progression hinders development of novel therapeutics. While the study of human tumors can benefit veterinary medicine, the rarity of the suggested orthologous disease (dendritic cell sarcoma) precludes this. This study aims to improve the understanding of underlying disease mechanisms using genome-wide DNA copy number and gene expression analysis of spontaneous HM across several dog breeds. Extensive DNA copy

Diagnostic of HS
use of genetic tools

- liquid biopsy:

PTPN11 mutations high specificity vs low sensitivity

OncoK9 :multi-cancer early detection (MCED) “liquid biopsy” test using next-generation sequencing (NGS)

overall sensitivity (detection rate) of 54.7% -
it detected cancer signal from 30 distinct cancer types in 433 cancer-diagnosed subjects evaluated

not specific of HS

Treatment of HS
use of genetic tools

FidoCure®

Identification of somatic mutations to prescribe targeted therapies (not specific of HS)

Histiocytic sarcoma

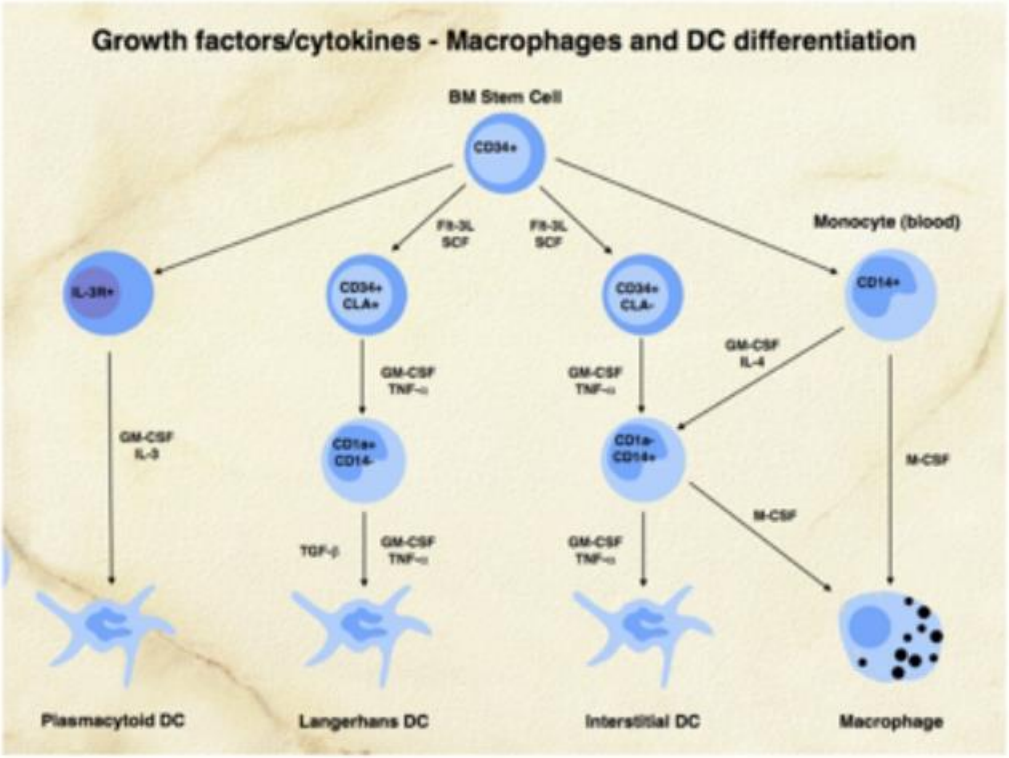


Fig. 1. Stem cell factors (SCF and Flt-3 Ligand) amplify histiocyte differentiation - especially to DCs. GM-CSF and TNF-alpha induce differentiation toward DCs (interstitial DCs and LCs); TGF-beta influences the terminal stages of LC differentiation. M-CSF induces macrophage differentiation.

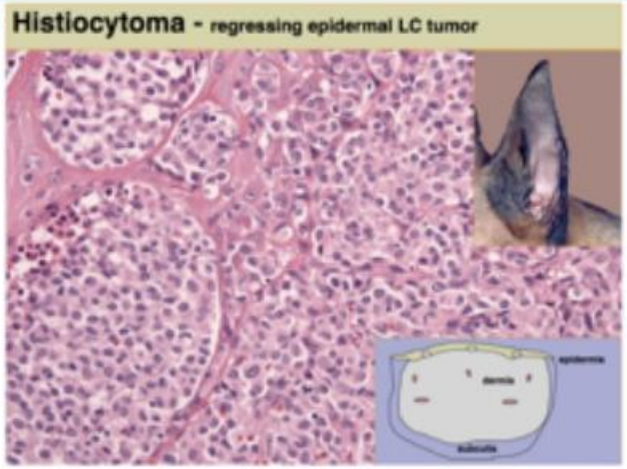
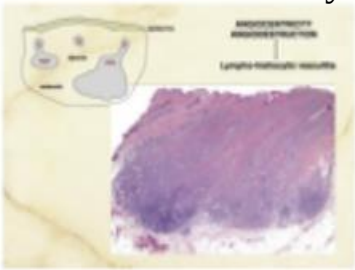


Fig. 3. Multiple histiocytomas on the margin of the pinna and in the ear canal; Drawing emphasizes the epidermal focus of histiocytomas; HE section of histiocytoma with intra-epidermal invasion

Reactive cutaneous/systemic histiocytosis



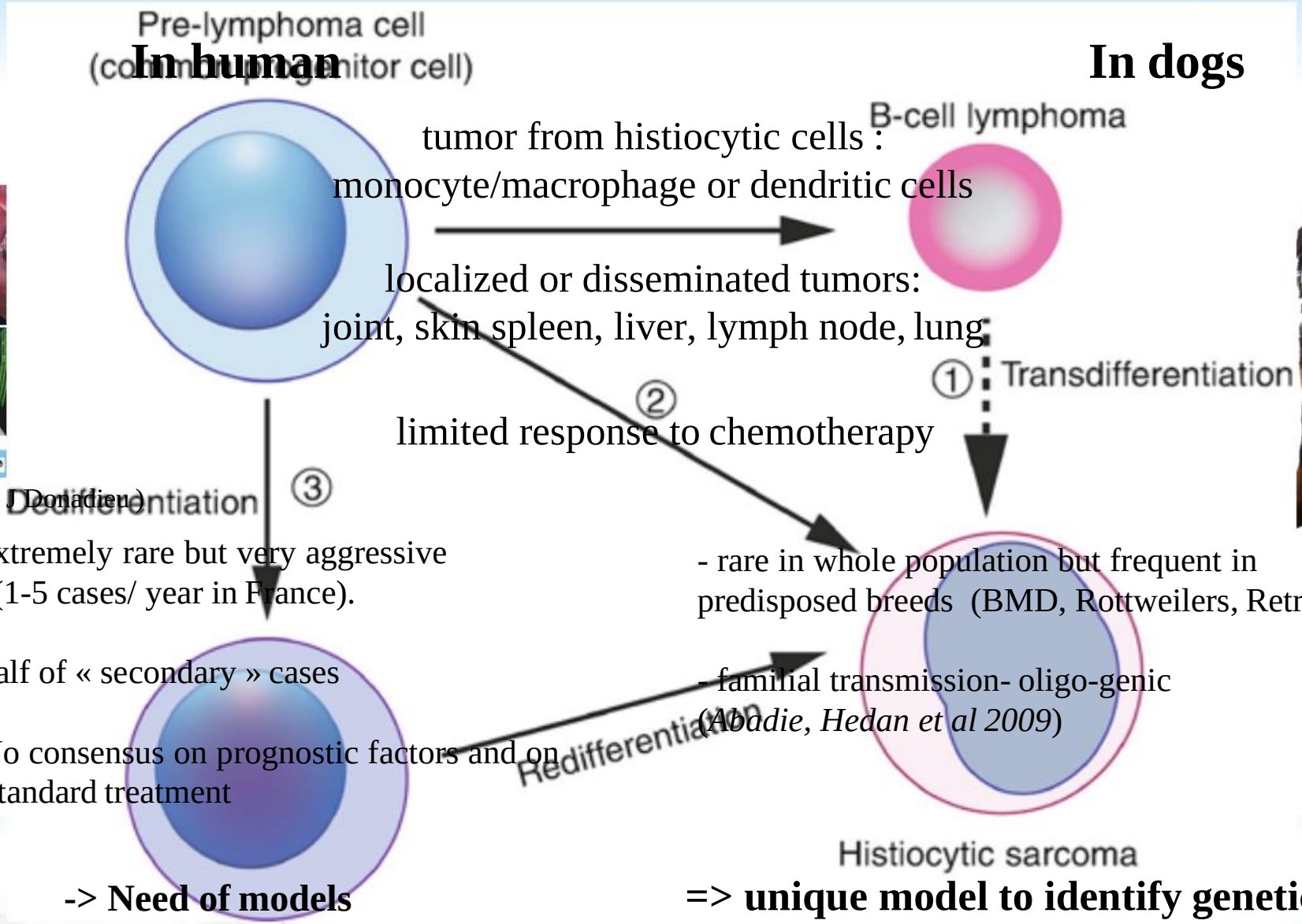
https://www.histiocytosis.ucdavis.edu/histiocytosis/ Peter F Moore

Histiocytic Sarcoma (HS)

In human

In dogs

(Credit : J Donadieu)



tumor from histiocytic cells :
monocyte/macrophage or dendritic cells

localized or disseminated tumors:
joint, skin spleen, liver, lymph node, lung

- extremely rare but very aggressive
  (1-5 cases/ year in France).

- half of « secondary » cases

- No consensus on prognostic factors and on standard treatment

-> Need of models

- rare in whole population but frequent in predisposed breeds (BMD, Rottweilers, Retrievers)

- familial transmission- oligo-genic
  (*Abadie, Hedan et al 2009*)

=> unique model to identify genetic bases of this cancer

Clinical presentations

previously called: malignant fibrous histiocytoma (MFH), malignant histiocytosis…

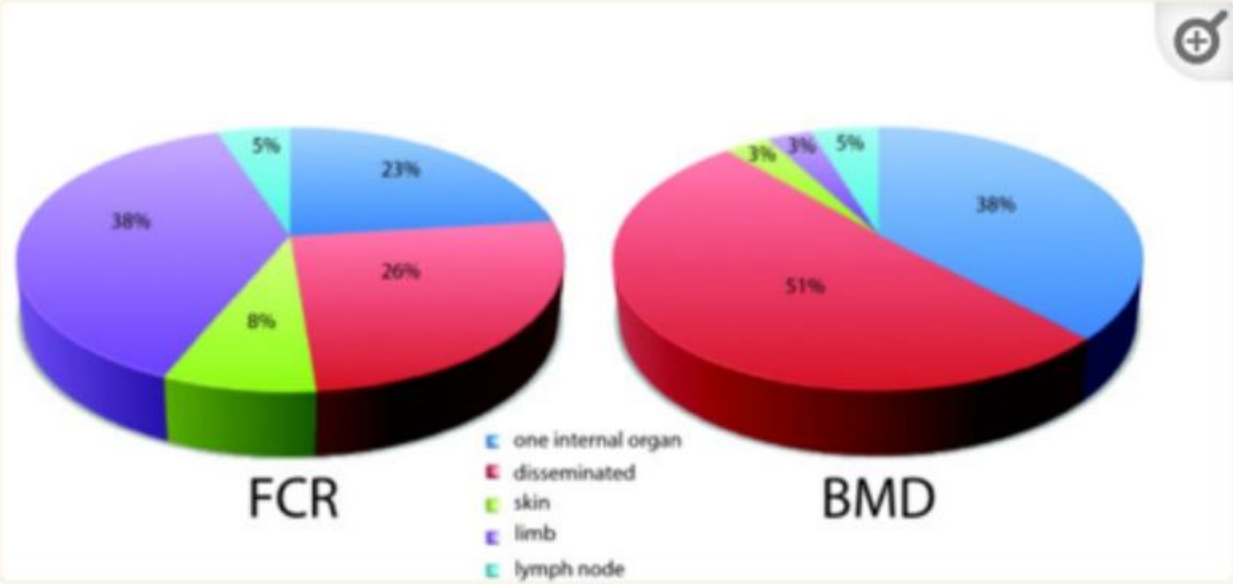
localized HS (LHS):

is considered to originate on a limb, skin, or within a single internal organ, commonly the spleen, the lung

disseminated HS (DHS)

is described as a multifocal disease with masses occurring simultaneously at multiple sites (most commonly spleen, lung, liver, and abdominal lymph nodes)

NB : hemophagocytic HS in spleen or bone marrow



Hedan et al 2011 BMC cancer

Figure 2

Anatomical distribution of histiocytic tumors in FCR and BMD. Anatomical location of HS is significantly different between the two breeds (p value < 0.001, Fisher Exact test).

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Clinical presentations

NOTE *Pathology*

Localized pulmonary histiocytic sarcomas in Pembroke Welsh Corgi

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ABSTRACT Nineteen cases of histiocytic sarcomas in Pembroke Welsh Corgi were examined histopathologically. Focal or multiple masses were detected in the lung or in regional lymph nodes, or in both lung and nodes. All neoplastic lesions had common histological features characterized by the proliferation of pleomorphic histiocytic cells combined with various inflammatory cells. Most of the pleomorphic neoplastic cells were immunopositive for human leukocyte antigen (HLA)-DR and Iba-1. The median survival time for all dogs was 133 days. In the present study, several prognostic factors, such as gender, age, single or multiple lesions, lymph node involvement at the time of diagnosis, surgical resection status and additional chemotherapy, were examined, although none of these factors approached statistical significance. Histiocytic sarcoma must be considered in the differential diagnosis of dogs with pulmonary masses, especially in the canine breed.
KEY WORDS histiocytic sarcoma, lung, Pembroke Welsh Corgi

doi: 10.1292/jvms.15-0284; *J. Vet. Med. Sci.* 77(12): 1659–1661, 2015

J Vet Diagn Invest 23:127–132 (2011)

CASE REPORTS

Pathological and immunohistochemical features of subdural histiocytic sarcomas in 15 dogs

Tetsuya Ide, Kazuyuki Uchida,[1] Yumiko Kagawa, Kazuhiko Suzuki, Hiroyuki Nakayama

Abstract. Subdural histiocytic sarcomas from 15 dogs (mean age: 7.8 years) were histopathologically examined. Among the 15 dogs, there was a marked breed predominance (toward Pembroke Welsh Corgi dogs 47%), but no gender predilection. Focal solitary subdural masses were detected in the cerebrum (12 cases) and spinal cord (1 case), whereas diffuse infiltrative lesions were observed in the cerebral leptomeninges in 2 cases. All neoplastic lesions had common histological features characterized by the proliferation of pleomorphic histiocytic cells combined with various inflammatory reactions. Multinucleated giant cells, phagocytosis, and atypical mitotic figures in the neoplastic cells were commonly observed. Most of the pleomorphic neoplastic cells in the present cases were immunopositive for monocytic, histiocytic, or both markers, such as human leukocyte antigen (HLA)-DR, ionized calcium-binding adaptor molecule 1 (Iba1), cluster of differentiation (CD)163, and CD204, except for the neoplastic cells in 2 focal and 2 diffuse histiocytic sarcomas. The findings suggest that differences in cell origin, molecular expression, or both patterns are responsible for the distribution patterns of canine subdural histiocytic sarcomas.

Key words: Dogs; histiocytic sarcomas; immunohistochemistry; leptomeninges.

Canine proliferative histiocytic diseases are implicat
a range of disorders with marked differences in clinical m
festations and pathologic features [1, 3, 8]. Canine histio
neoplasia occurs as a localized tumor or a disseminated
plastic process [1, 8]. Disseminated histiocytic sarcoma
first described as malignant histiocytosis of Bernese M
tain Dog [1, 8]. Localized histiocytic sarcomas develop
a single site [1, 5]. They are locally invasive and metast
to draining lymph nodes. They arise most often in the su
tis, but may be seen in other primary locations, such a
lung, spleen and liver. The prevalence in dogs is prim
found within a narrow range of breeds, especially the Ber
Mountain Dog (for systemic HS) and Flat-Coated Retri
(for localized type) [1, 2, 5, 8]. Pembroke Welsh Corgi i
of the most popular breeds in Japan; for several years,
has been a suspicion of increased neoplasia in this bree
7, 10]. Although pulmonary involvement has been recogn
in dogs with disseminated (secondary) HS, published re

The World Health Organization classification for brain tumors in domestic animals includes malignant histiocytosis,[6] and the involvement of systemic central nervous

during the last 5 years (2005–2009). The breed, sex, age, major neurological signs, tumor location, and distribution patters are shown in Table 1. Among the 15 dogs were 7 Pembroke Welsh Corgis (47%), 2 Bernese Mountain Dogs (13%), 1 Wire Fox Terrier (7%), 1 Rottweiler (7%), 1 Newfoundland (7%), 1 Norfolk Terrier (7%), 1 Chihuahua (7%), and 1 Miniature Schnauzer (7%). No gender redilection was detected. The mean age of the dogs was 8 years (5 years 10 months to 11 years). The majority of the dogs had a history of neurological signs, including tumors, postural reaction deficits, and paresis. Focal dilatory subdural masses were found in the cerebrum (12 ases) and spinal cord (1 case), whereas diffuse infiltrative sions were observed in the cerebral leptomeningies in 2 ses. Clinicopathological evaluation of the cases revealed o involvement of other visceral organs.

Tissue lesion samples were fixed in 10% phosphate uffered formalin solution before being embedded in araffin. The paraffin sections (3-4 μm thick) were then ained with hematoxylin and eosin. Histological classifi- tion of the canine histiocytic sarcomas was performed ccording to whether the tumor was distributed locally or iffusely. Immunohistochemistry was performed using the extran polymer method.[5] The primary antibodies used and heir positive reactions in canine tissues are listed in

Journal of Veterinary Internal Medicine

Open Access

J Vet Intern Med 2015;29:607–613

Histiocytic Sarcoma with Central Nervous System Involvement in Dogs: 19 Cases (2006–2012)

C.L. Mariani, M.K. Jennings, N.J. Olby, L.B. Borst, J.C. Brown Jr, I.D. Robertson, G.S. Seiler, and E. MacKillop

Background: Reports of histiocytic sarcoma (HS) involving the central nervous system (CNS) are sparse and consist mainly of case reports describing 1–3 animals.

Objective: The objective of this study was to report the signalments, clinical signs, clinicopathologic and diagnostic imaging findings, treatment, and outcome of a series of dogs with HS and CNS involvement.

Animals: Nineteen dogs with HS examined at veterinary referral hospitals.

Methods: Retrospective case series. Medical records were reviewed and cases with a histopathological diagnosis of CNS HS were included in the study. Diagnostic imaging studies of the CNS were evaluated and histopathologic samples were reviewed to confirm the diagnosis.

Results: Retrievers and Pembroke Welsh Corgis were overrepresented in this cohort of dogs. Tumors involved the brain in 14 dogs and the spinal cord in 5. In 4 dogs, HS was part of a disseminated, multiorgan process whereas it appeared confined to the CNS in 15 dogs. Diagnostic imaging had variable appearances although extraaxial masses predominated in the brain. There was meningeal enhancement in all dogs that was often profound and remote from the primary mass lesion. Pleocytosis was present in all dogs with CSF evaluation. Median survival was 3 days.

Conclusions and Clinical Importance: Breed predispositions appear to vary from reports of HS in other organ systems. Some unique imaging and clinicopathologic characteristics, particularly brain herniation, profound meningeal enhancement, and pleocytosis in combination with 1 or more mass lesions, might help to differentiate this neoplasm from others involving the CNS, although this requires further study.

Key words: Brain tumor; Malignant histiocytosis; Round cell tumor; Spinal cord tumor.

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Clinical presentations

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F SMALL ANIMAL PRACTICE

PAPER

Histiocytic sarcoma in 14 miniature schnauzers – a new breed predisposition?

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Objectives: To describe a series of miniature schnauzers diagnosed with histiocytic sarcoma and assess for possible breed predisposition.

Materials and Methods: Medical records of miniature schnauzers with a diagnosis of histiocytic sarcoma between January 2008 and April 2015 were reviewed. Data collected included signalment, body weight, presenting complaint, date of diagnosis, clinicopathologic and diagnostic imaging findings, treatment, therapeutic response, date of death or last follow-up and necropsy findings. Breed predisposition was assessed with odds ratios, using breed-matched dogs without histiocytic sarcoma admitted during the study period as controls. Pedigree analysis was performed for dogs with available registration information.

Results: Fourteen miniature schnauzers were diagnosed with histiocytic sarcoma during the study period, making them over-represented among the hospital population (odds ratio=4·8, P=0·0009). Disease was considered localised in ten dogs and disseminated in four. Of the dogs with localised disease, nine were diagnosed with primary pulmonary histiocytic sarcoma based on the presence of a large pulmonary mass with (n=7) or without (n=2) evidence of intra-thoracic metastasis, and one had gastric histiocytic sarcoma with nodal metastasis. Treatments varied, but an aggressive clinical course was found in most patients. Pedigree analysis revealed a recent common ancestor for a subset of the dogs assessed.

Clinical Significance: Miniature schnauzers were over-represented among dogs with histiocytic sarcoma in this patient population. Pedigree analysis supports an inherited risk factor, which has not previously been suggested in the breed. Primary pulmonary involvement with or without intra-thoracic metastasis was common in this cohort.

BSAVA
SMALL ANIMAL PRACTICE

PAPER

Histiocytic sarcoma in miniature schnauzers: 30 cases

K. Purzycka[1,2,3], L. M. Peters[4], J. Elliott[5], C. R. Lamb[1*], S. L. Priestnall[3], A. Hardas[3], C. A. Johnston[4] and I. Rodriguez-Piza[6]

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Objectives: To summarise the clinical presentation and outcomes in a series of miniature schnauzers diagnosed with histiocytic sarcoma.

Materials and Methods: Retrospective review of medical records of miniature schnauzers diagnosed with histiocytic sarcoma between 2008 and 2019 at two referral centres in the UK. Signalment, clinical signs at initial presentation, imaging results and clinico- and histopathological findings, treatment type and outcome were recorded. Progression-free survival and overall survival time were calculated.

Results: Thirty dogs were included. Twenty-four of 29 dogs undergoing imaging of the thorax had lung and/or mediastinal involvement. The median overall survival time for dogs that were not euthanased within 3 days of diagnosis was 117 days (range 10 to 790). Three dogs underwent surgery; 13 received treatment with lomustine as a sole therapy - with partial responses documented on imaging in five of six dogs and 11 of 13 showing clinical improvement.

Clinical Significance: Histiocytic sarcoma should be considered as a differential diagnosis for miniature schnauzers with pulmonary masses. Although responses to treatment were common, they were usually short-lived because of the aggressive nature of the disease.

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Accepted: 14 March 2020

INTRODUCTION

Canine histiocytic tumours encompass a group of neoplasms with

et al. 2015, Mariani *et al.* 2015, Dervisis *et al.* 2017, Marlowe *et al.* 2018) while the [illegible] organs involvement.

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Canine histiocytic sarcoma:
Somatic mutations : applications for veterinary medicine

Received: 10 January 2022 | Revised: 9 March 2022 | Accepted: 9 March 2022
DOI: 10.1111/vco.12812

ORIGINAL ARTICLE WILEY

mRNA sequencing analysis and growth inhibitory effects of palbociclib on cell lines from canine histiocytic proliferative disorders

loss in >67.7% of HS

Miyuki Hirabayashi[1,2] | James K. Chambers[1] | Akiyoshi Tani[3] |
Hirotaka Tomiyasu[3] | Tomoki Motegi[4] | Kenji Rimpo[2] | Hiroyuki Nakayama[1] |
Kazuyuki Uchida[1]

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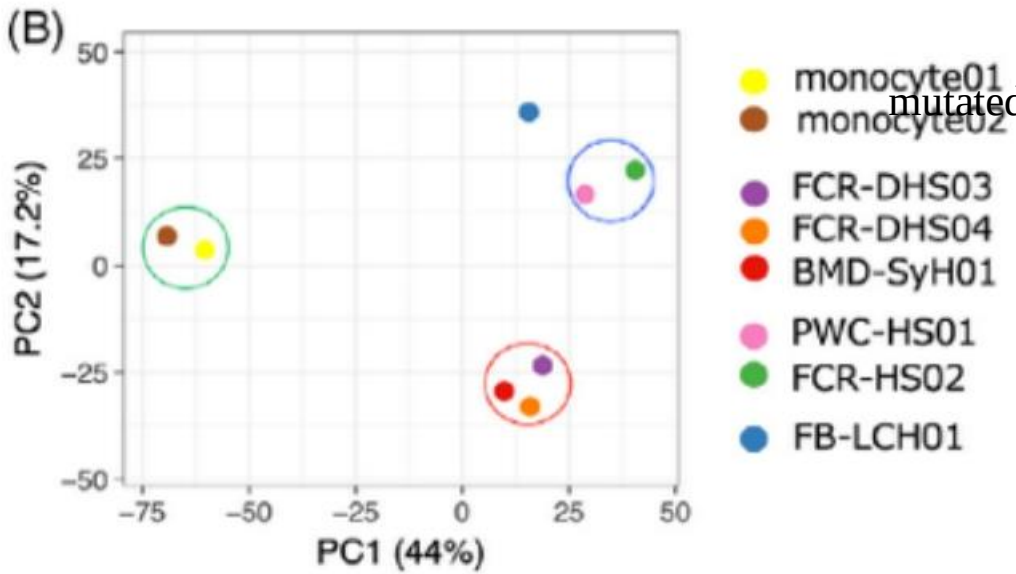
Abstract
Canine histiocytic proliferative disorders include aggressive and fatal diseases, such

mutated/deleted in >50% of HS

Palbociclib



mutated/deleted in >50% of HS

CDK4/6 inhibitor, histiocytic sarcoma, Langerhans cell histiocytosis, palbociclib, RNA sequencing, systemic histiocytosis

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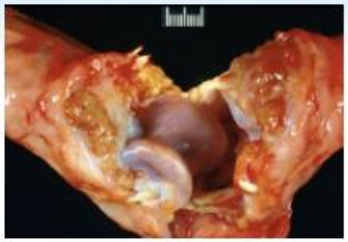

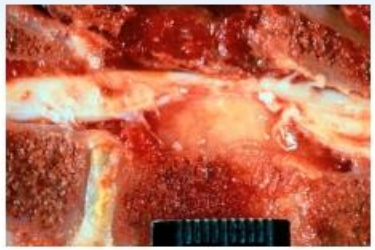

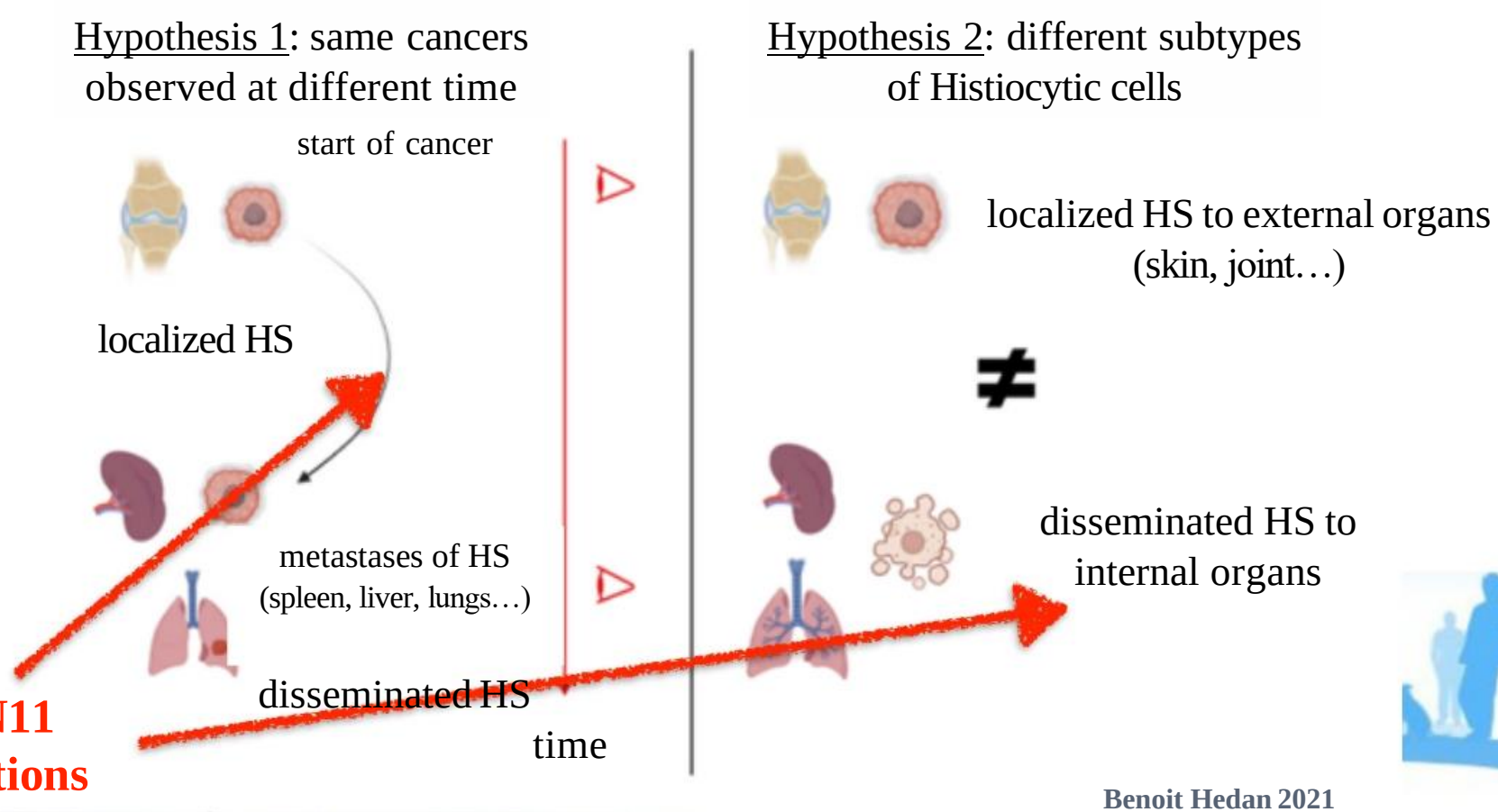

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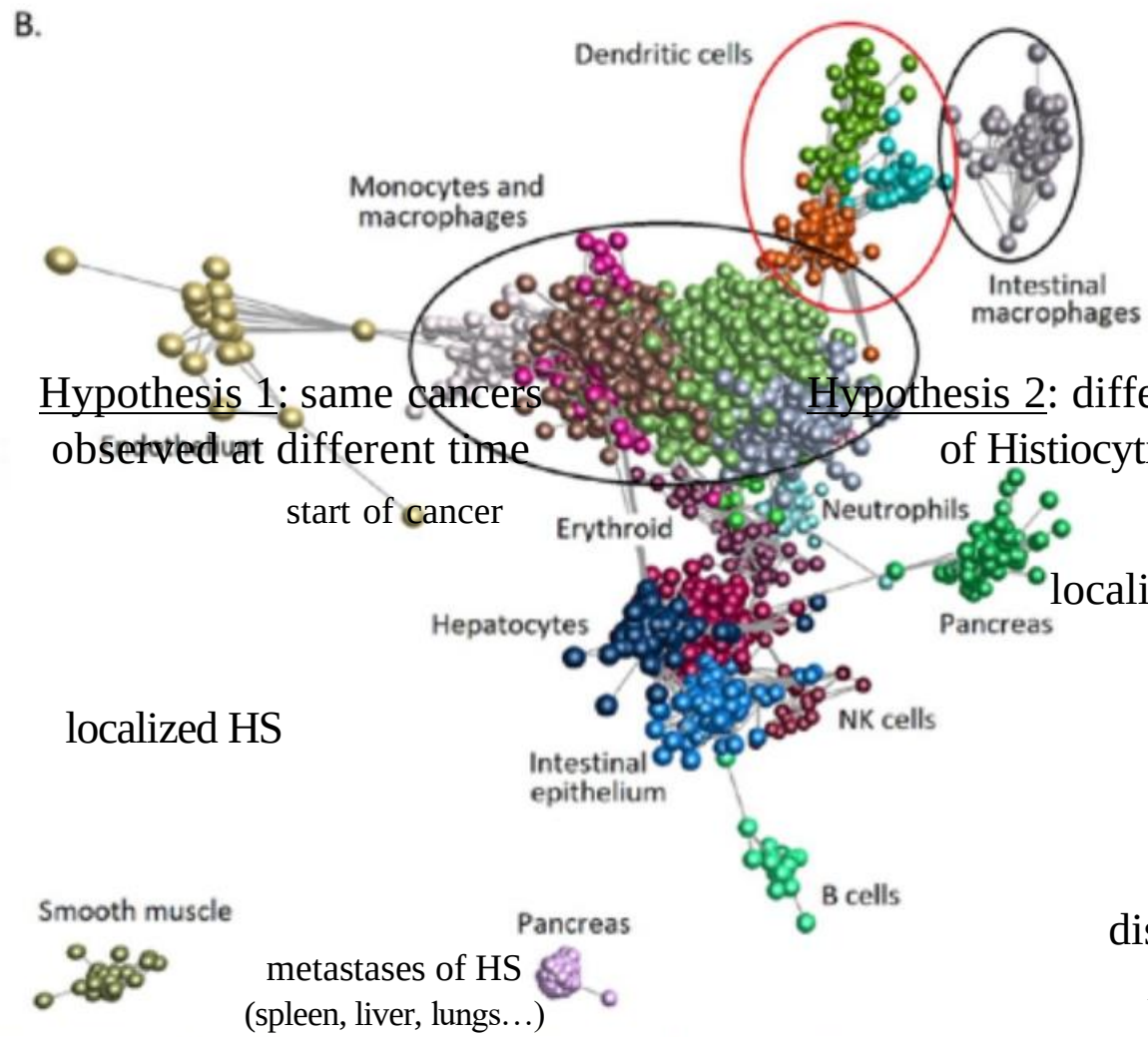


Hypothesis 1: same cancers observed at different time

start of cancer

Hypothesis 2: different subtypes of Histiocytic cells

localized HS to external organs (skin, joint…)

localized HS

disseminated HS to internal organs

metastases of HS (spleen, liver, lungs…)

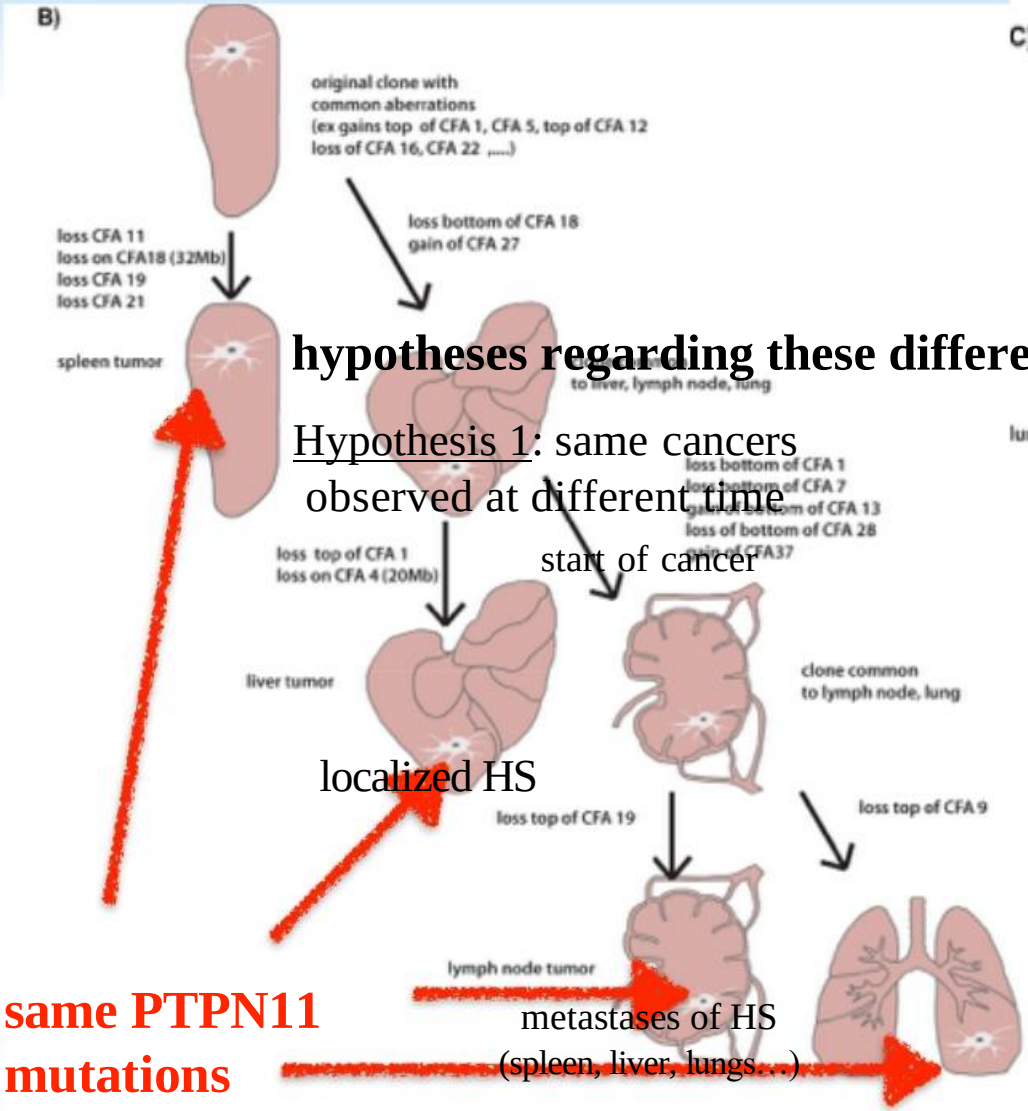
Fig 5. GCN analysis of gene expression in MPS cell populations. Each sphere (node) represents a gene, and lines between them (edges) show Pearson correlations between them of ≥0.75. Nodes were grouped into clusters with related expression pat[illegible] rithm with an inflation value of 1.7. Lists of genes and expression profiles of clusters are presented in S2 Data. (A) The network generated by the BioLayout analysis. Elements with ≥5 nodes are shown. Nodes are coloured by MCL cluster. Lists of genes and average expression profiles for all clusters are presented in S2 Data. Monocyte and macrophage genes (black ovals), DC genes (red oval). (B) Network showing only major clusters of monocyte and macrophage genes (black ovals), DC genes (red oval), and other cell types. DC

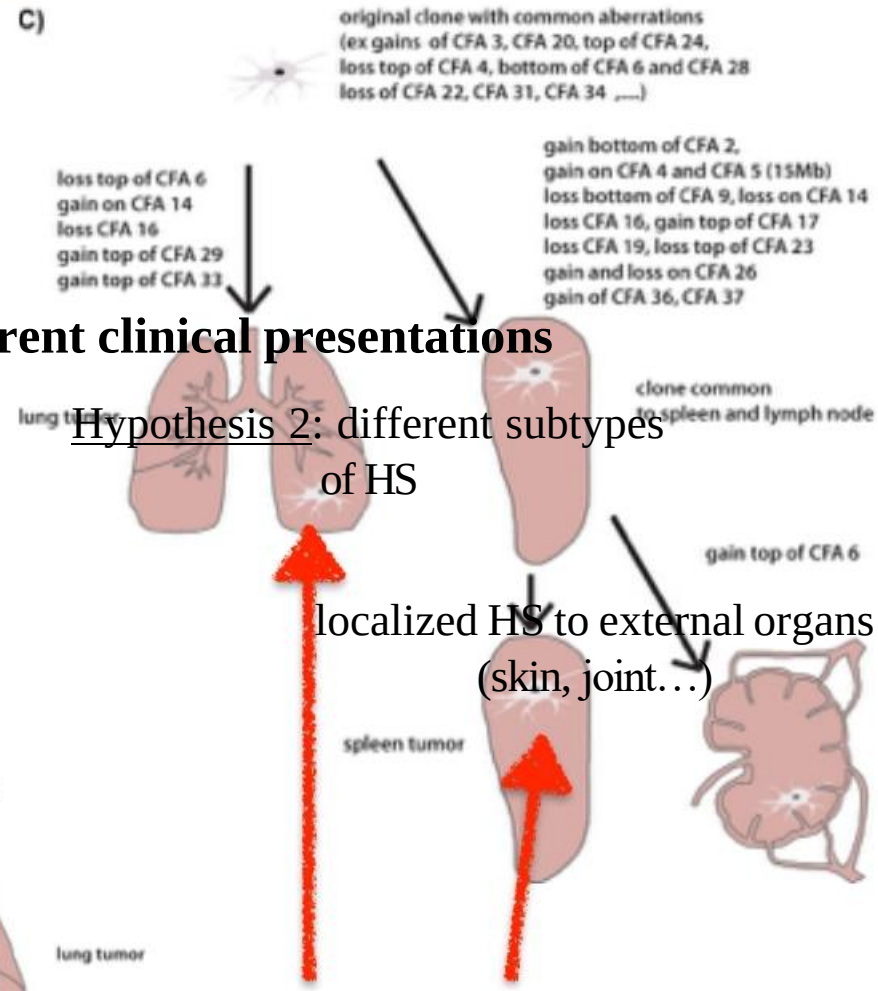
disseminated HS

time

PTPN11 mutations

Copy Number aberrations





hypotheses regarding these different clinical presentations

<u>Hypothesis 1</u>: same cancers observed at different time

start of cancer

localized HS

metastases of HS
(spleen, liver, lungs…)

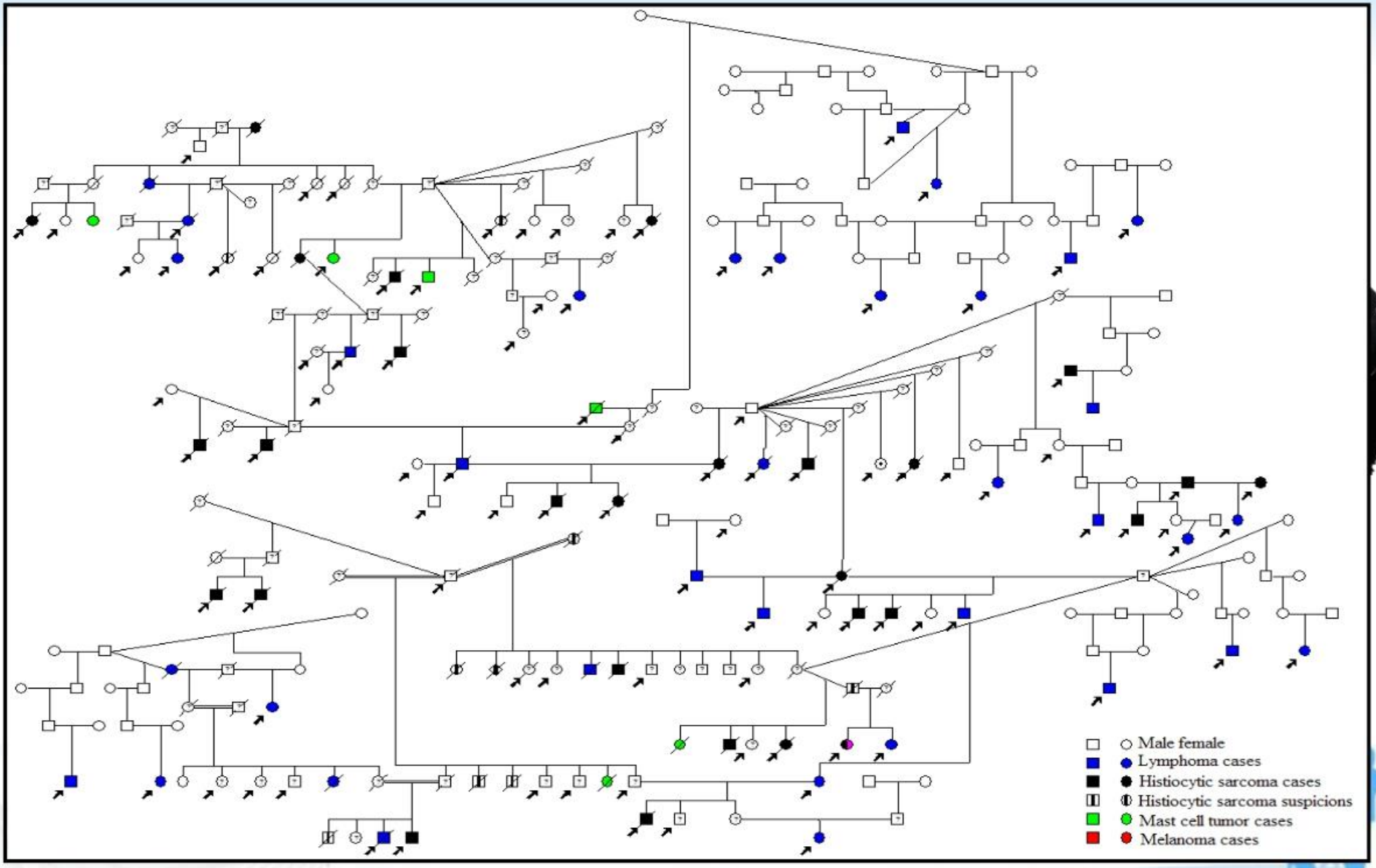
disseminated HS

same PTPN11 mutations

CGH analyses - Matthew Breen (NCSU)

<u>Hypothesis 2</u>: different subtypes of HS

localized HS to external organs (skin, joint…)

different PTPN11 mutations

Histiocytic Sarcoma