

# Molecular basis of canine histiocytic sarcoma in dogs:

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DVM - PhD

Canine Genetics Team

Catherine André

IGDR UMR6290 CNRS Univ.Rennes1

FRANCE

## In human

tumor from histiocytic cells :  
monocyte/macrophage or dendritic cells

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

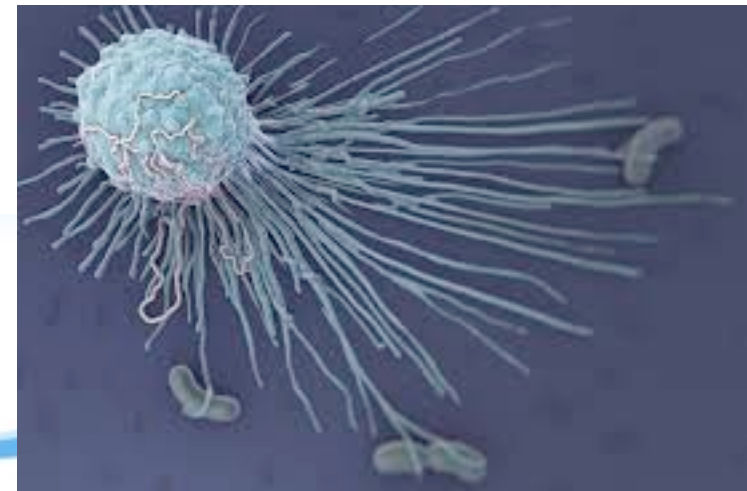
limited response to chemotherapy



KINDERSPITAL ZÜRICH  
FÖRDERUNGSZENTRUM  
FÜR DAS KINZ  
Universität  
Zürich

(Credit : J Donadieu )

## In dogs





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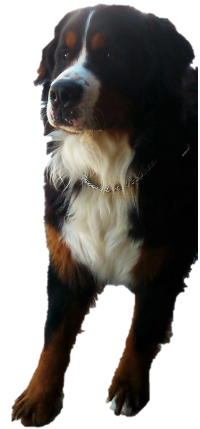
KINDERSPITAL ZÜRICH | FÖRDERZENTRUM FÜR GUT UND BLUT | UNIVERSITÄT ZÜRICH

(Credit : J Donadieu )

- 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**

## In dogs



- 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**

## In human

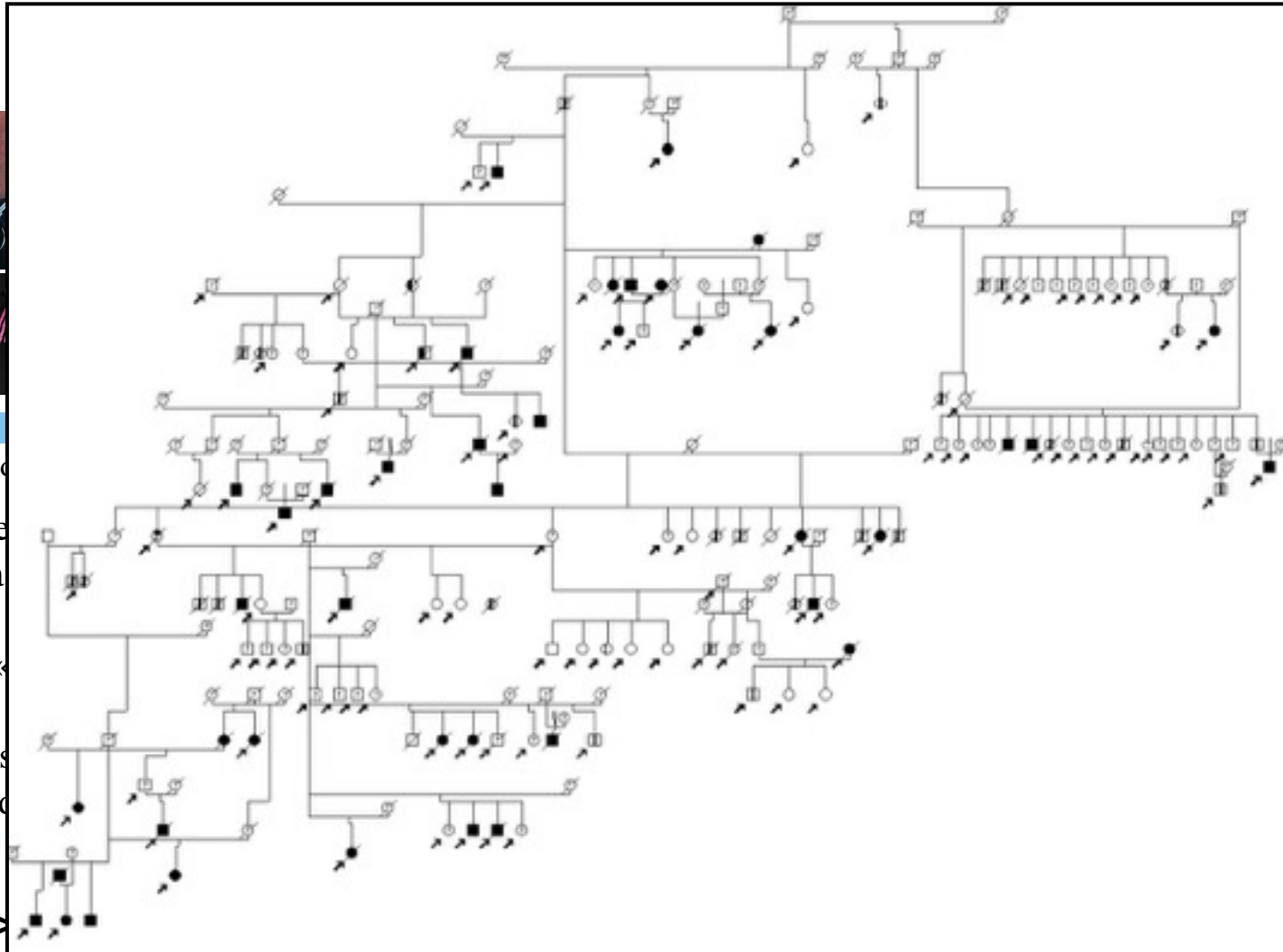
## In dogs



KINDERSPITAL ZÜRICH

(Credit : J Donac)

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bases of this cancer

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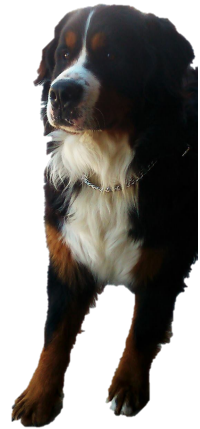
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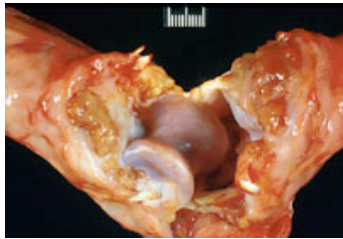


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**=> unique model to identify genetic bases of this cancer**

## in dogs

localized HS (LHS):  
skin/joint



[www.histiocytosis.ucdavis.edu/sarcoma.html](http://www.histiocytosis.ucdavis.edu/sarcoma.html)

Retrievers



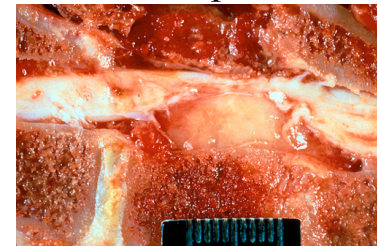
disseminated HS (DHS)



BMD



subdural HS:  
Cerebrum/spinal cord

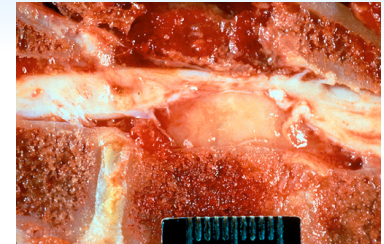
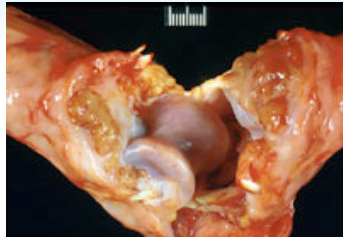


[www.histiocytosis.ucdavis.edu/sarcoma.html](http://www.histiocytosis.ucdavis.edu/sarcoma.html)

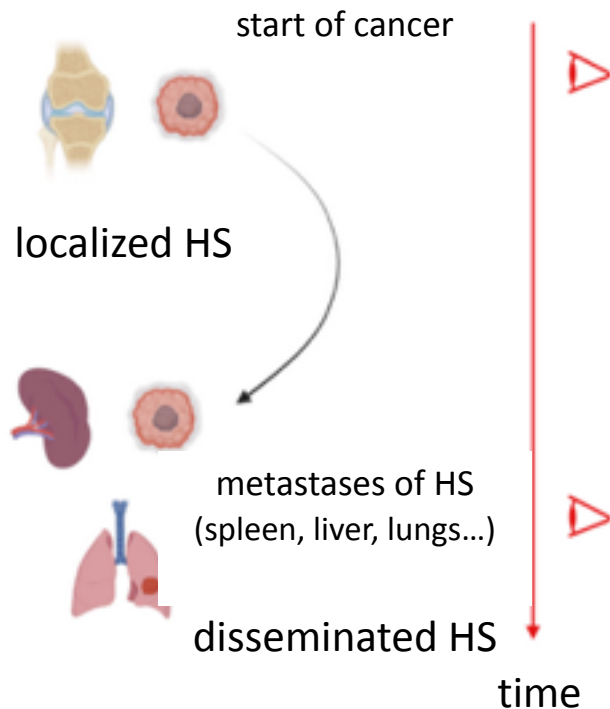
Pembroke Welsh Corgi



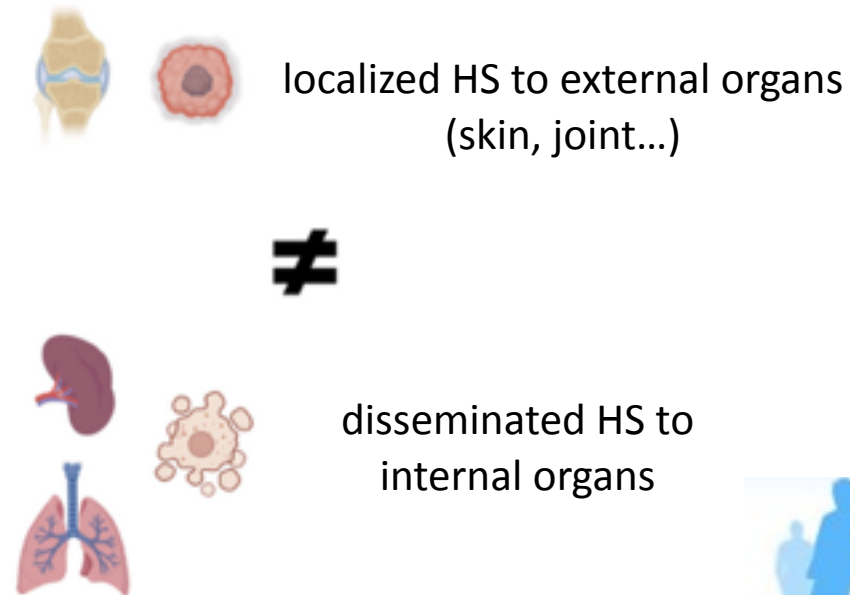
## regarding these different clinical presentations



Hypothesis 1: same cancers  
observed at different time

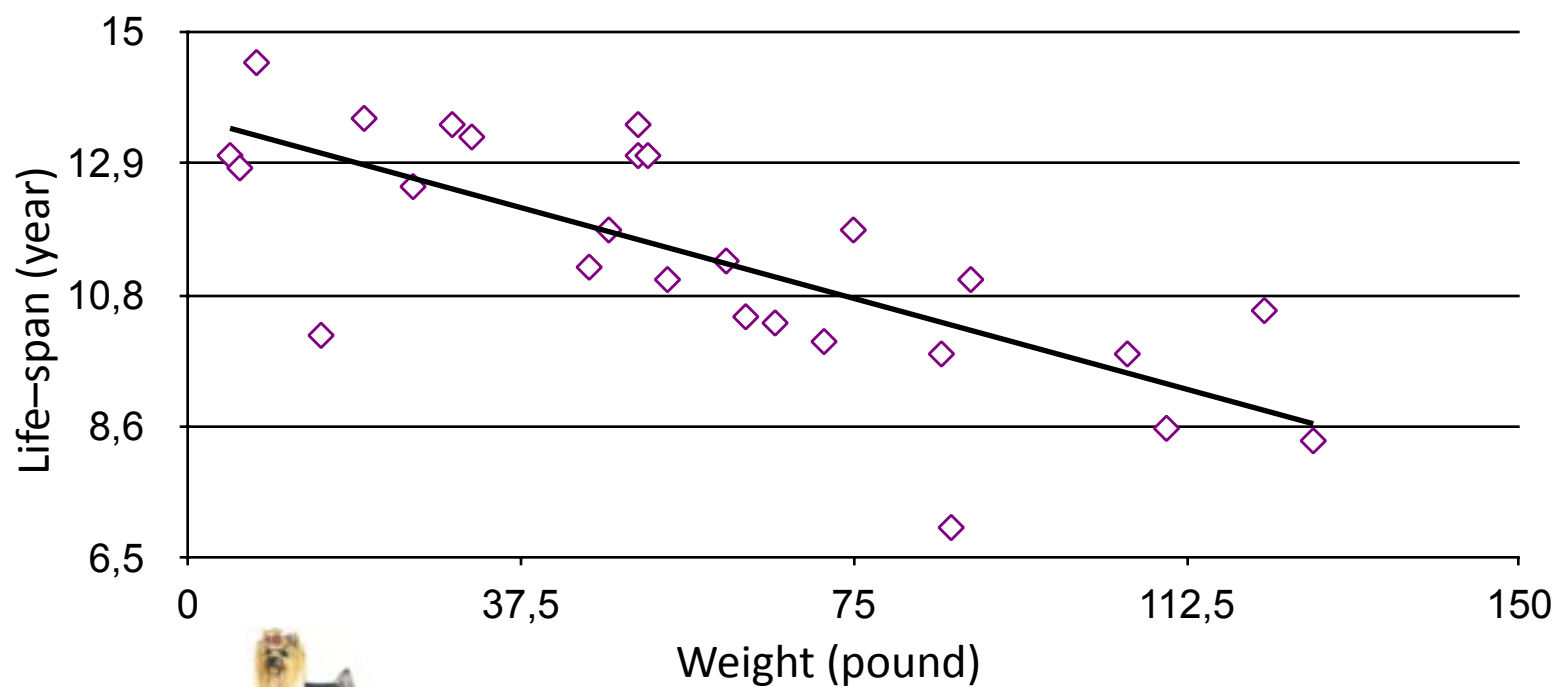


Hypothesis 2: different subtypes  
of Histiocytic cells





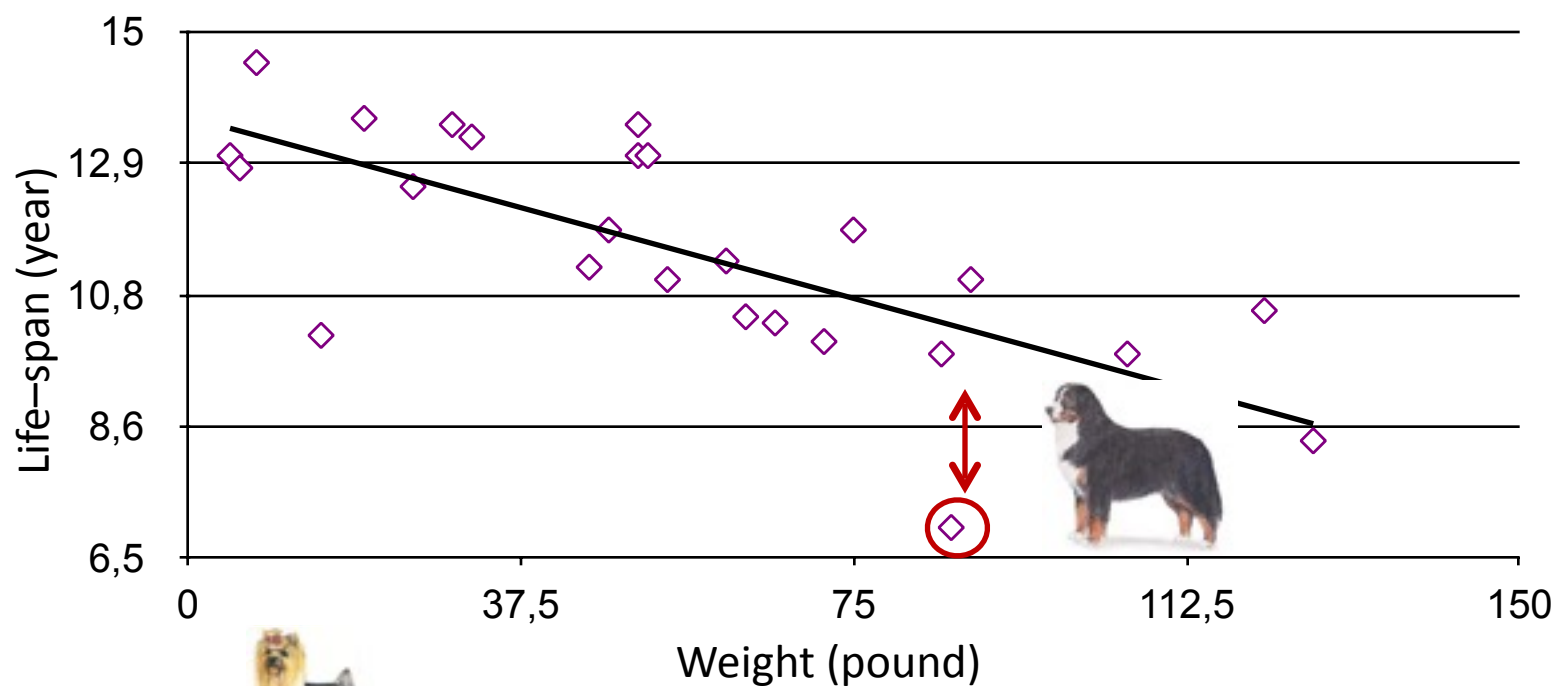
in dogs



www.



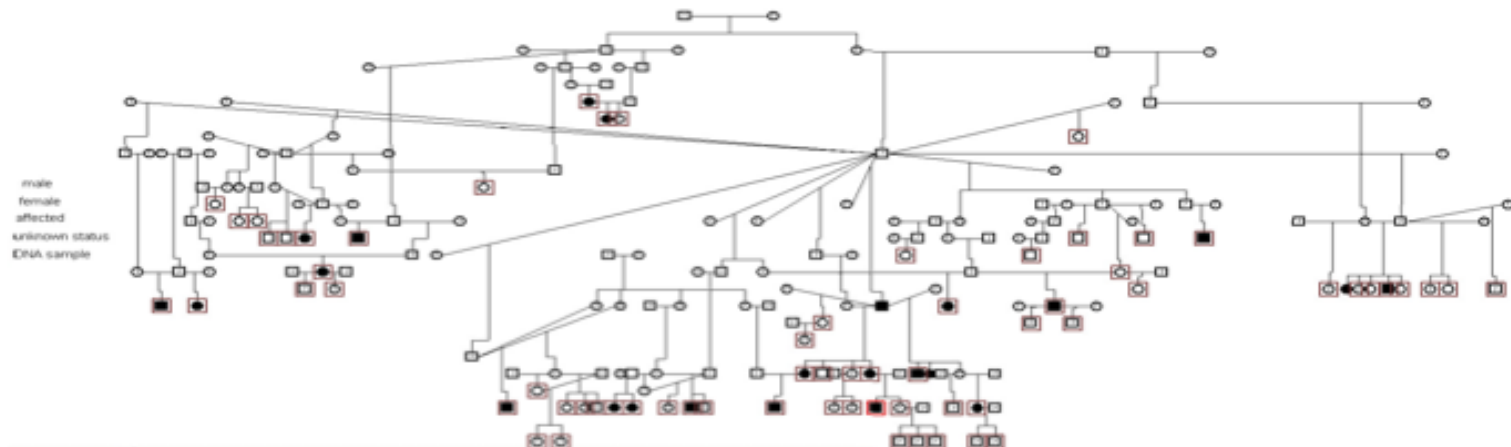
in dogs



www

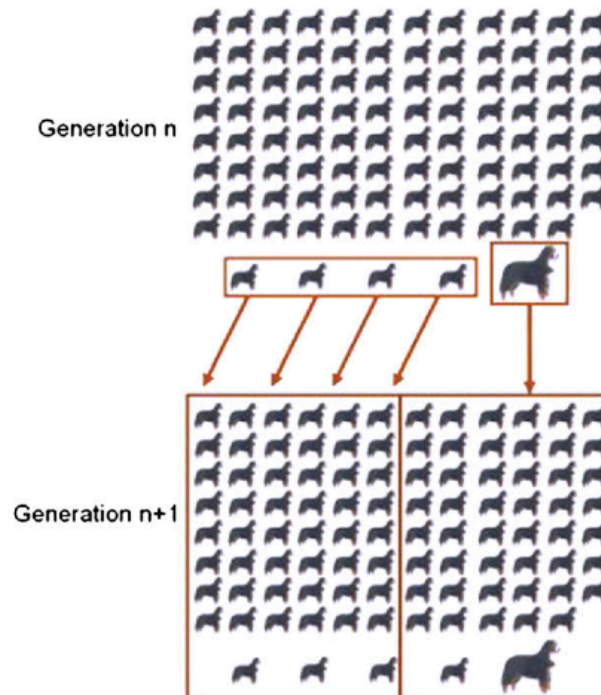
Breed practices could increase transmission of risk alleles:

- Breed = isolated population  $\leftrightarrow$  reduced diversity
- Consanguinity and sire effect increase frequency of inherited diseases



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*Abadie, Hedan et al. 2009*

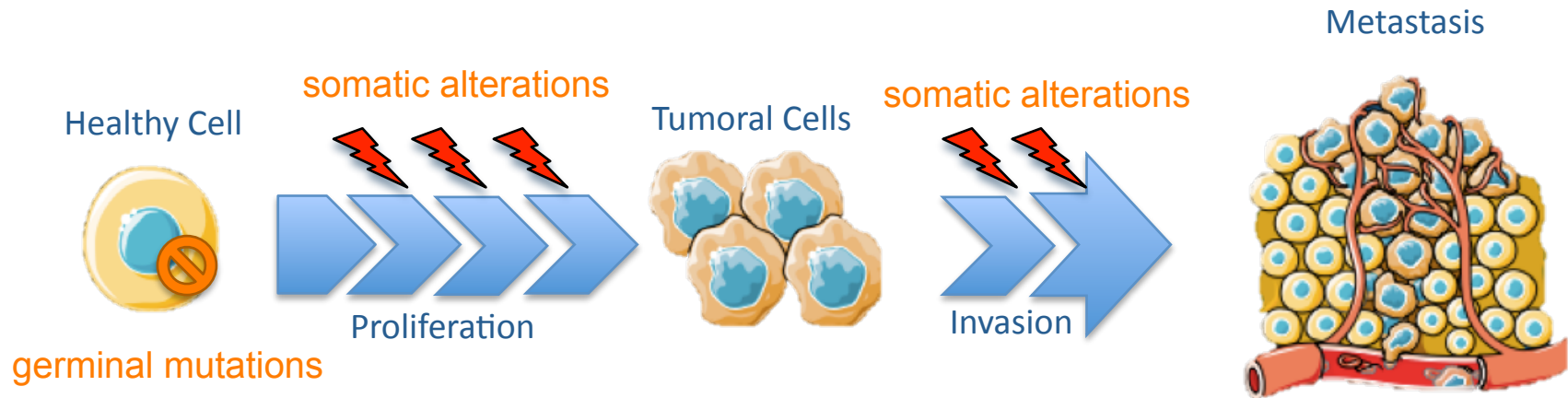
# A long term goal : Identify genetic mechanisms involved in HS

**Cancer = genetic disease**



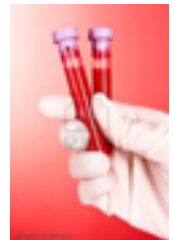
Hanahan et Weinberg. Cell. 2011

# A long term goal : Identify genetic mechanisms involved in HS



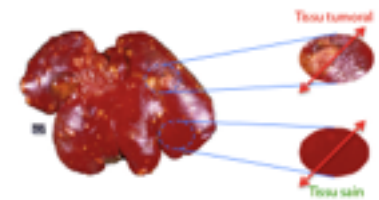
- Identify predisposing genes and risk alleles

-> Blood collection



- Identify recurrent somatic alterations associated with tumor progression

-> Tissue collection

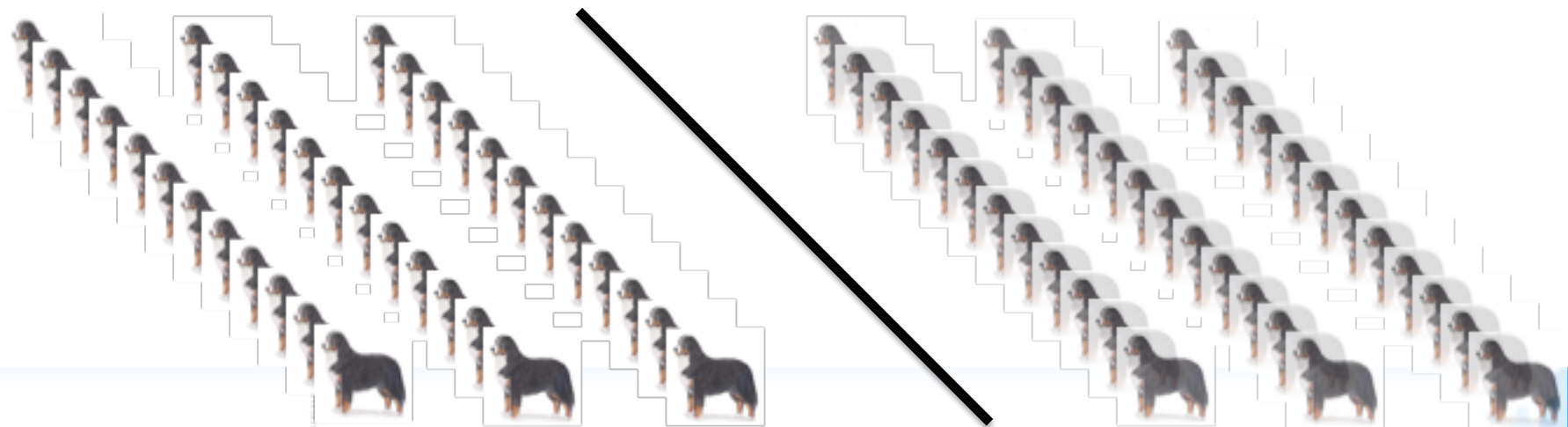




# Looking for predisposing genes: Research project

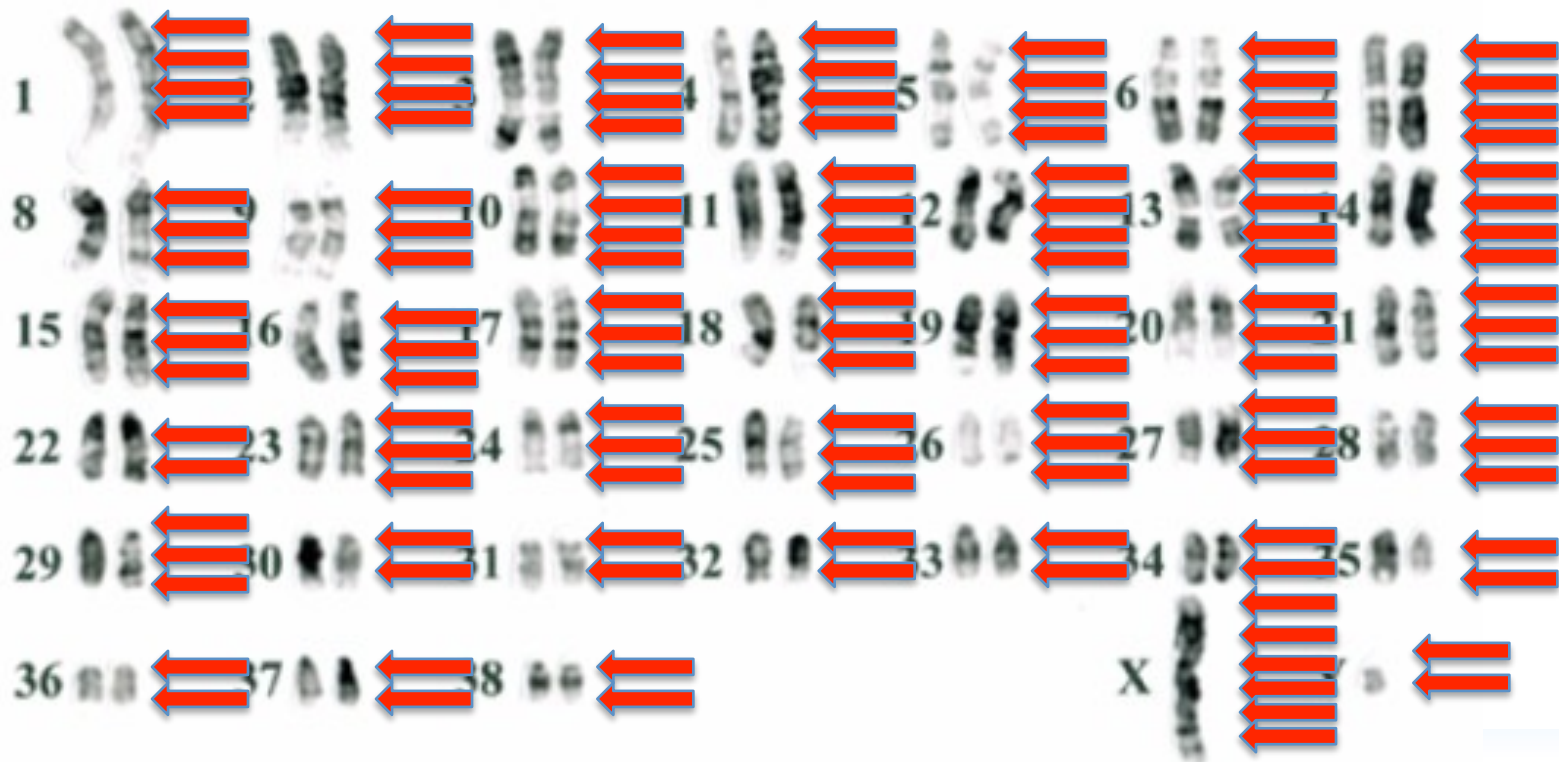
- Since 2002 - Cani-DNA biobank
  - > 4000 blood samples and > 400 tissue samples
  - > 450 HS cases with histopathological report
- Characterization of the Disease:  
*Abadie, Hedan et al. 2009*
- In coll. with E. Ostrander's lab (NIH, Bethesda)

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



# Looking for predisposing genes: Research project

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



comparaison of 22 000 markers

# Looking for predisposing genes: Research project

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



Markers differs between unaffected and affected BMDs

# Looking for predisposing genes: Research project

➤ Since 2002 - Cani-DNA biobank (dog.genetics.genouest.org)

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

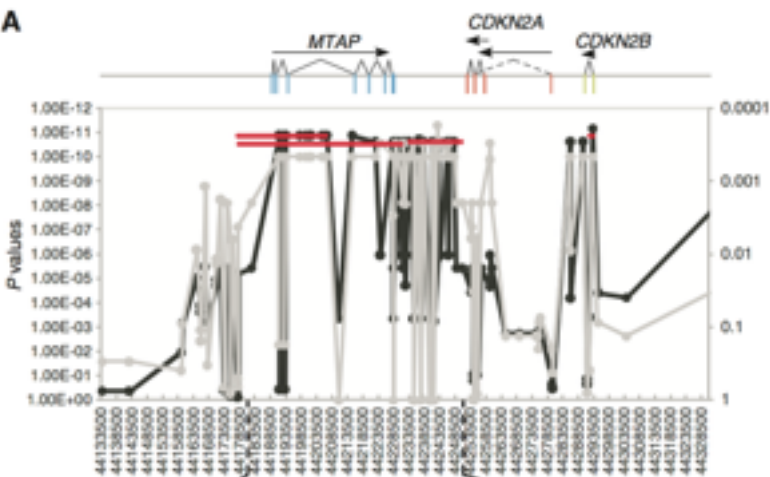
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GWAS: 232 unaffected and 244 affected BMDs (American/European)

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

A



Research Article

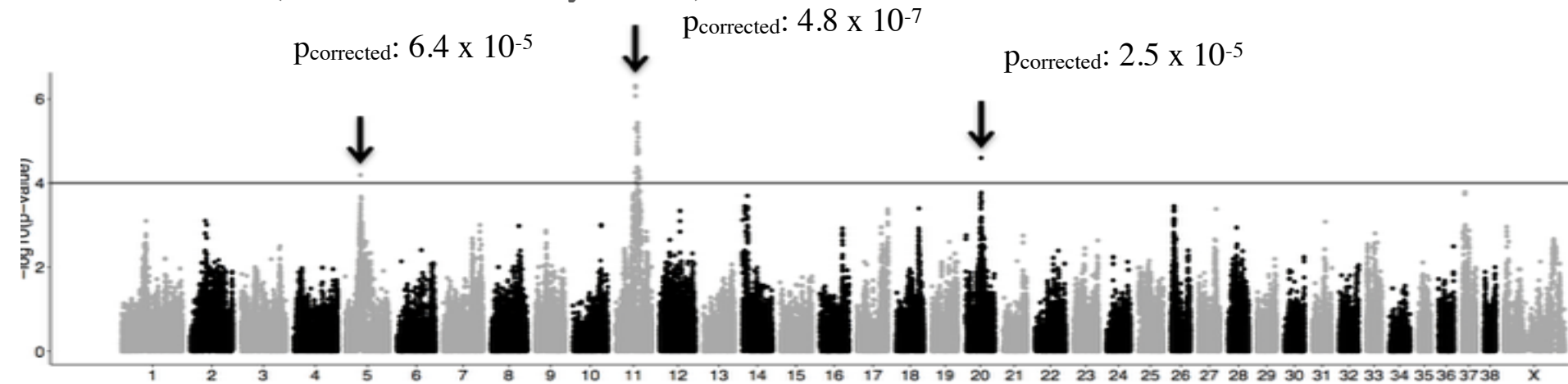
## The MTAP-CDKN2A Locus Confers Susceptibility to a Naturally Occurring Canine Cancer

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

Cancer  
Epidemiology,  
Biomarkers  
& Prevention

# Looking for predisposing genes: Research project

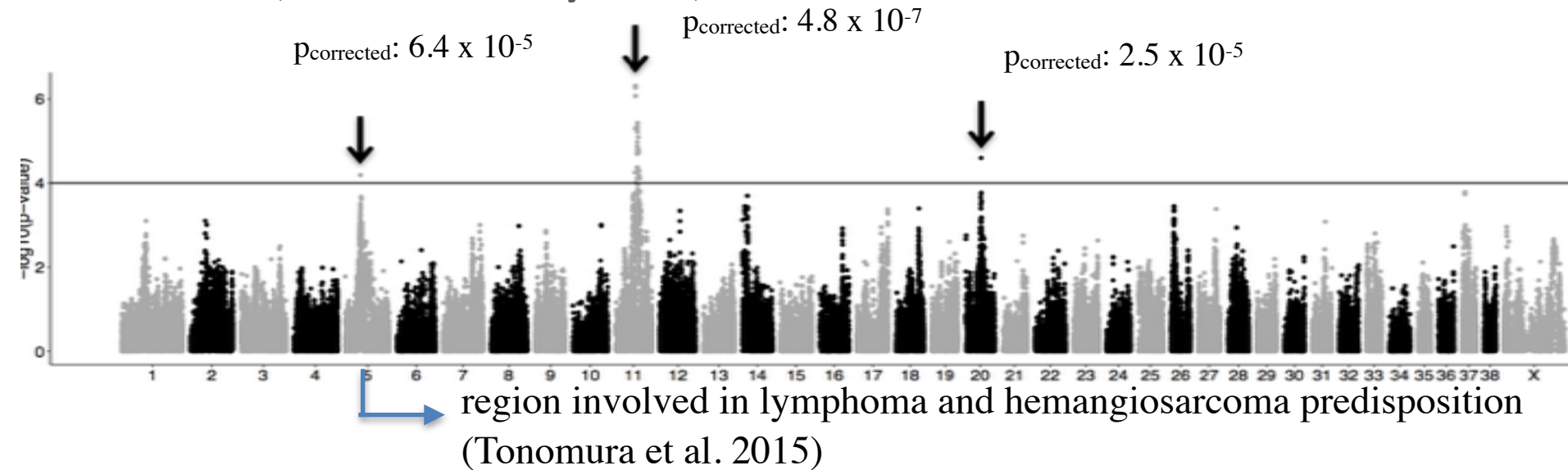
➤ New GWAS (Illumina SNP array 170K) 2011-2012: 154 unaffected and 172 affected BMDs





# Looking for predisposing genes: Research project

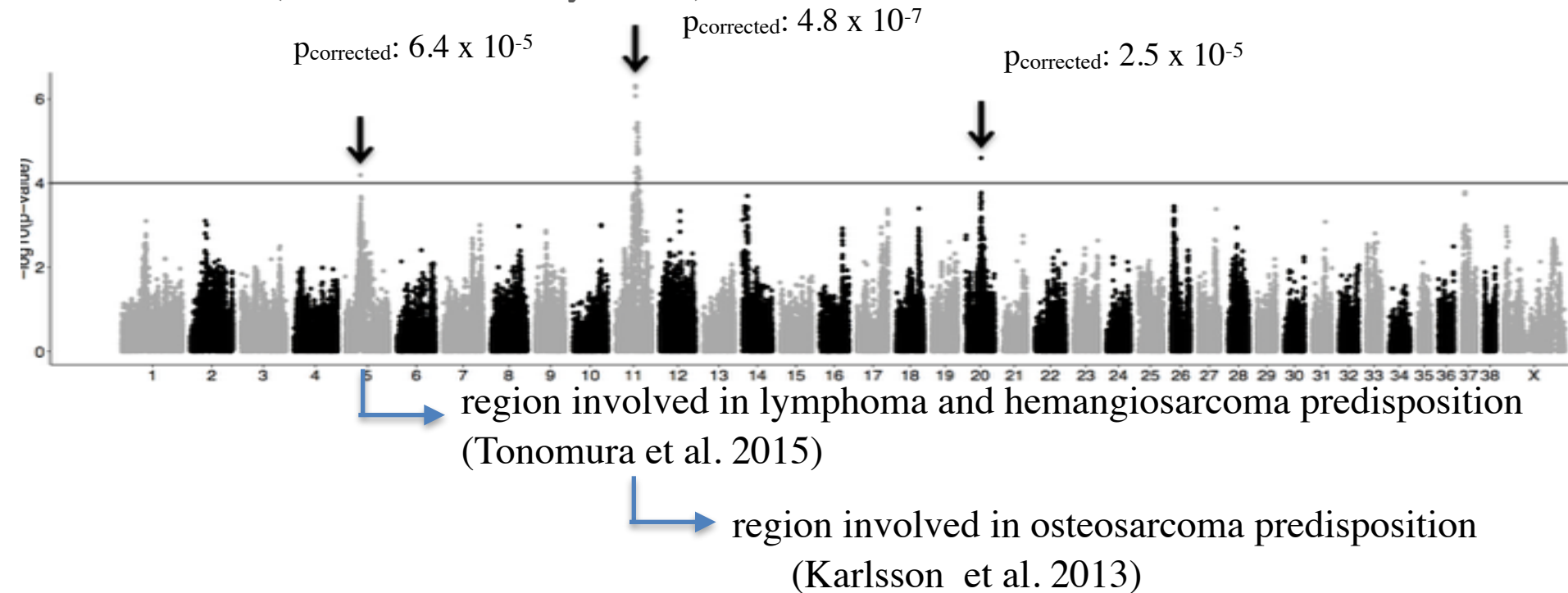
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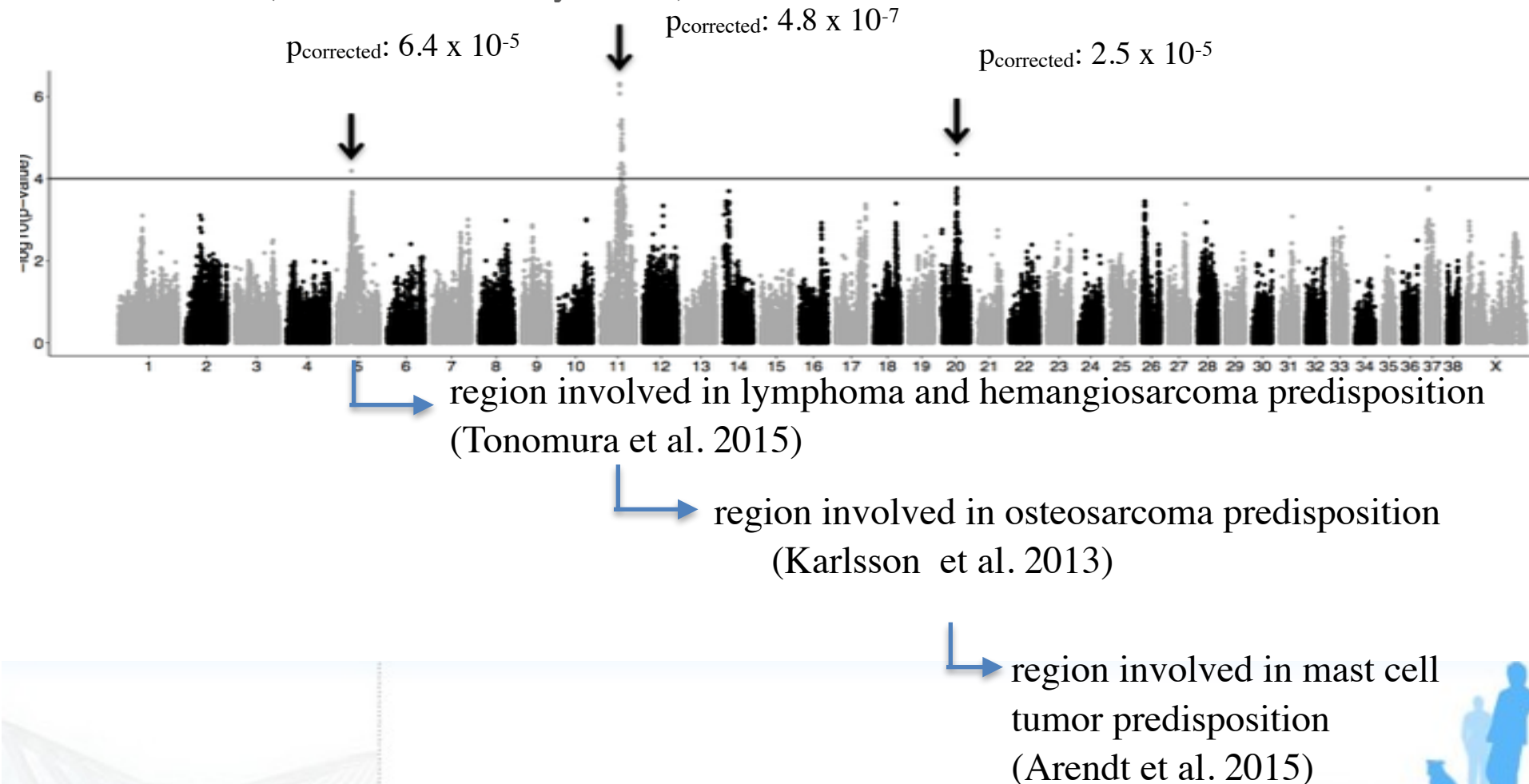
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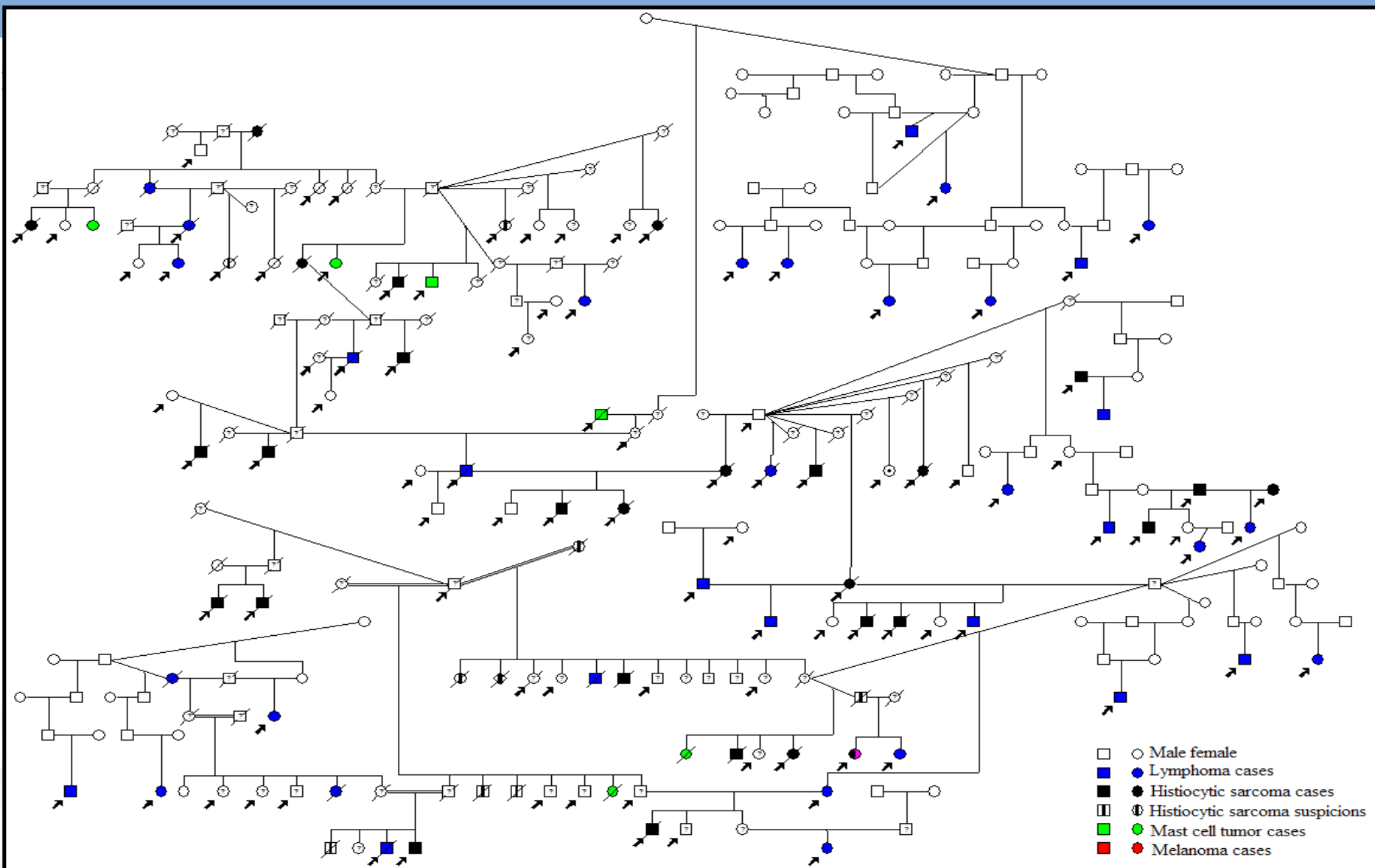


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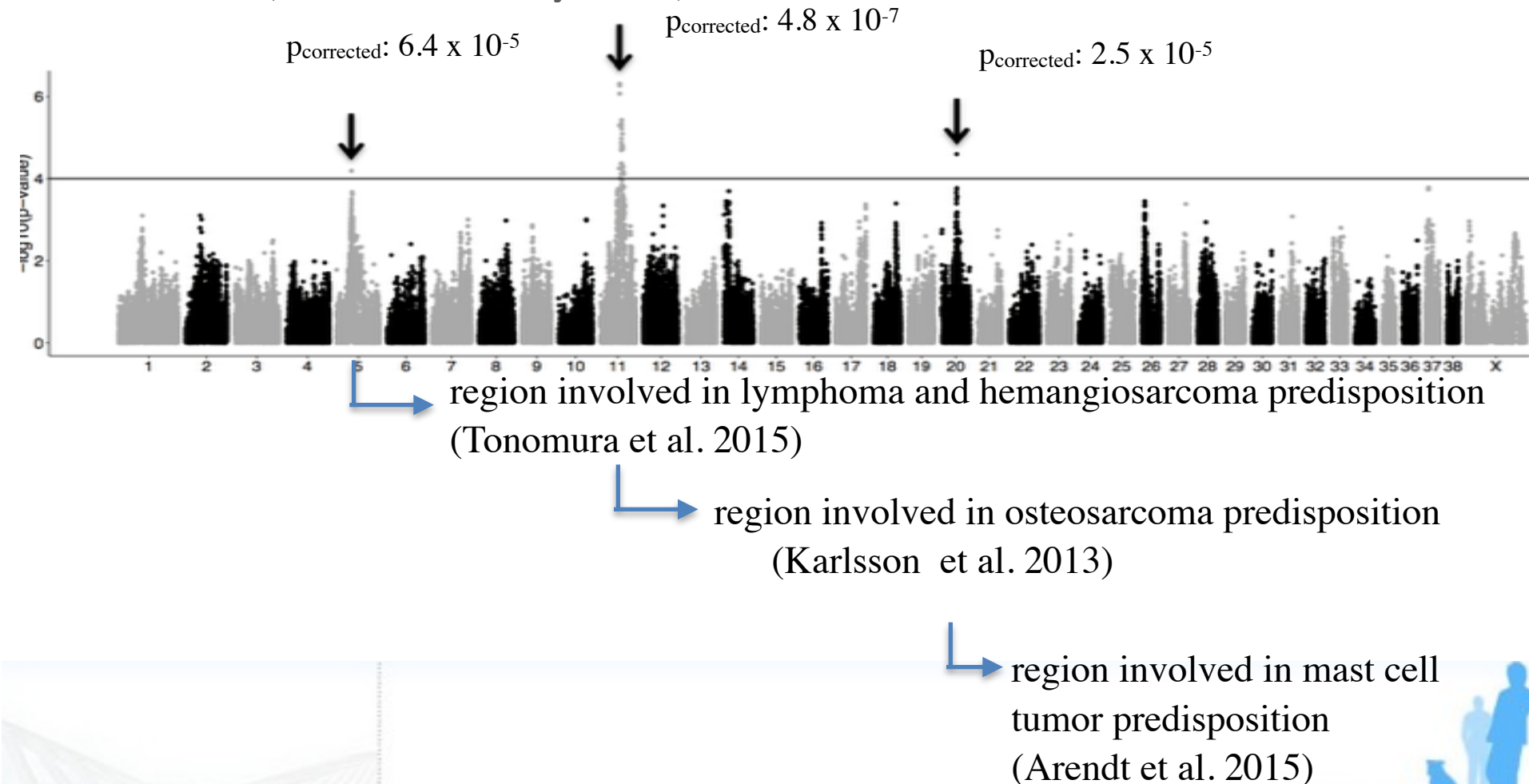


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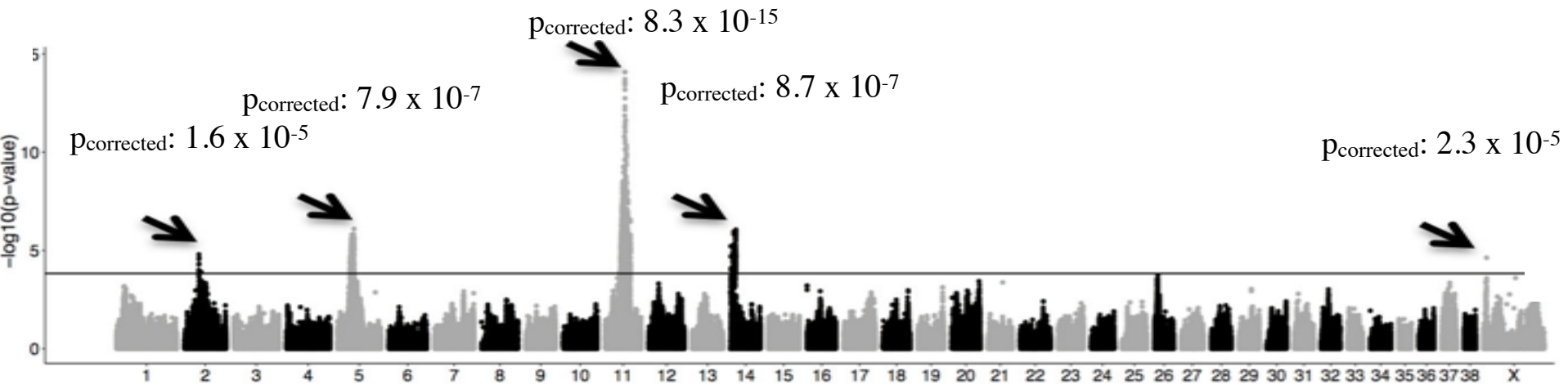
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# Looking for predisposing genes: Research project

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

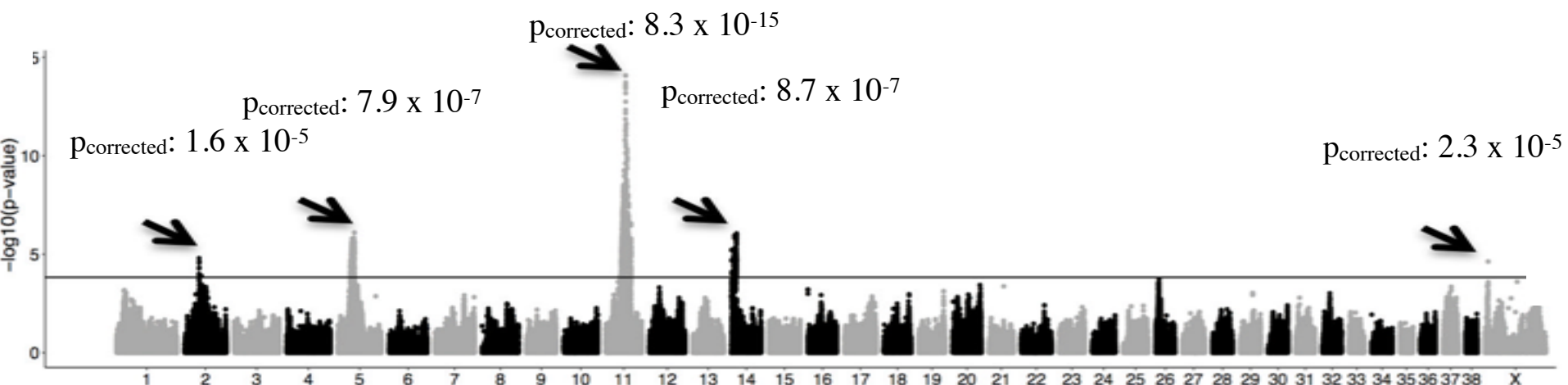


3 main regions on CFA 5, 11 and 14 :

- 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

# Looking for predisposing genes: Research project

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



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

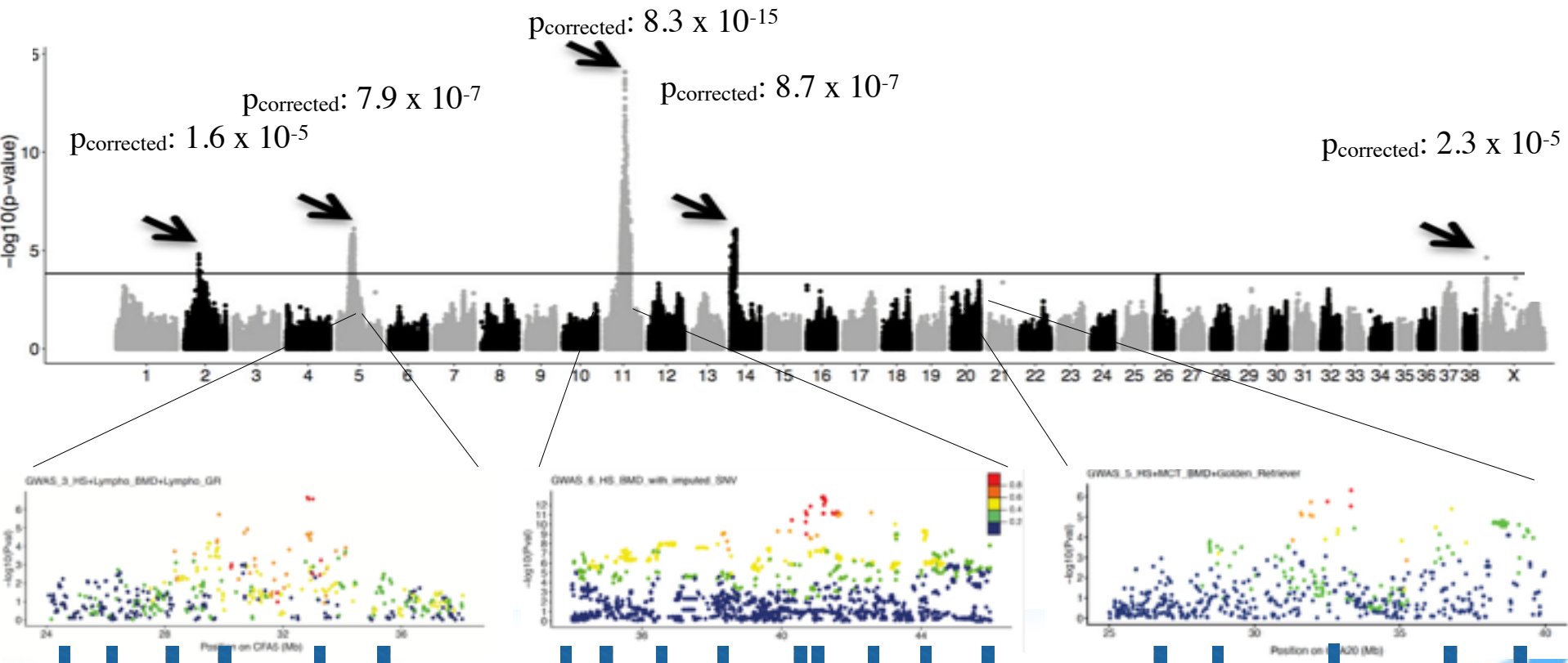
| frequencies of risk alleles in the 3 breeds |          |            | Odds Ratio |             |                        |
|---------------------------------------------|----------|------------|------------|-------------|------------------------|
| number of risk alleles                      | affected | unaffected | Odds Ratio | interval    | pval                   |
| ≥5 risk alleles                             | 0,7      | 0,27       | 5.41       | [4.04-7.24] | 1.14x10 <sup>-31</sup> |
| 4 risk alleles                              | 0,23     | 0,34       | 0.5        | [0.37-0.67] | 2.74x10 <sup>-6</sup>  |
| ≤3 risk alleles                             | 0,07     | 0,29       | 0.15       | [0.1-0.23]  | 2.17x10 <sup>-21</sup> |





# Looking for predisposing genes: Research project

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



**Predisposition -> accumulation of many regions and subregions**

RESEARCH ARTICLE

# Identification of common predisposing loci to hematopoietic cancers in four dog breeds

Benoit Hédan<sup>1\*</sup>, Édouard Cadieu<sup>1</sup>, Maud Rimbault<sup>1</sup>, Amaury Vayssie<sup>1</sup>, Caroline Dufaire de Citres<sup>2</sup>, Patrick Devauchelle<sup>3</sup>, Nadine Botheref<sup>1</sup>, Jérôme Abadie<sup>4</sup>, Pascale Guignon<sup>1</sup>, Thomas Derrien<sup>1</sup>, Catherine André<sup>1</sup>

**1** Univ Rennes, CNRS, IGDR (Institut de Génétique et Développement de Rennes)—UMR6290, Rennes, France, **2** Antagene, La Tour-de-Salvagny, France, **3** Mico Vet, Créteil, France, **4** Oniris, Laboratoire—Department of Biology, Pathology and Food Sciences, Nantes, France

\* benoit.hedan@univ-rennes1.fr

## Abstract

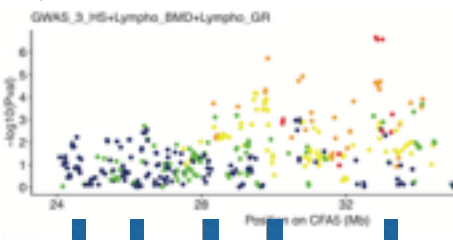
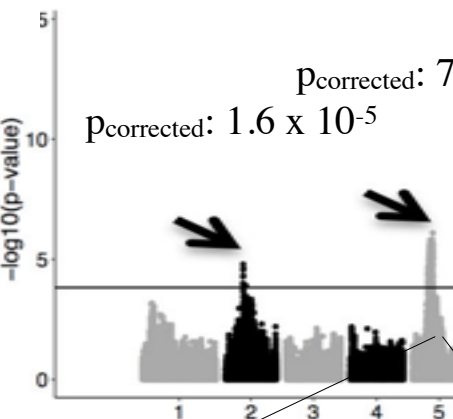
Histiocytic sarcoma (HS) is a rare but aggressive cancer in both humans and dogs. The spontaneous canine model, which has clinical, epidemiological, and histological similarities with human HS and specific breed predispositions, provides a unique opportunity to unravel the genetic basis of this cancer. In this study, we aimed to identify germline risk factors associated with the development of HS in canine-predisposed breeds. We used a methodology that combined several genome-wide association studies in a multi-breed and multi-cancer approach as well as targeted next-generation sequencing, and imputation. We combined several dog breeds (Bernese mountain dogs, Rottweilers, flat-coated retrievers, and golden retrievers), and three hematopoietic cancers (HS, lymphoma, and mast cell tumor). Results showed that we not only refined the previously identified HS risk *CDKN2A* locus, but also identified new loci on canine chromosomes 2, 5, 14, and 20. Capture and targeted sequencing of specific loci suggested the existence of regulatory variants in non-coding regions and methylation mechanisms linked to risk haplotypes, which lead to strong cancer predisposition in specific dog breeds. We also showed that these canine cancer predisposing loci appeared to be due to the additive effect of several risk haplotypes involved in other hematopoietic cancers such as lymphoma or mast cell tumors as well. This illustrates the pleiotropic nature of these canine cancer loci as observed in human oncology, thereby reinforcing the interest of predisposed dog breeds to study cancer initiation and progression.

## Author summary

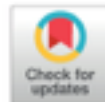
Because of specific breed structures and artificial selection, pet dogs suffer from numerous genetic diseases, including cancers and represent a unique spontaneous model of human cancers. Here, we focused on histiocytic sarcoma (HS), a rare and highly aggressive cancer in humans. In this study, we have used spontaneous affected and unaffected dogs from three predisposed dog breeds to identify loci involved in HS predisposition. Through genetic analyses, we showed that these canine cancer predispositions are due to the additive effect of several risk haplotypes also involved in the predisposition of other

coated retriever)

$p_{\text{corrected}}: 2.3 \times 10^{-5}$



Predisposition ->



## OPEN ACCESS

**Citation:** Hédan B, Cadieu É, Rimbault M, Vayssie A, Dufaire de Citres C, Devauchelle P, et al. (2021) Identification of common predisposing loci to hematopoietic cancers in four dog breeds. *PLoS Genet* 17(4): e1009395. <https://doi.org/10.1371/journal.pgen.1009395>

**Editor:** Carlos Alvarez, Abigail Winer Research Institute at Nationwide Children's Hospital, UNITED STATES

**Received:** July 28, 2020

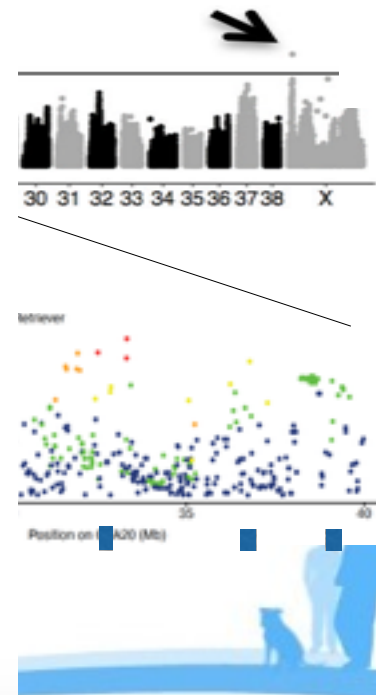
**Accepted:** February 3, 2021

**Published:** April 1, 2021

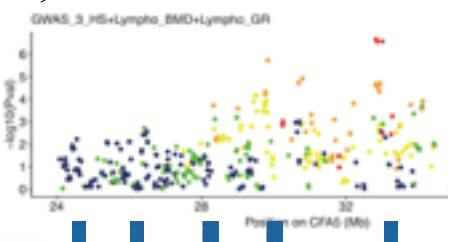
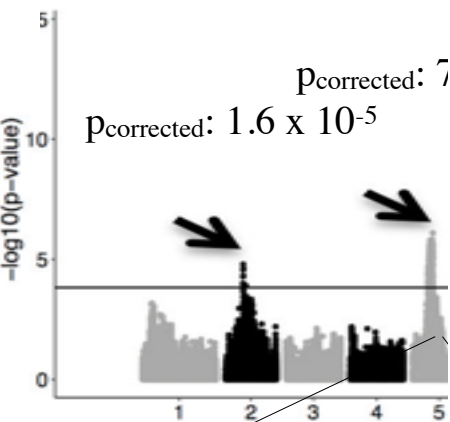
**Peer Review History:** PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pgen.1009395>

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**Data Availability Statement:** All genotyping data is available at doi:10.5061/dryad/hv3ffgq4.



➤ New GWAS on 455 affected et 408



Predisposition ➔



#### OPEN ACCESS

**Citation:** Evans JM, Parker HG, Rutteman GR, Plassais J, Grünwis GDM, 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

**Received:** January 13, 2021

**Accepted:** April 12, 2021

**Published:** May 13, 2021

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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 GED (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 UK

#### RESEARCH ARTICLE

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

Jacquelyn M. Evans<sup>1</sup>, Heidi G. Parker<sup>2</sup>, Gerard R. Rutteman<sup>2</sup>, Jocelyn Plassais<sup>3</sup>, Guy C. M. Grünwis<sup>4</sup>, Alexander C. Harris<sup>1</sup>, Susan E. Lana<sup>4</sup>, Elaine A. Ostrander<sup>1\*</sup>

**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

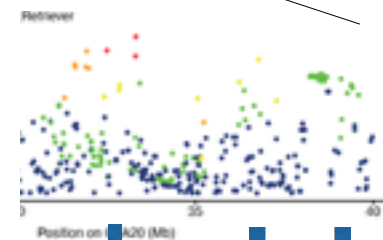
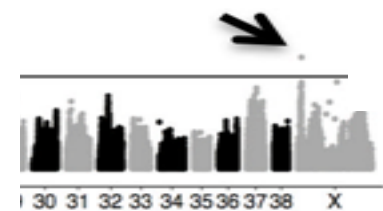
\* [eostrand@mail.nih.gov](mailto:eostrand@mail.nih.gov)

### 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 ( $P_{\text{lead}} = 4.83 \times 10^{-6}$ ) and 19 ( $P_{\text{lead}} = 2.25 \times 10^{-7}$ ). 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 *PW3R6* 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.

coated retriever)

$p_{\text{corrected}}: 2.3 \times 10^{-5}$



# 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

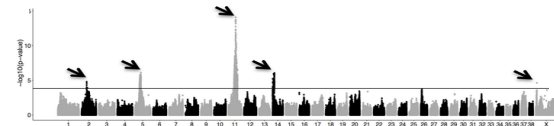


- Based on our first GWAS data, selection of 140 markers associated with HS
- Genotyping of 1081 French BMDs ( 256 affected dogs + 165 unaffected dogs)
  - > selection of the 9 markers more significantly associated with risk to develop HS



In 2012

# How these data could be useful for breeders?



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

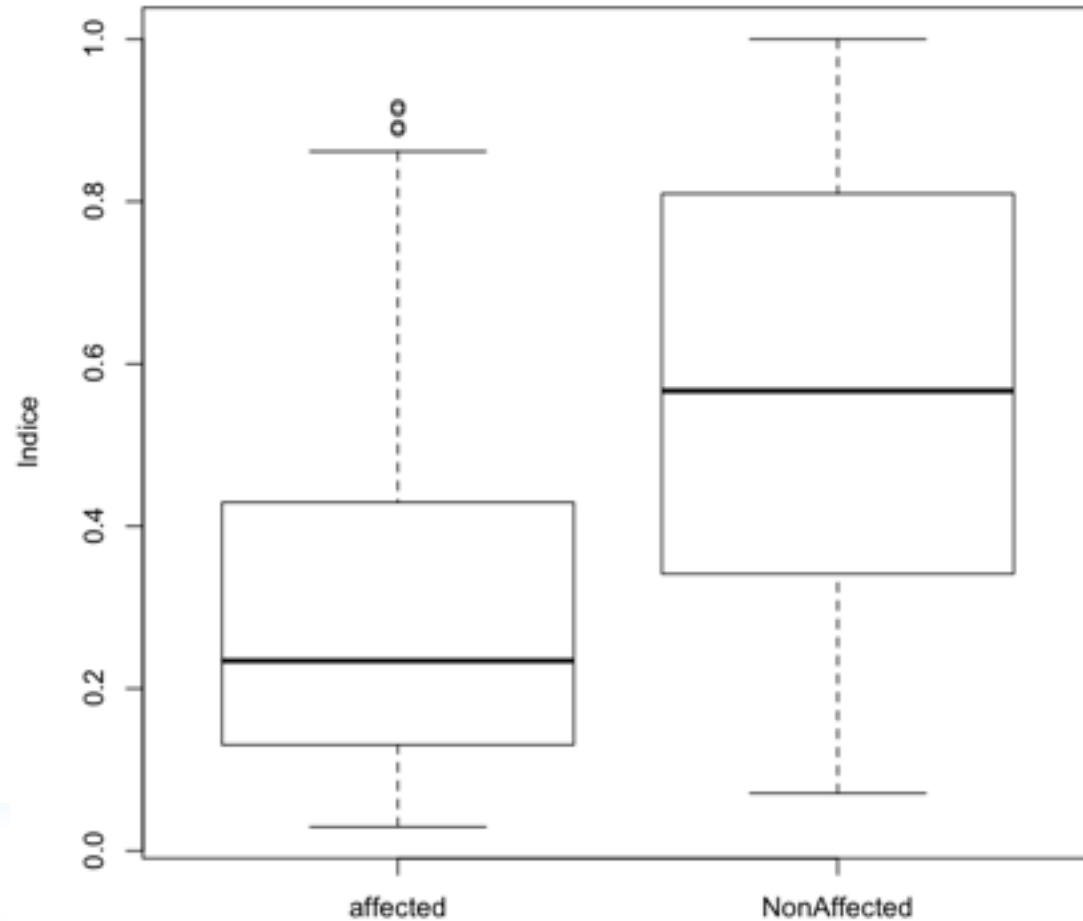
$$\text{Risk} = \frac{\exp(\sum_{i=1}^n (\alpha_i \text{SNP}_i))}{1 + \exp(\sum_{i=1}^n (\alpha_i \text{SNP}_i))}$$

Index



# Index development

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

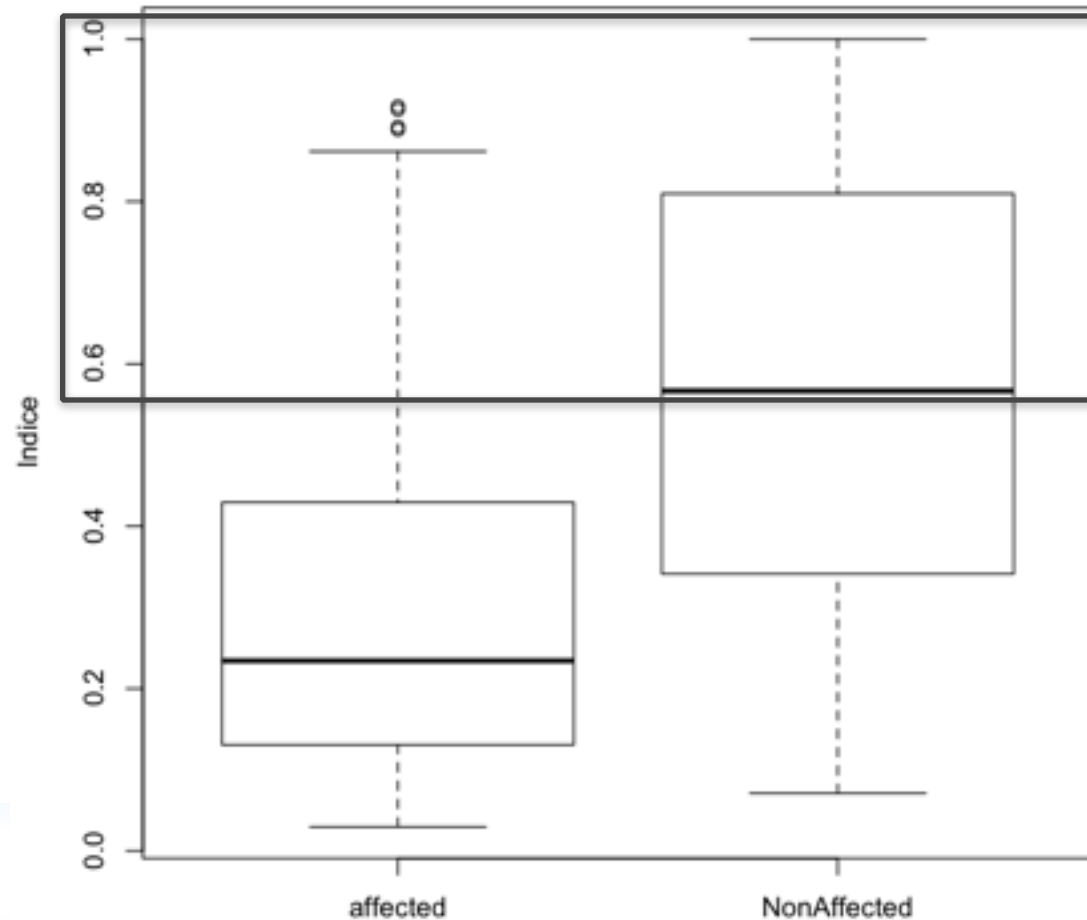


p-value :  $1.754 \times 10^{-16}$  (Mann Whitney test)

# Index development

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

Category A



47 % of healthy  
10% of affected

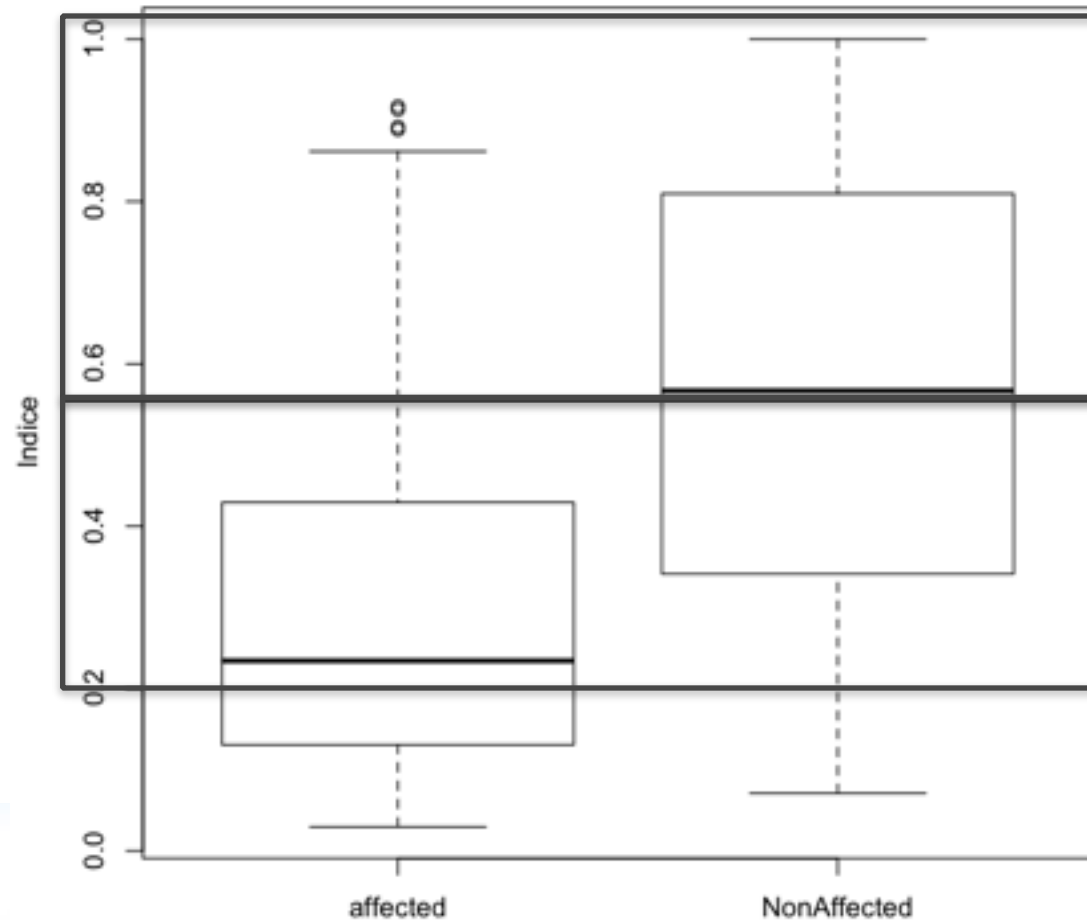
p-value :  $1.754 \times 10^{-16}$  (Mann Whitney test)

# Index development

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

Category A

Category B



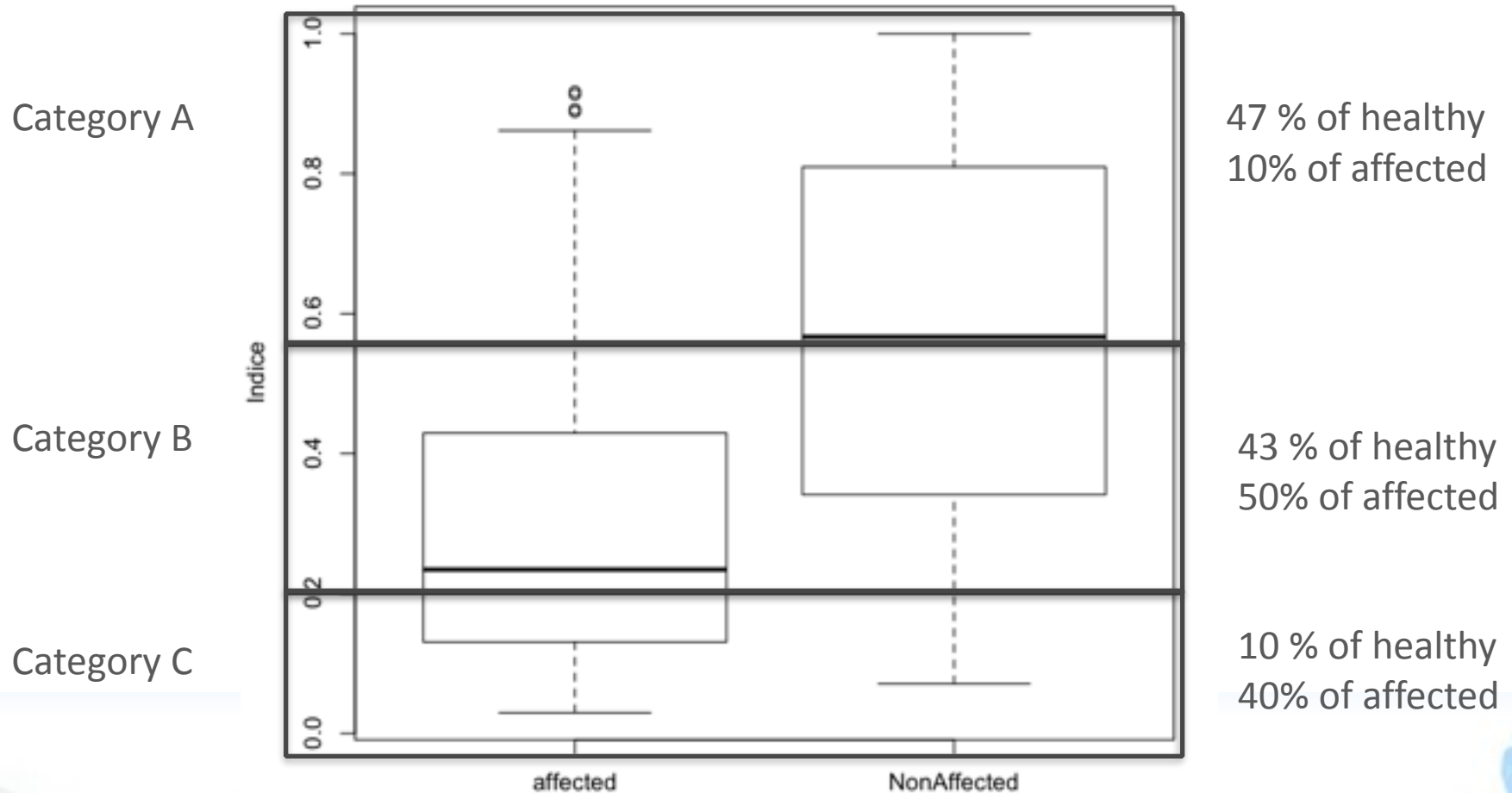
47 % of healthy  
10% of affected

43 % of healthy  
50% of affected

p-value : 1.754 10<sup>-16</sup> (Mann Whitney test)

# Index development

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



p-value :  $1.754 \times 10^{-16}$  (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     |
|------------------------|-------|-------|-------|
| <i><b>Affected</b></i> | 14%   | 43.1% | 42.9% |
| <i><b>Healthy</b></i>  | 41.5% | 46.5% | 11.9% |

➤ **Statistics :** 88% p-value : 2.1 10<sup>-24</sup> (Xi<sup>2</sup> test)

Index A to detect healthy dogs :

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

Index C to detect affected dogs :

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

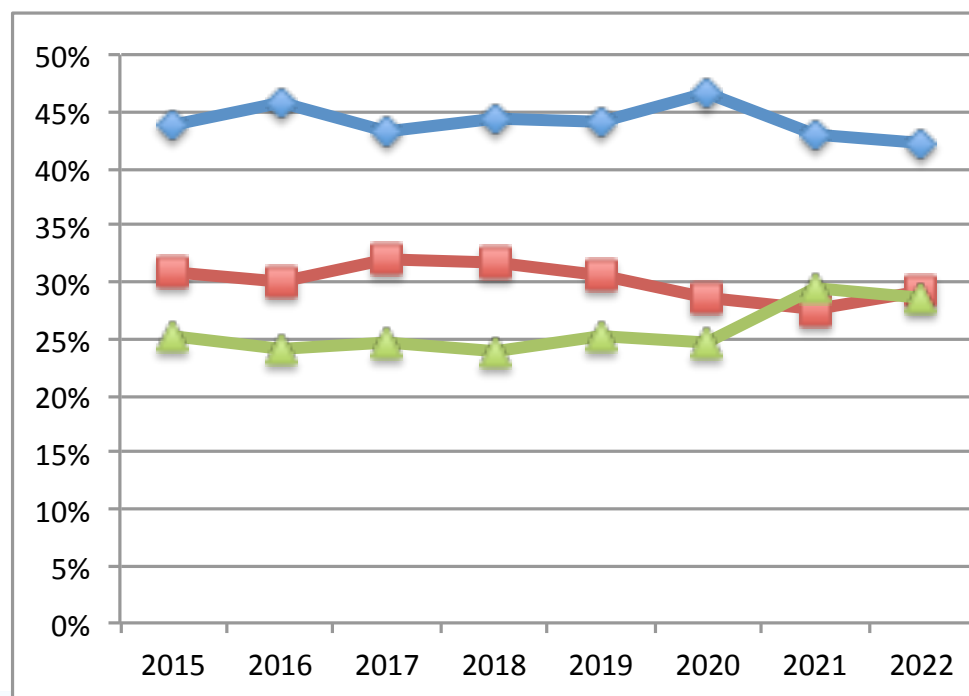
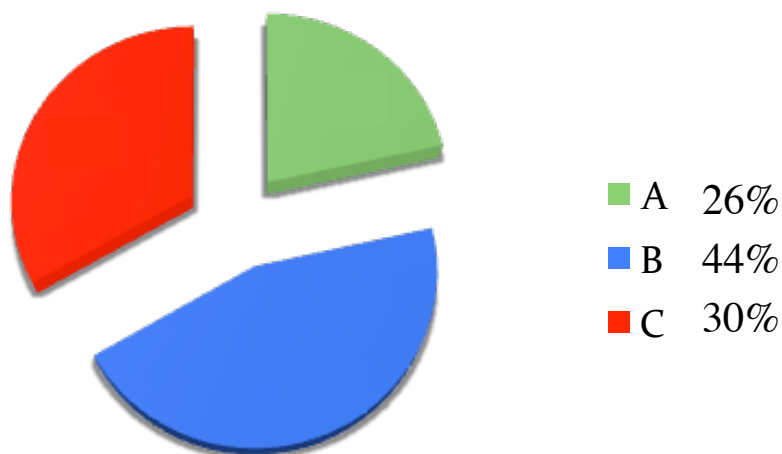


# Summary of HS tests performed by Antagene



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

distribution of index in this population:

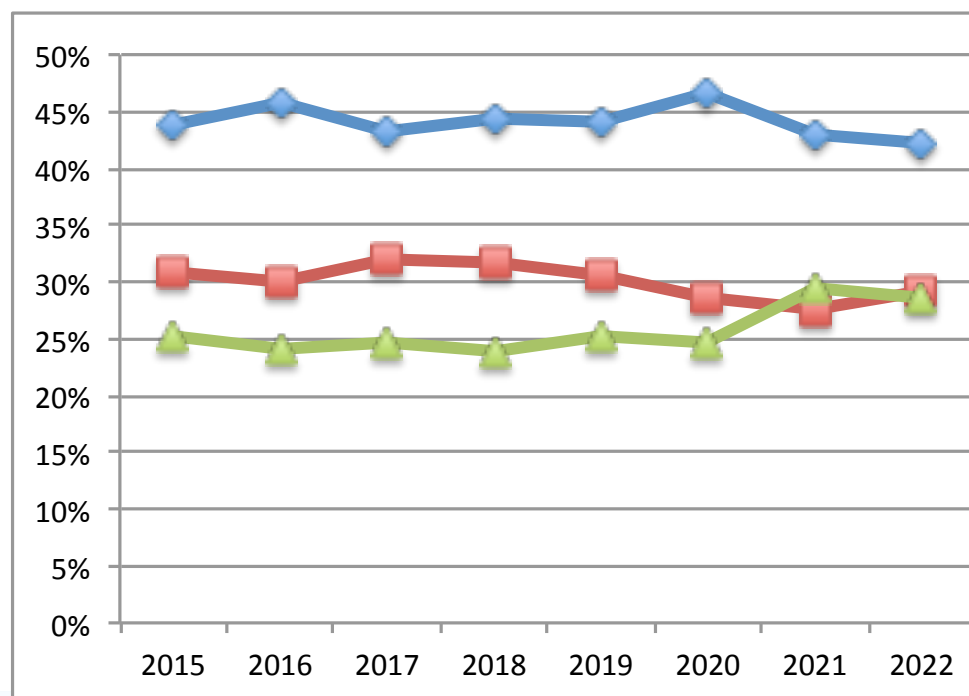
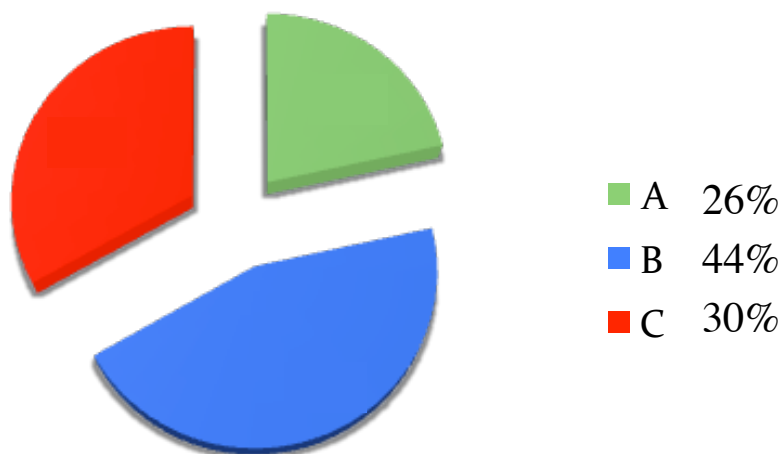


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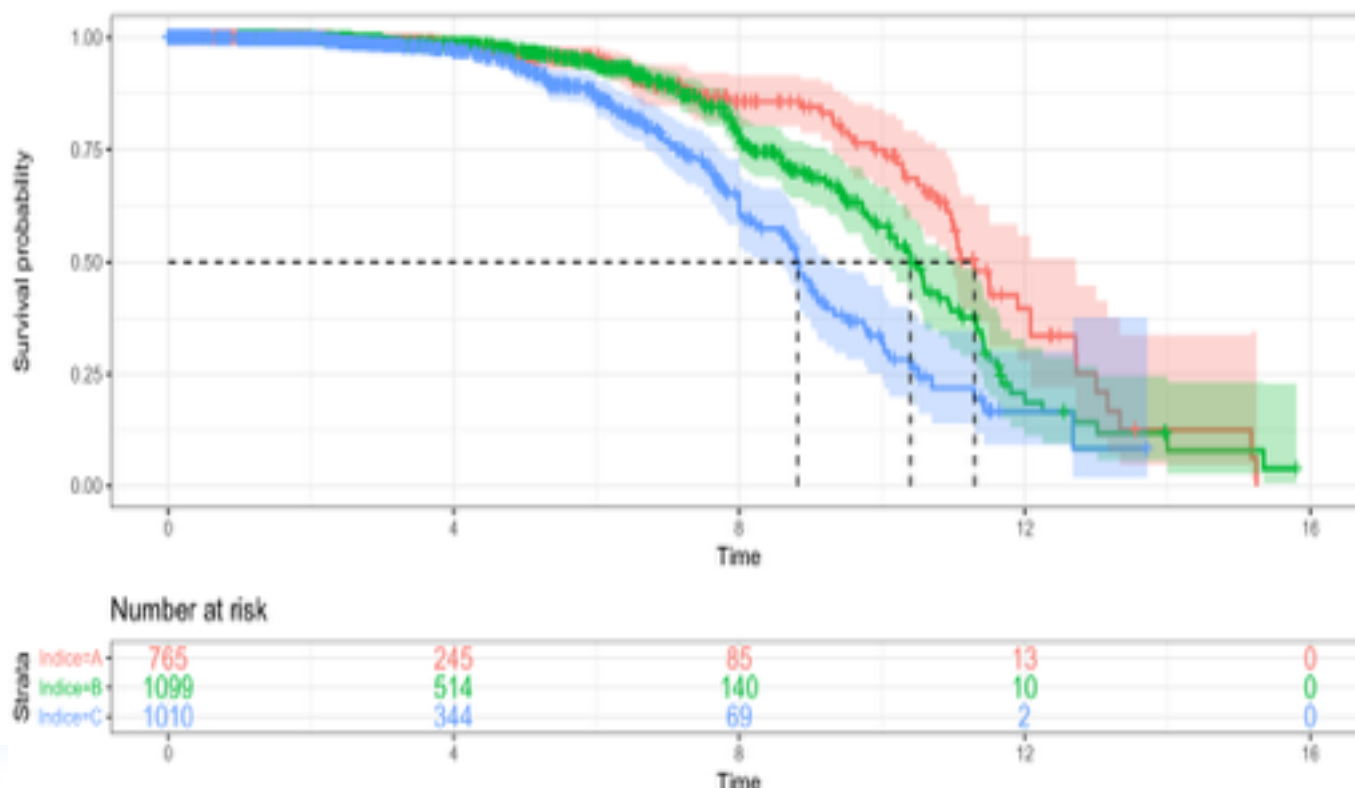
In Sweden : 481 tested dogs

23% of A , 42,6% of B and 34,4% of C

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Since 2012 , Antagene has tested >6700 dogs (unknown phenotypes):  
follow up of >5 years old dogs (PhD Eléonore Thiery)



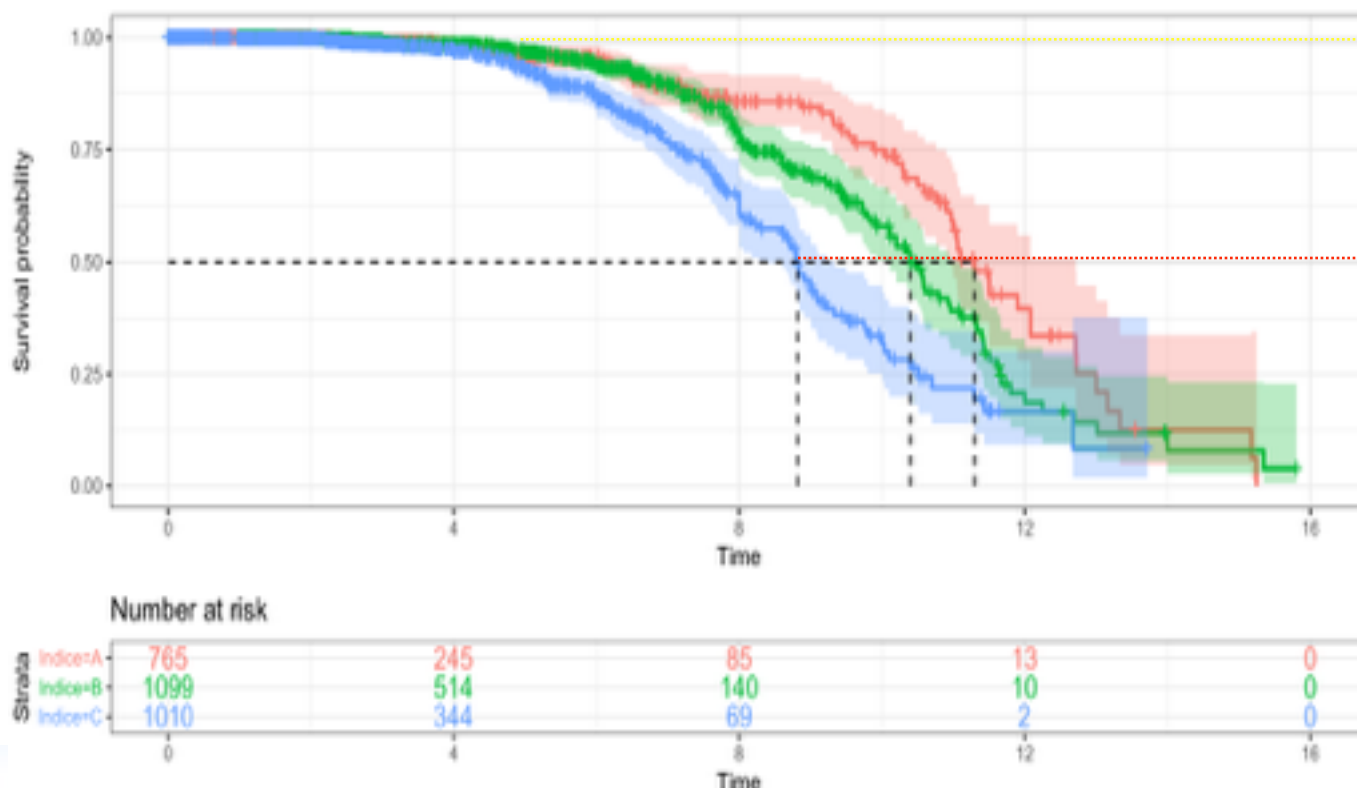
pvalue=  $4 \times 10^{-10}$

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

# Summary of HS tests performed by Antagene



Since 2012 , Antagene has tested >6700 dogs (unknown phenotypes):  
follow up of >5 years old dogs (PhD Eléonore Thiery)



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

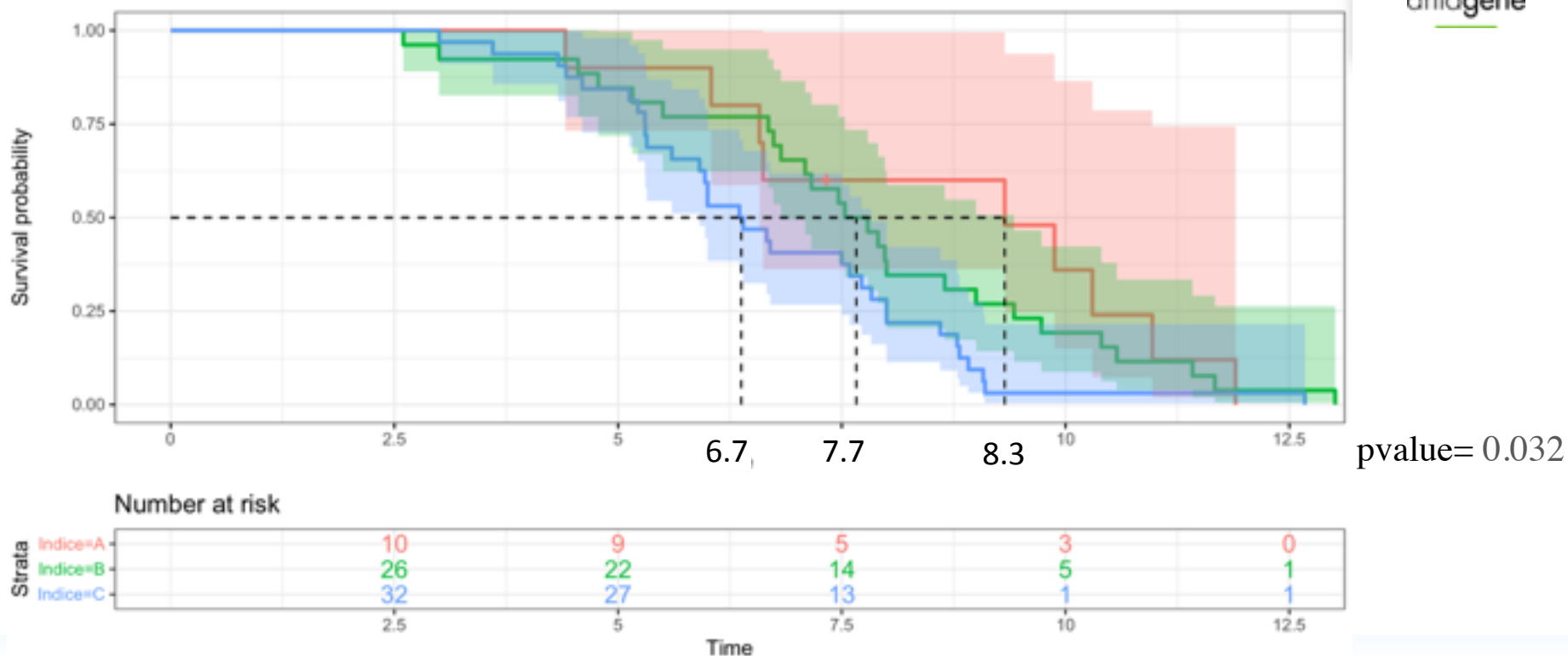
pvalue=  $4 \times 10^{-10}$

-> 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**):



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)

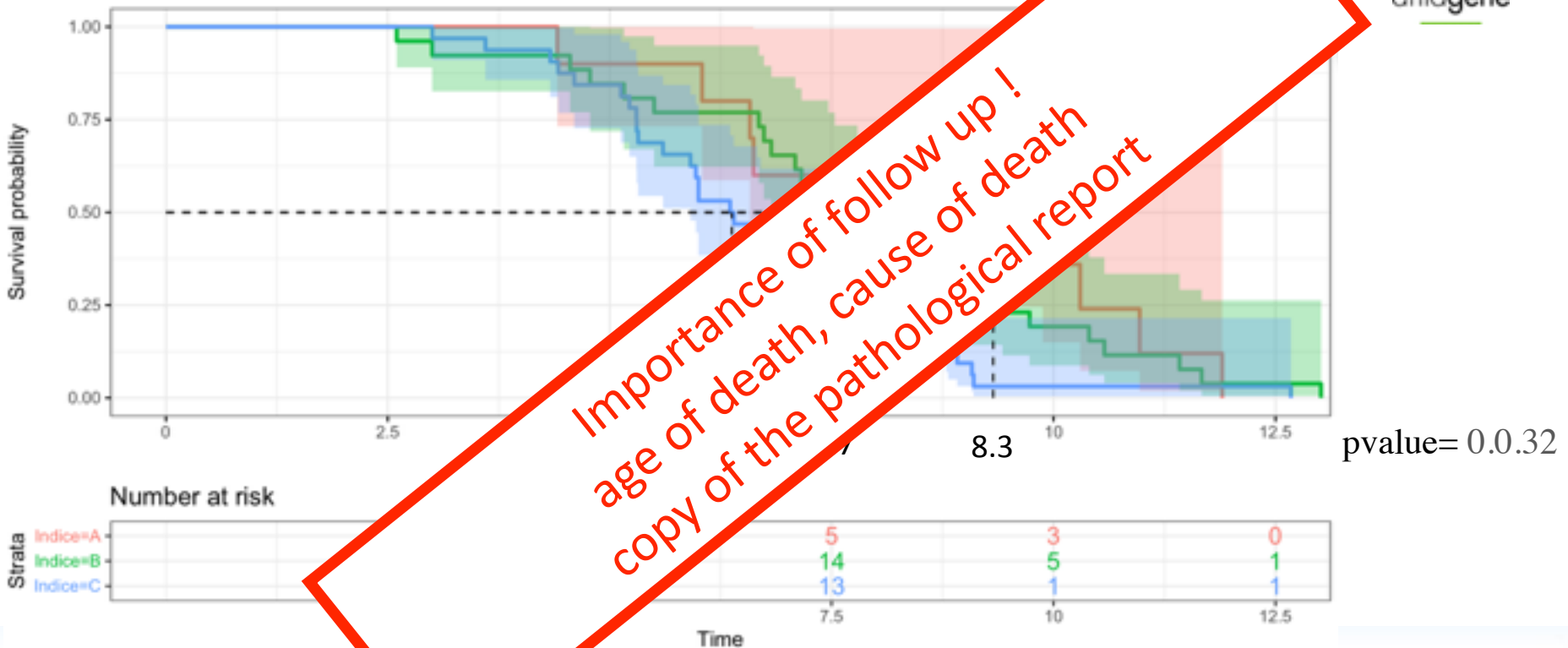
# Summary of HS tests performed by Antagene



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

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

Importance of follow up !  
age of death, cause of death  
copy of the pathological report



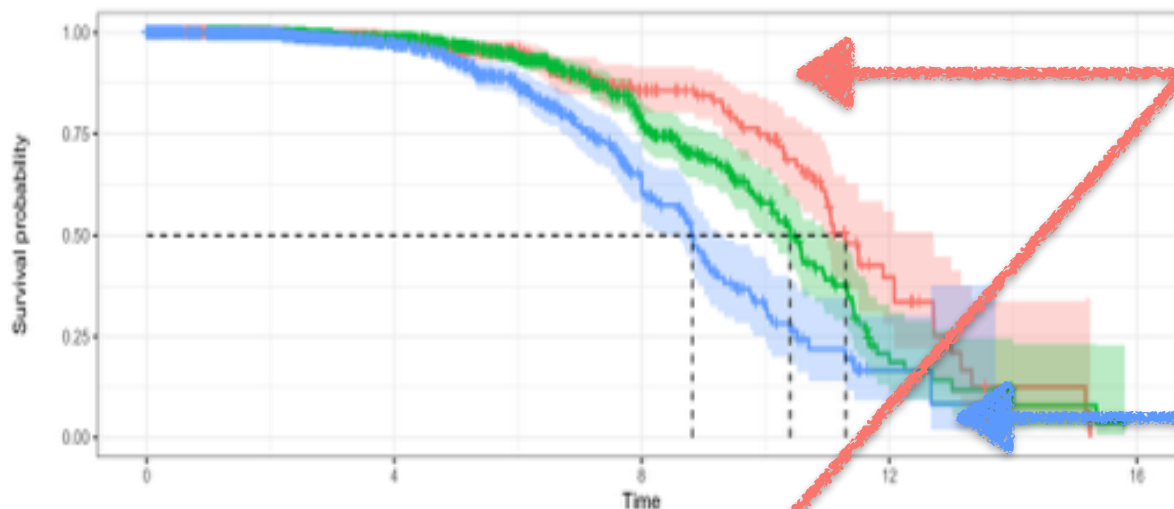
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# Summary of HS tests performed by Antagene



Since 2012 , Antagene has tested >6700 dogs (unknown phenotypes):  
follow up of >5 years old dogs (PhD Eléonore Thiery)



Why ~14 % of affected are A?

Why ~12% of unaffected are C?

|                 | A            | B            | C            |
|-----------------|--------------|--------------|--------------|
| <b>Affected</b> | <b>14%</b>   | <b>43.1%</b> | <b>42.9%</b> |
| <b>Healthy</b>  | <b>41.5%</b> | <b>46.5%</b> | <b>11.9%</b> |

# 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)



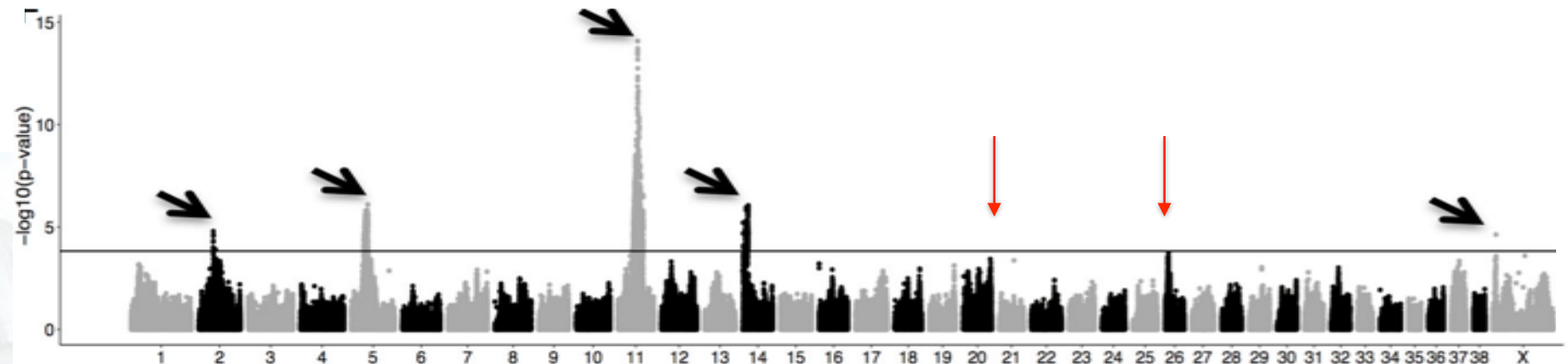
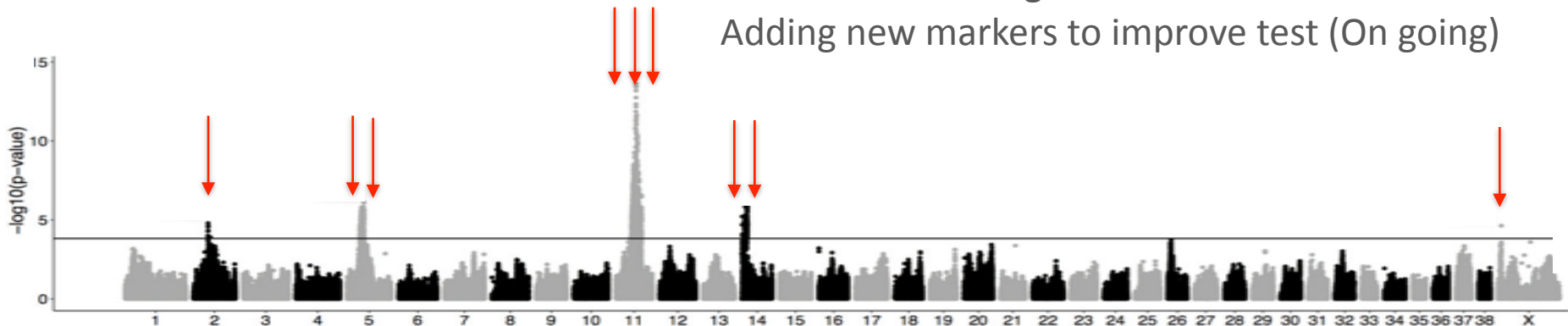
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Adding new markers to improve test (On going)



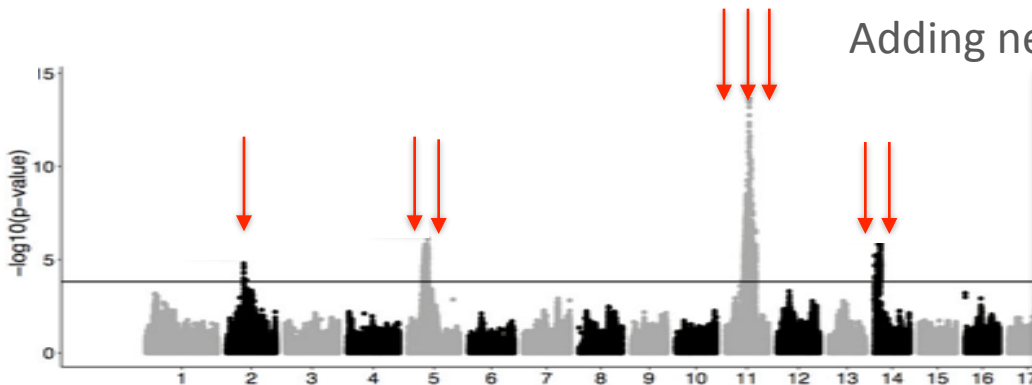
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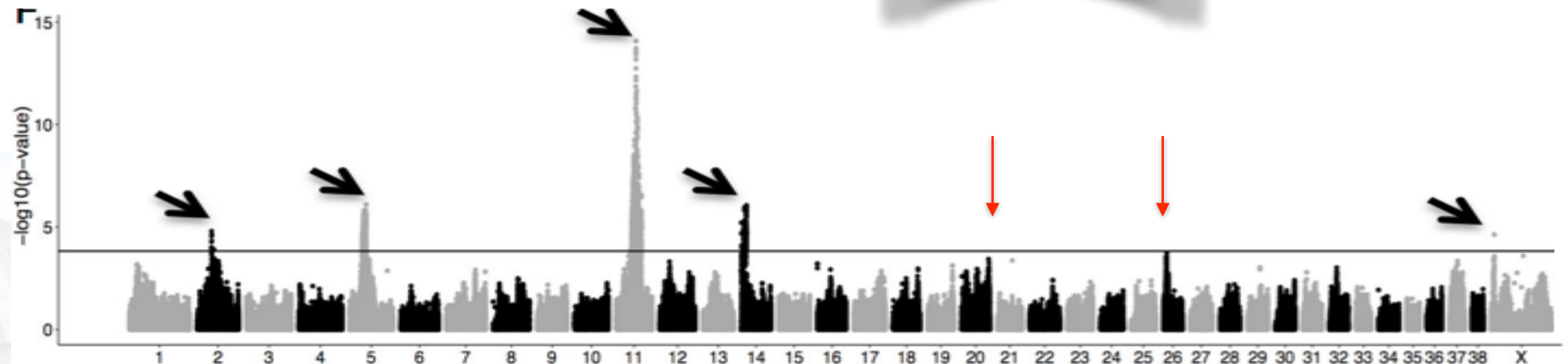
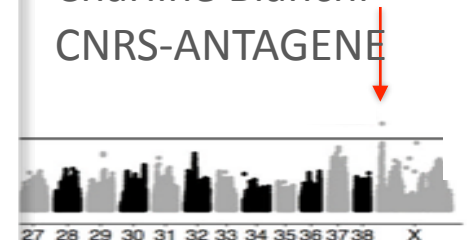
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Charline Bianchi  
CNRS-ANTAGENE



# 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)

➤ environmental factors :

- sexual hormones

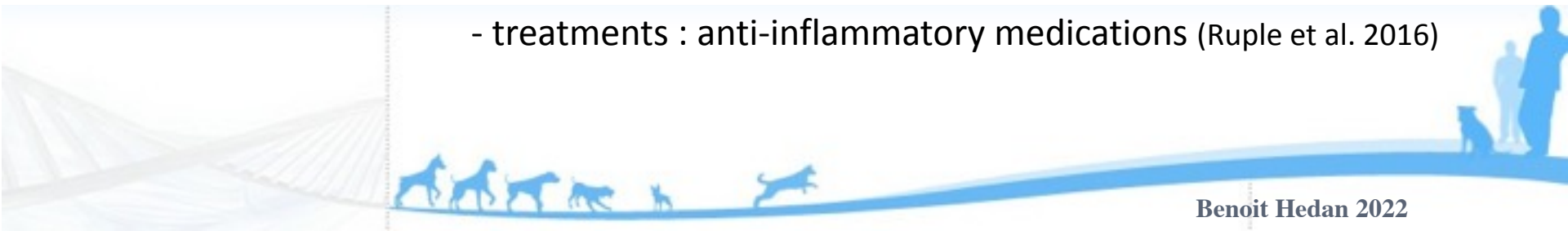
- inflammation : orthopaedic conditions...

(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

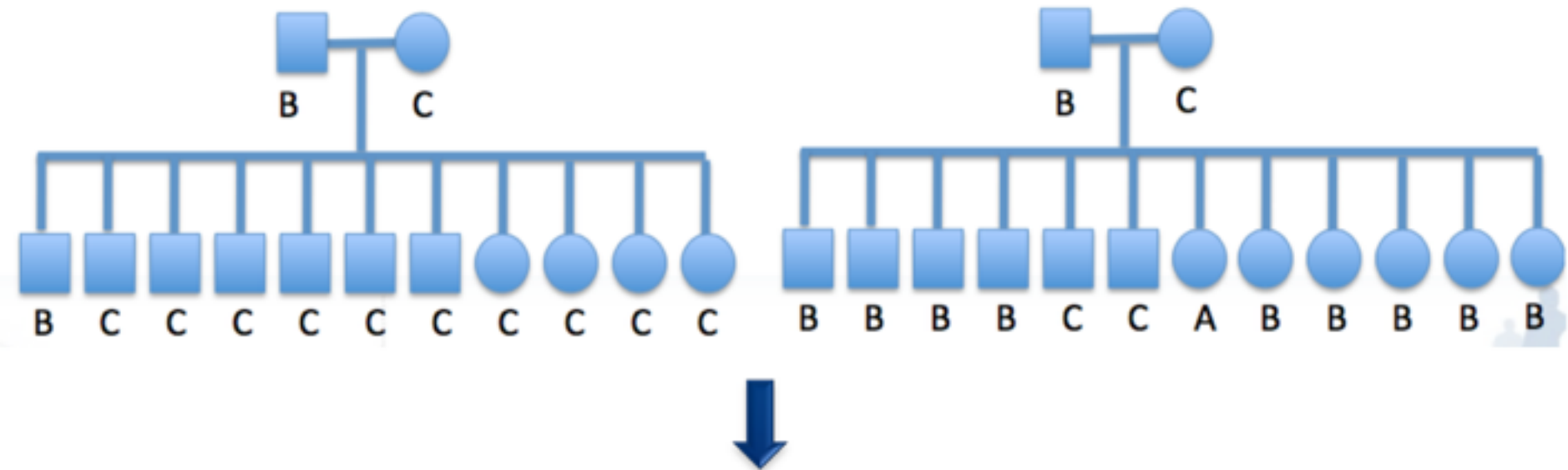
Ruple et al 2016

- 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

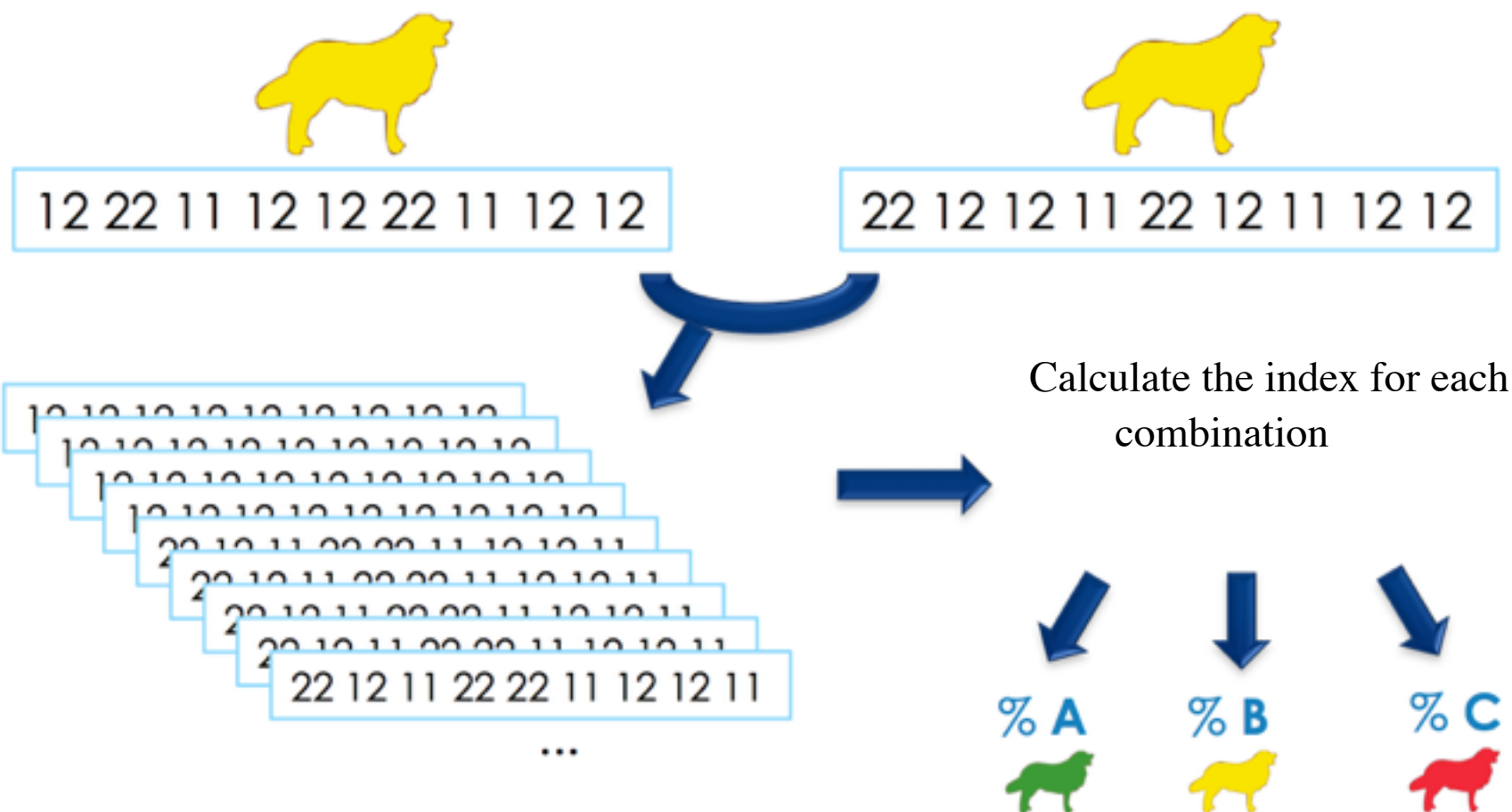
4123 sires and dams used for simulations

around 50 000 simulations per year



# Histiocytic Sarcoma Index Mate Selection (HSIMS)

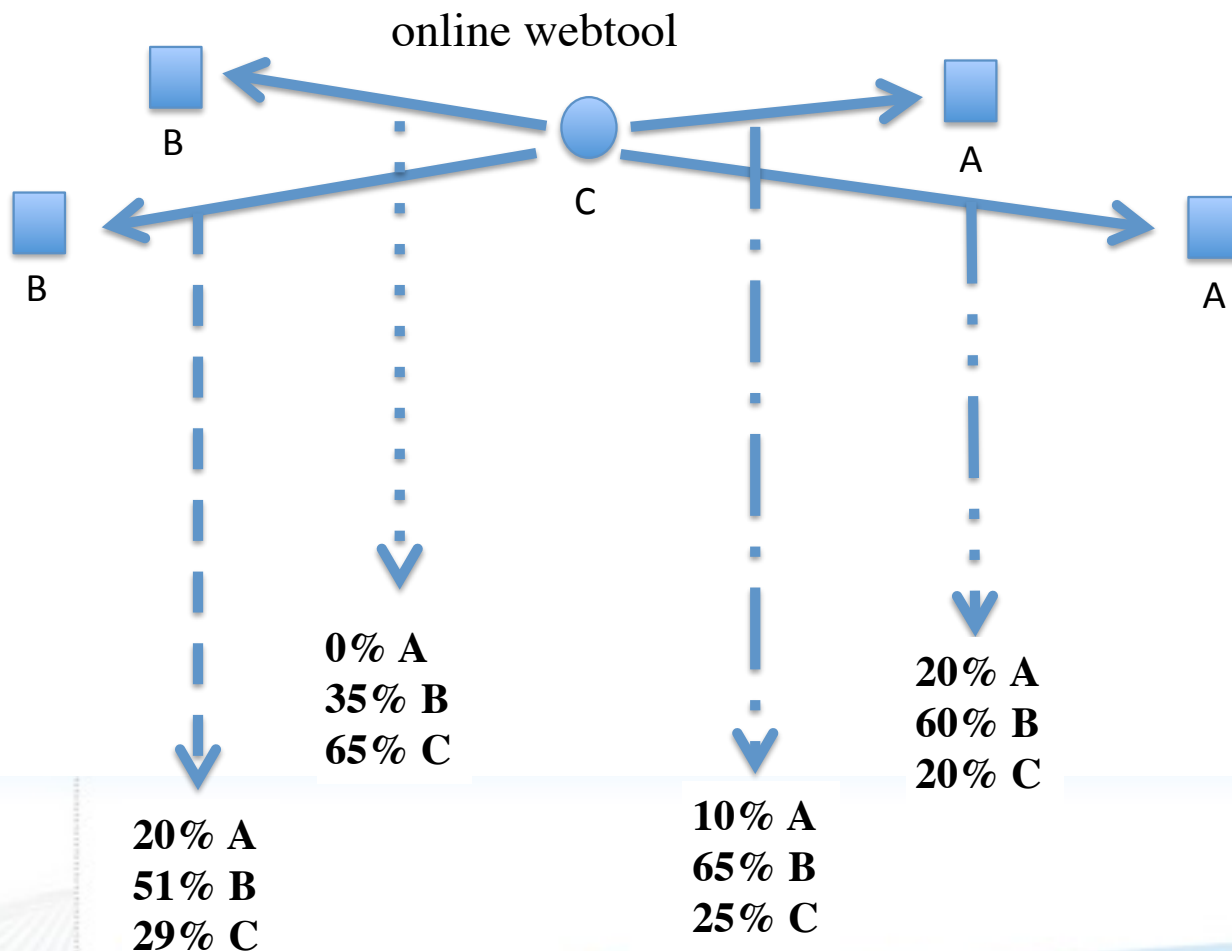
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# Histiocytic Sarcoma Index Mate Selection (HSIMS)

How to use this test ?

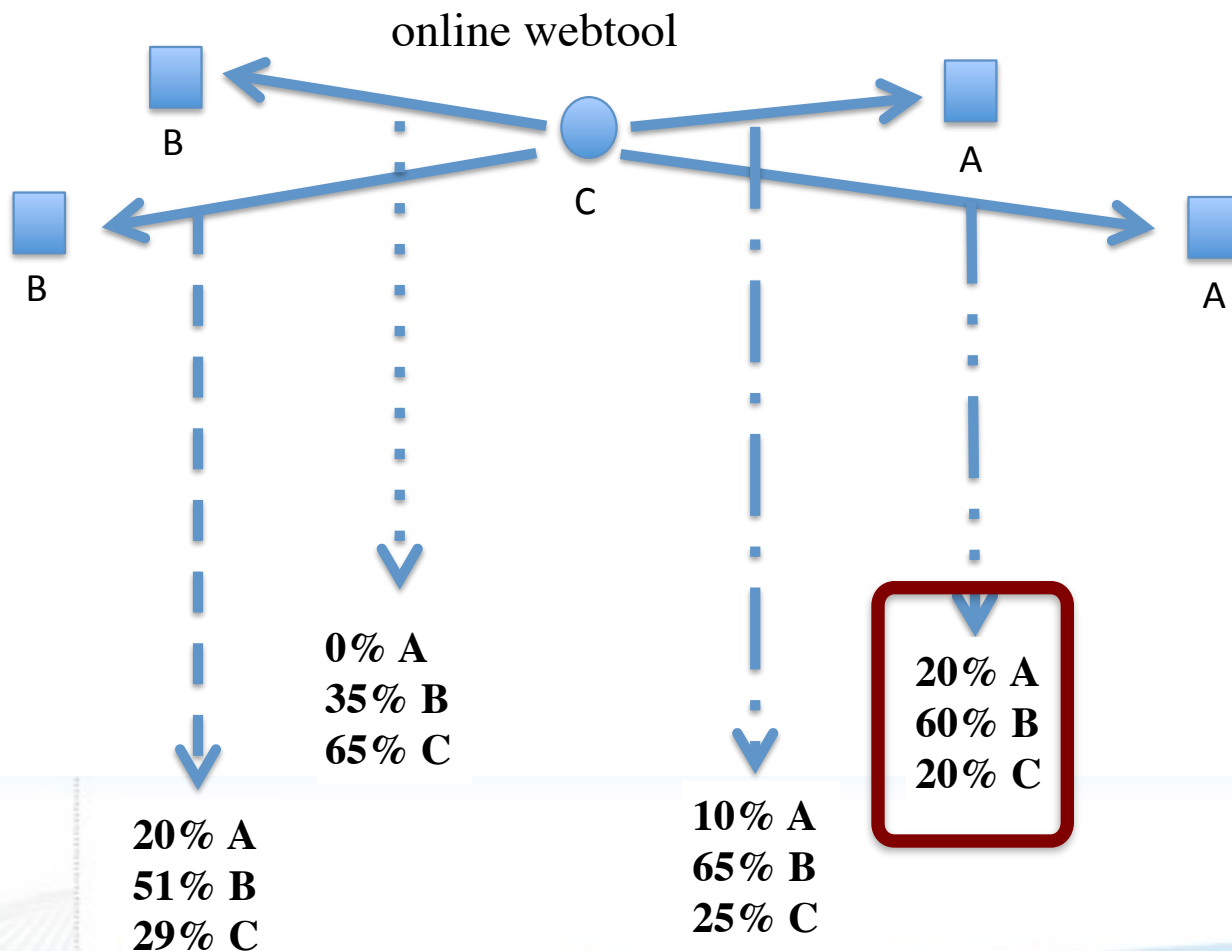
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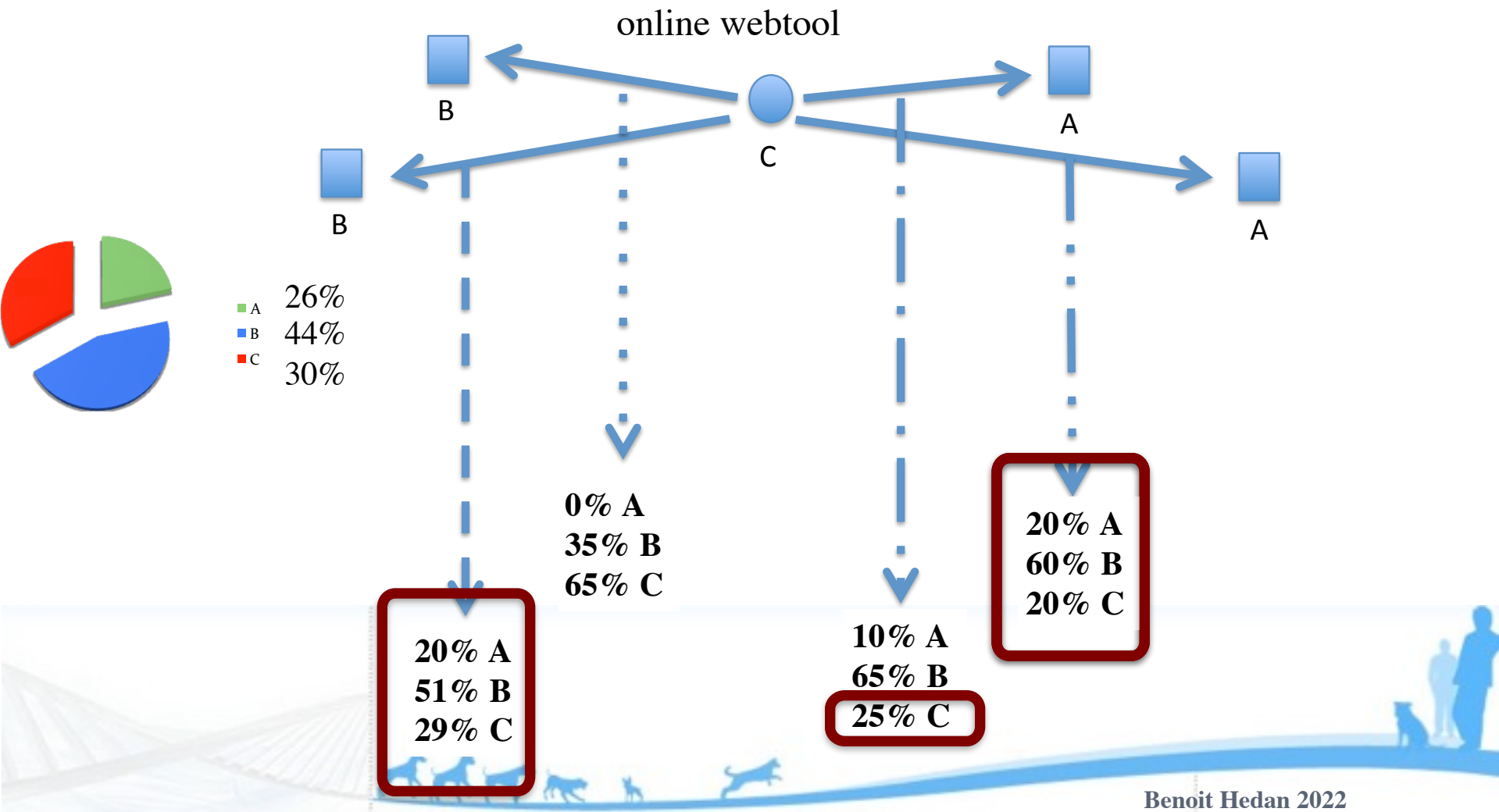
How to use this test ?

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## How to use this test ?

### Development of Histiocytic Sarcoma Index Mate Selection (HSIMS)



# Histiocytic Sarcoma Index Mate Selection (HSIMS)

## Recommendation from SKK and SShK

Dog with risk index C should not be mated with another C dog or with untested dog. For a safer risk assessment, the breeding tool HSIMS should be used, if possible, and C should be below 35% (in France they say 31%). **It is not mentioned that many A's are recommended.**

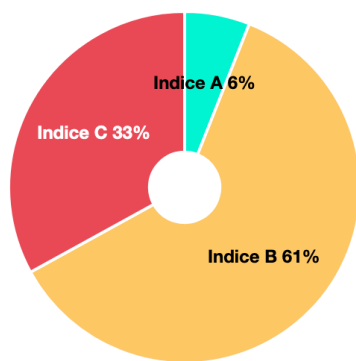
## Question regarding the use of the test:

### My question is.

**Why is this combination preferable,**

it does have C as the normal population, but A has 17% worse than the normal population.

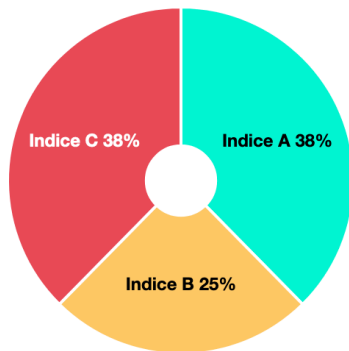
**Bitch C and Male B**



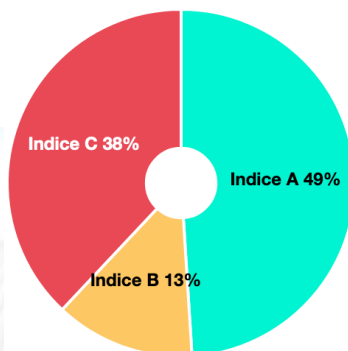
**Why should you not use these combinations,**

here C is certainly 3% above recommended, but in one case A is 15% and in the other case 26% above the normal population. **Aren't these two combinations better than the first mentioned.**

**Bitch C and Male A**



**Bitch B and Male B**





# Histiocytic Sarcoma Index Mate Selection (HSIMS)

## Recommendation from SKK and SShK

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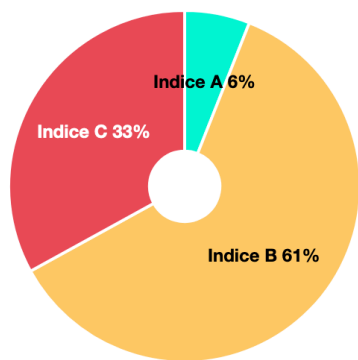
**Why is this combination preferable,**

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Bitch C and Male B



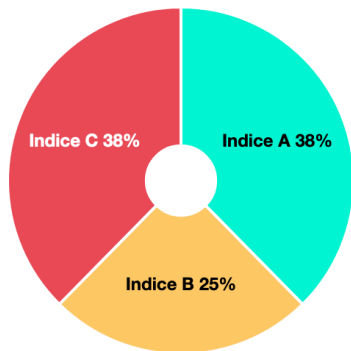
We focused on C dogs because breeders focus on A dogs:

- overuse of some A sire
- remove C dogs from reproduction

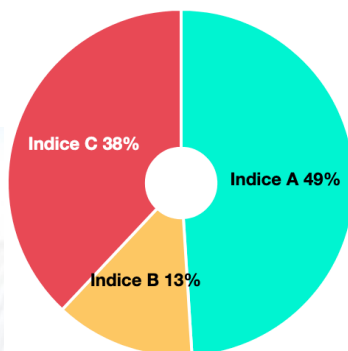
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Bitch B and Male B



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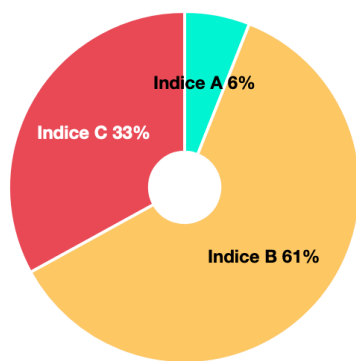
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**Bitch C and Male B**



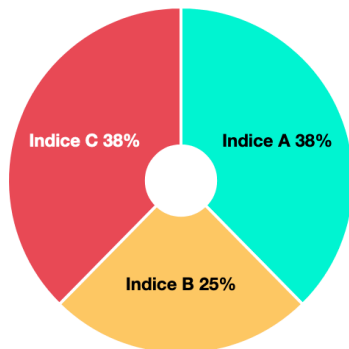
In a litter of 5 dogs : we expect  
1 to 2 C dogs, 3 B dogs, 0 to 1 A dog

In a litter of 10 dogs : we expect  
3 to 4 C dogs, 6 B dogs, 0 to 1 A dog

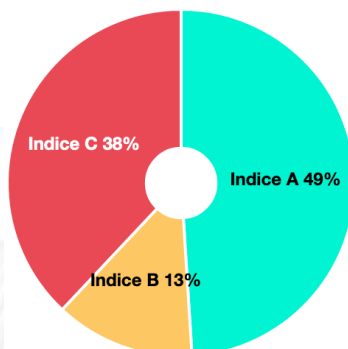
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**Bitch C and Male A**



**Bitch B and Male B**



In a litter of 5 dogs : we expect  
2 C dogs, 0 to 1 B dog, 2 to 3 A dogs

In a litter of 10 dogs : we expect  
4 C dogs, 1 to 2 B dogs, 5 A dogs

# Summary of the HS test

## How to use this test ?

- help for selection and mating based on **actual knowledge**  
other important unknown genetic factors



# Summary of the HS test

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- help for selection and mating based on **actual knowledge**  
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# Summary of the HS test

## How to use this test ?

- help for selection and mating based on **actual knowledge**  
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- a helpful criteria in association with other criteria : **Life span, health, inbreeding...**
- **keep diversity and lineages ! Use the C dogs (30%) and not overused the A dogs!**



# Summary of the HS test

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C



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compatible sire    C  
(A,B,C)

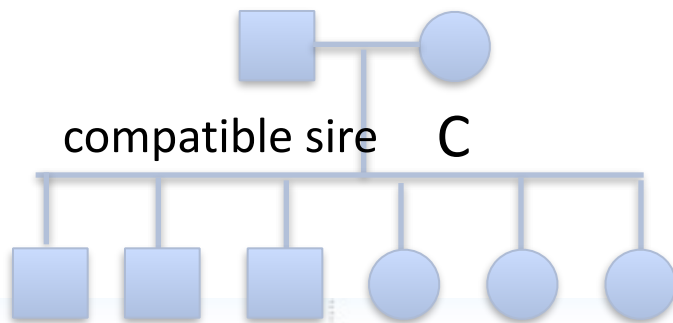




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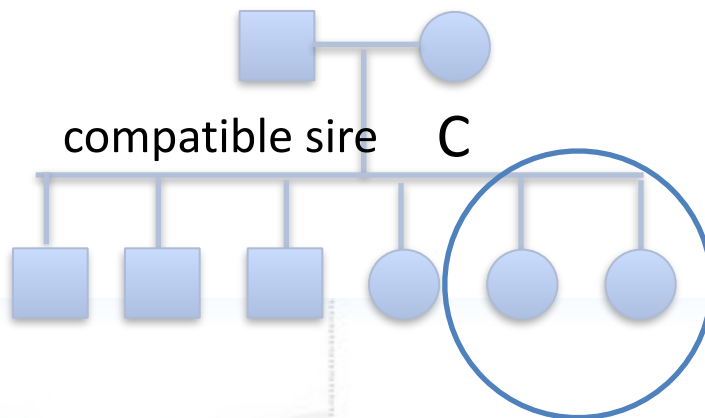
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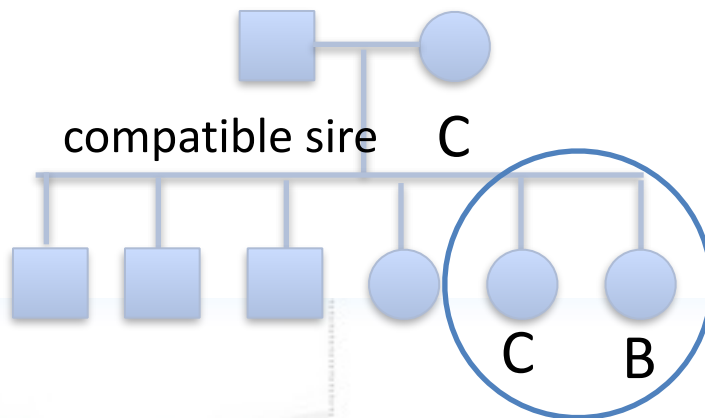


Selection of your best candidate futur dams

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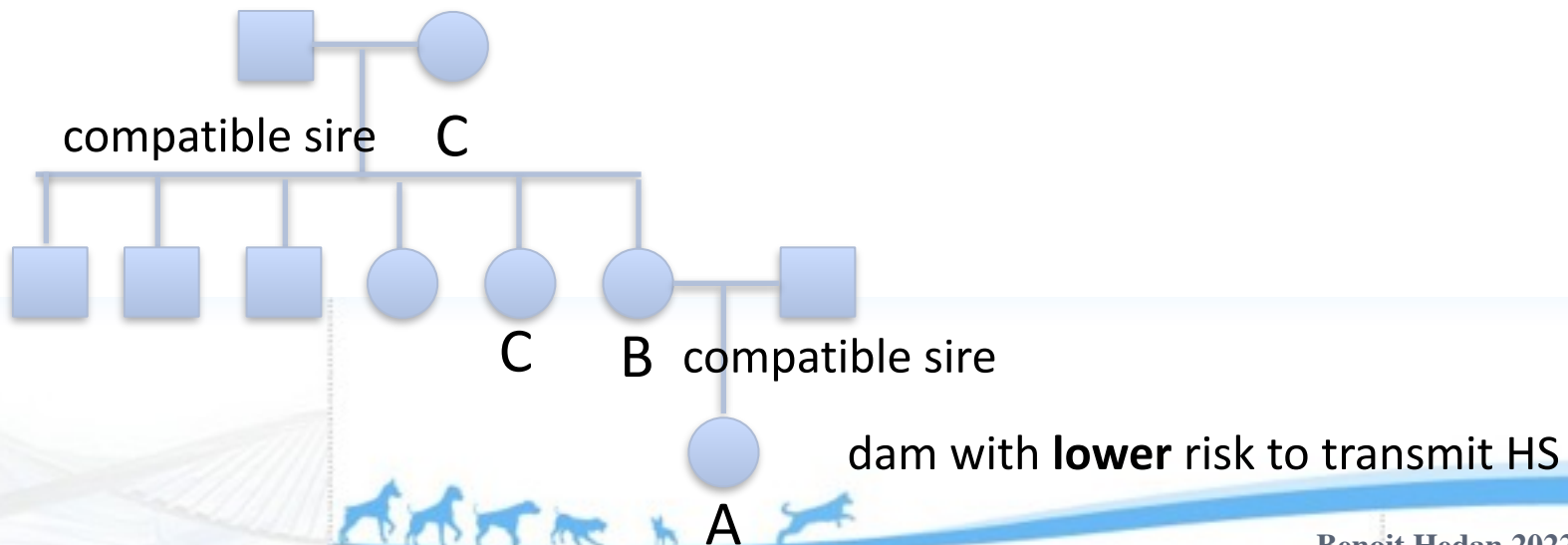


Selection of your best candidate futur dams  
TEST SH

# Summary of the HS test

## How to use this test ?

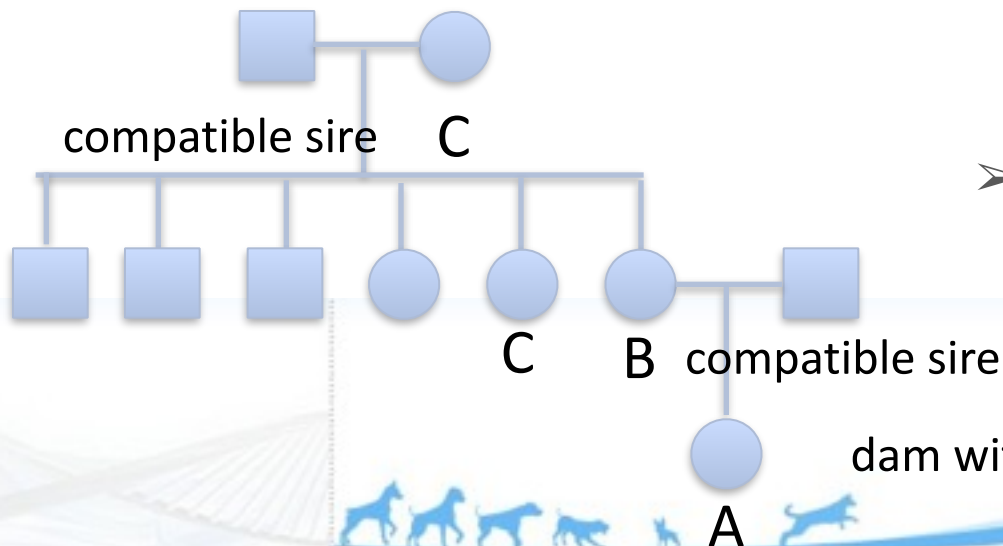
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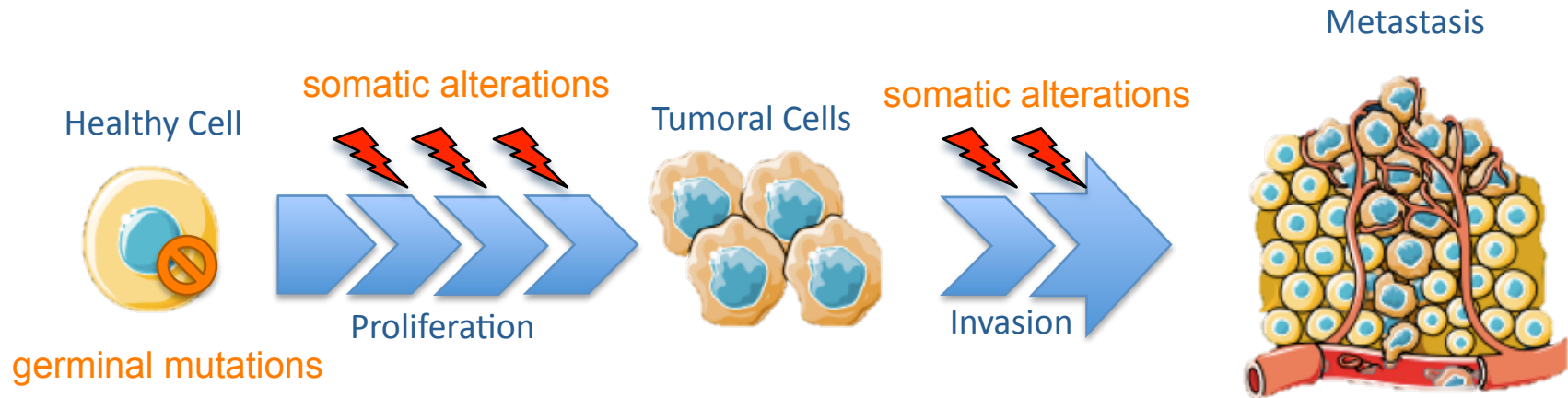
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- help for selection and mating based on **actual knowledge**  
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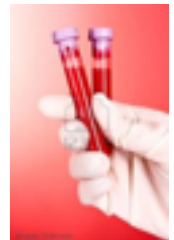
➤ **keep diversity and select slowly**

# A long term goal : Identify genetic mechanisms involved in HS



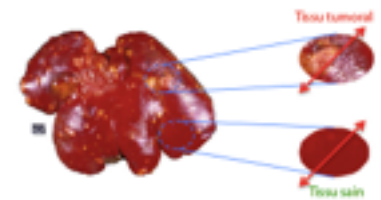
- Identify predisposing genes and risk alleles

-> Blood collection



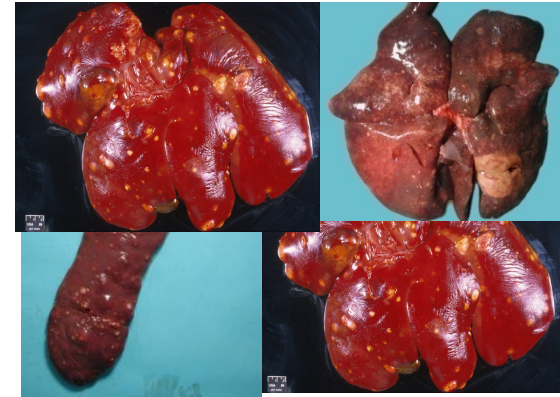
- Identify recurrent somatic alterations associated with tumor progression

-> Tissue collection



# Canine histiocytic sarcoma: Somatic mutations

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

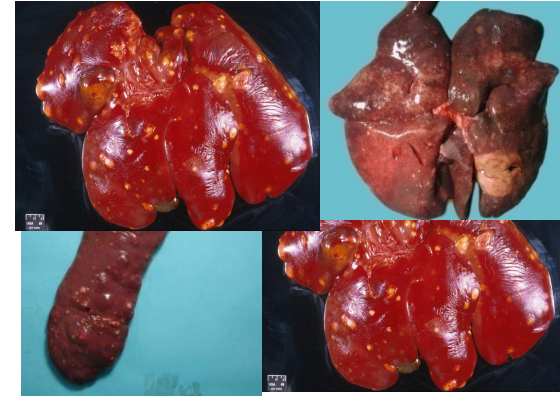




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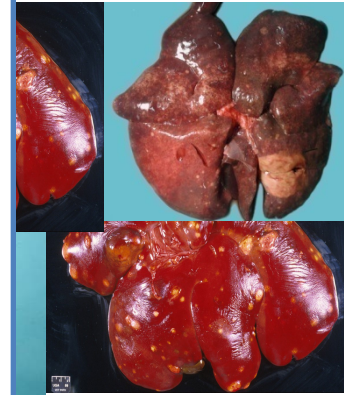
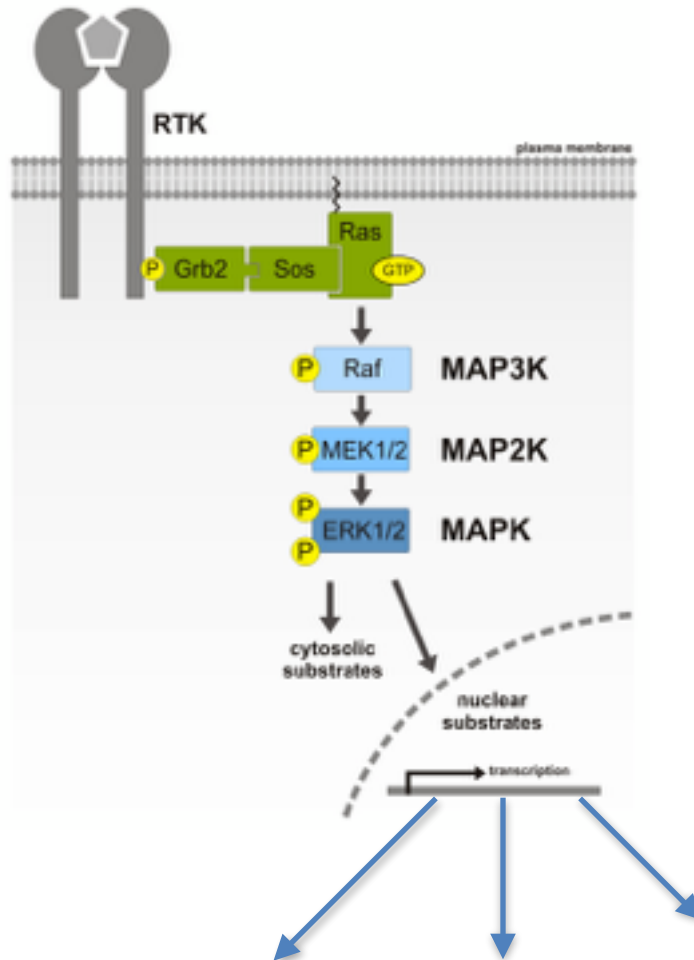
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Gene mutated in human myelomonocytic leukemia



# Canine histiocytic sarcoma:

Recurrent somatic mutations  
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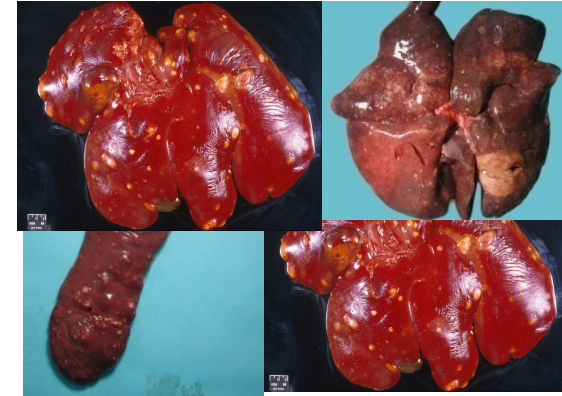


Proliferation survival differentiation....

# 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

**-> major event for HS development**



ORIGINAL ARTICLE

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

T. Thaiwong<sup>1</sup> | S. Sirivisoot<sup>1,2</sup> | M. Takada<sup>3,4</sup> | V. Yuzbasiyan-Gurkan<sup>3,4</sup> | M. Klupel<sup>1,4,5</sup>

<sup>1</sup>Veterinary Diagnostic Laboratory, Michigan State University, Lansing, Michigan

<sup>2</sup>Department of Pathology, Faculty of Veterinary Sciences, Chulalongkorn University, Bangkok, Thailand

<sup>3</sup>Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan

<sup>4</sup>Comparative Medicine and Integrative Biology Program, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan

<sup>5</sup>Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan

Correspondence

M. Klupel, Veterinary Diagnostic Laboratory, Michigan State University, 4125 Beaumont Road, Room 152A, Lansing, MI 48950.  
Email: klupel@vet.msu.edu

Histiocytic sarcoma (HS) is an aggressive malignant neoplasm of dendritic cell origin that is 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*<sup>E76K</sup> in HS of BMDs. Amplification of exon 3 of the *PTPN11* gene followed by Sanger sequencing was used to detect the mutation and estimate the prevalence in HS from 30 BMDs, 13 Golden Retrievers and 10 other dog breeds. The overall prevalence of *PTPN11*<sup>E76K</sup> in HS of BMDs was 36.67% 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*<sup>E76K</sup> suggests that a gain-of-function might be mediated by the ERK and AKT pathways. These data suggest *PTPN11*<sup>E76K</sup> as an important driver mutation of HS in BMDs. This information may not only aid in unravelling the tumorigenic events associated with HS in BMDs, but also help in identifying more promising therapeutic strategies.

KEYWORDS

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

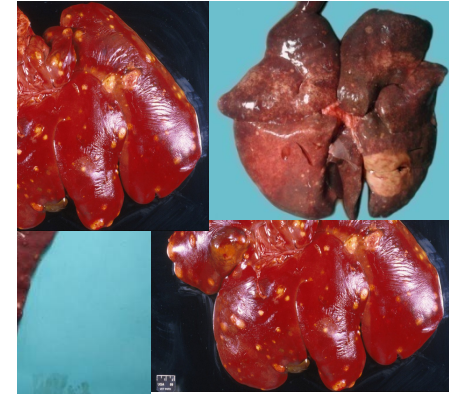
## 1 | INTRODUCTION

Histiocytic sarcoma (HS) is the most aggressive entity in the spectrum of histiocytic diseases in dogs.<sup>1</sup> HS was first recognized in Bernese Mountain Dogs (BMDs)<sup>2</sup> as a neoplasm derived from cells with the phenotypic profile of interstitial dendritic cells.<sup>3</sup> 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.<sup>4–6</sup> 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.<sup>7</sup> Therefore, it has been suggested that the potential breed-specific genetic background may be responsible for the tumorigenesis of HS in BMDs.<sup>1–7</sup>

The genomic loci involved in HS in BMDs and Flat-Coated Retrievers have been studied extensively,<sup>8,9</sup> but not definitively.

Evaluation of genome-wide copy number aberrations (CNAs) revealed recurrent CNAs common to HS. CNAs in tumour suppressor gene loci (*CDKN2A/B*, *FBX1* 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.<sup>8</sup> 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* and is present in 96% of affected BMDs.<sup>9</sup> While impaired expression of *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.

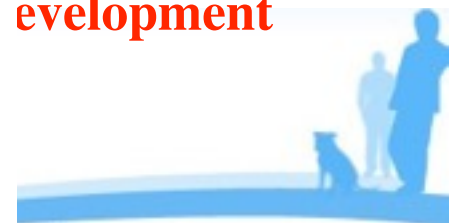
In humans, HS is a rare neoplasm that tends to have an aggressive course and responds poorly to treatment.<sup>10</sup> The most frequent mutations in human HS cases were those identified in the *BRAF* gene,



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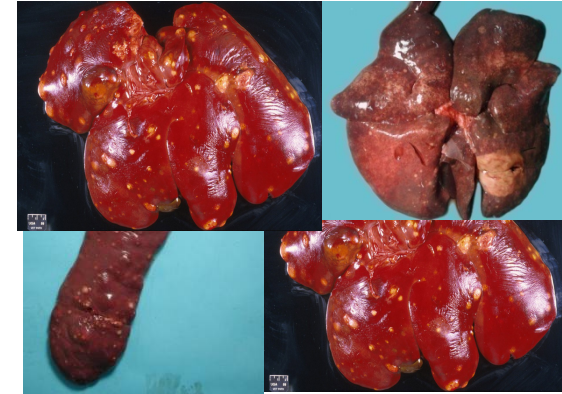
enoit Hedan 2022



# 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)

Recurrent somatic mutations in the genes *TP53* and *PTEN*

Gene mutated in 100% of cases

Sequencing of PTEN and TP53 in 100 cases

Mutations linked to clinical presentation (J Donnadieu)

## Short Report

### PTPN11 mutations in canine and human disseminated histiocytic sarcoma

Benoit Hédan<sup>1</sup>, Mélanie Rault<sup>2</sup>, Jérôme Abadie<sup>3</sup>, Ronan Ulv<sup>4</sup>, Nadine Botheret<sup>5</sup>, Patrick Devauchelle<sup>6</sup>, Christiane Cople-Bergman<sup>4,5</sup>, Edouard Cadieu<sup>1</sup>, Marie Parrens<sup>6</sup>, Julia Alten<sup>7</sup>, Emmanuelle L. Zaïzman<sup>8</sup>, Gunnar Carlö<sup>9</sup>, Gandhi Dama<sup>10</sup>, Karima Mekhtari<sup>10</sup>, François Le Loarer<sup>11</sup>, Aurone Coulomb-Lhermine<sup>12</sup>, Thomas Derrien<sup>1</sup>, Christophe Hitté<sup>1</sup>, Laura Bachelot<sup>13</sup>, Matthew Green<sup>13</sup>, David Gilon<sup>1</sup>, Jean Y. Blay<sup>13</sup>, Jean Donnadieu<sup>14</sup> and Catherine André<sup>1</sup>

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<sup>4</sup>Assistance Publique-Hôpitaux de Paris, Département de Pathologie, Groupe Henri-Mondor-Albert-Chenevier, Critériol, France

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<sup>6</sup>Department of Pathology, CHU de Bordeaux, Hôpital du Haut Lévy, INSERM U1095, Université de Bordeaux, Bordeaux, France

<sup>7</sup>Pediatric Oncology/Hematology, University Hospital Schleswig-Holstein, Kiel, Germany

<sup>8</sup>Department of Neuropathology, GHU Paris Psychiatrie Neurosciences, Sainte-Anne Hospital, Paris, France

<sup>9</sup>Haematology Institute, CHU de Caen and Centre François Baclesse, Caen, France

<sup>10</sup>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 Escourrou, Paris, France

<sup>11</sup>Department of Medical Oncology, Centre Léon Bérard, Lyon, France

<sup>12</sup>Department of Pathology, Trousseau Hospital, Paris, France

<sup>13</sup>Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, CVM Research Building, Raleigh, NC

<sup>14</sup>Department of Hematology, APHP, Trousseau Hospital, Paris, France

In humans, histiocytic sarcoma (HS) is an aggressive cancer involving histiocytes. Its rarity and heterogeneity explain that 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 concentrated at hotspots common to human cancers. Recurrent *PTPN11* mutations are associated to visceral disseminated HS subtype in dogs, the most aggressive clinical presentation. 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 results 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*

DOI: 10.1002/ijc.32191

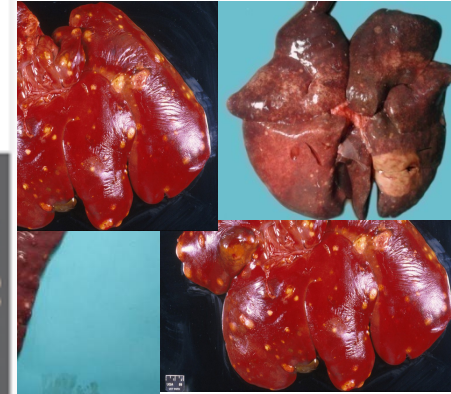
History: Received 20 Dec 2019; Accepted 20 Feb 2020;

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

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

Cancer Genetics and Epigenetics



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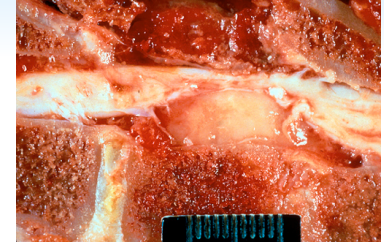
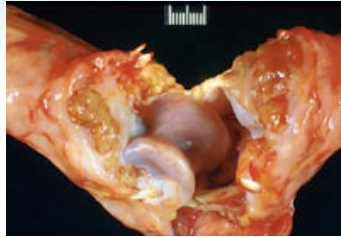
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20

Fisher test)

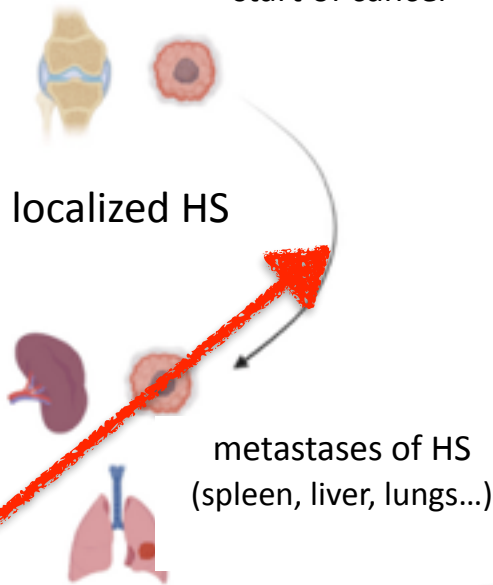
Benoit Hédan 2022





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...)

≠

disseminated HS to  
internal organs



**PTPN11  
mutations**

PTPN11 mutations probably occur in late stages of the tumor





# 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 )

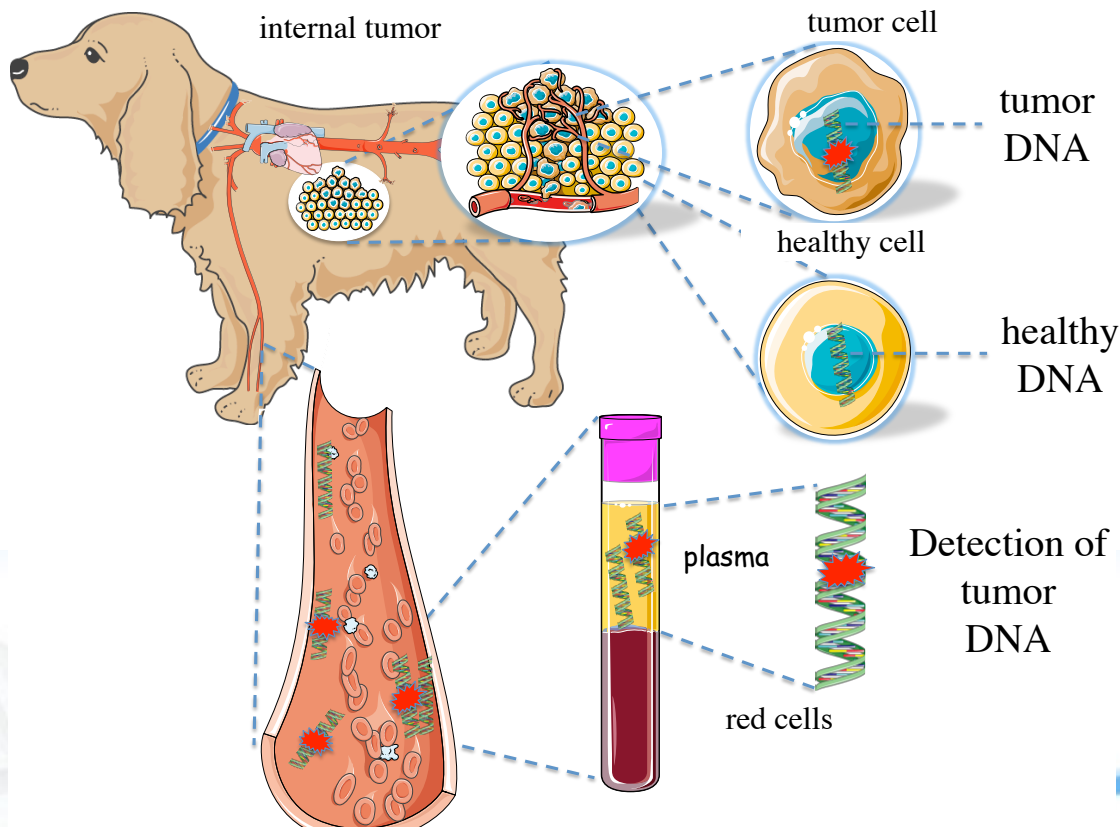


# Canine histiocytic sarcoma:

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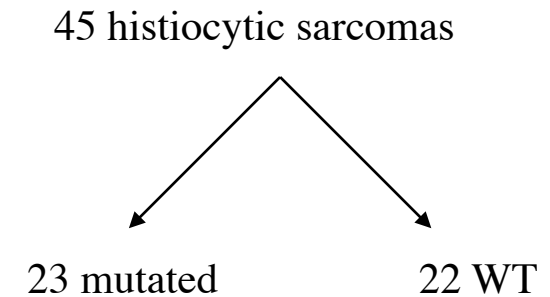
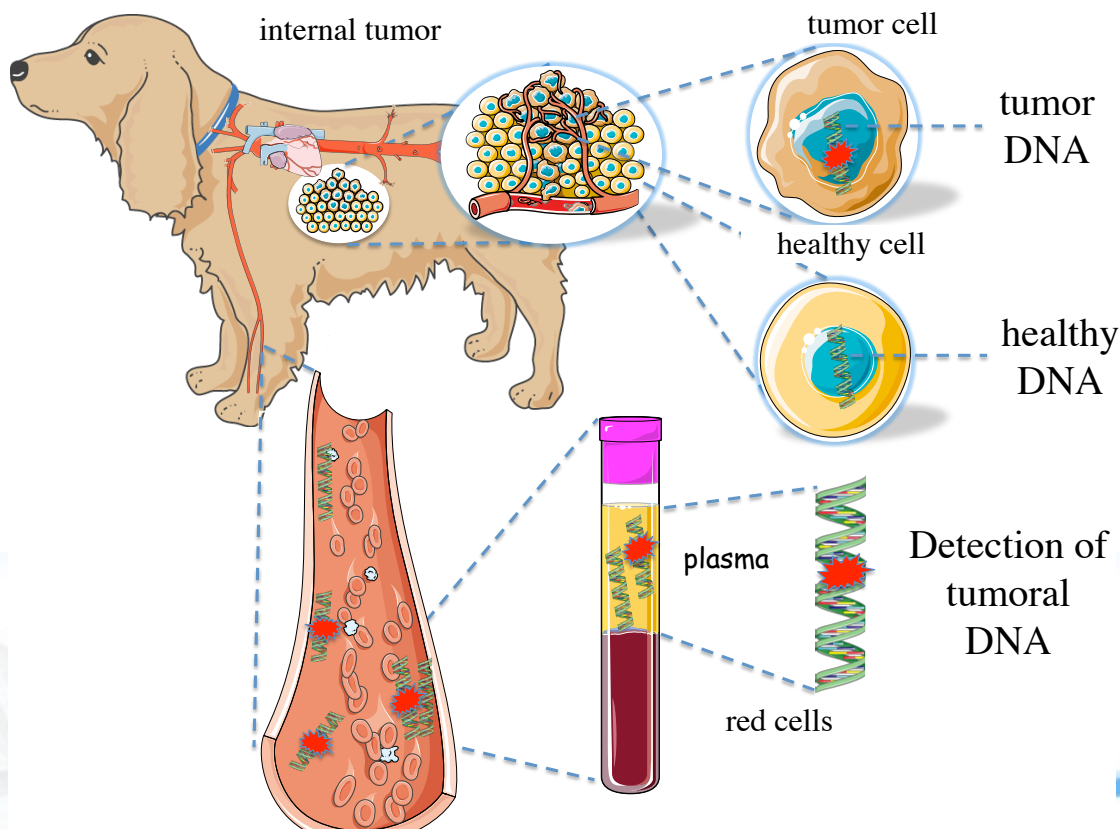


# Canine histiocytic sarcoma:

## Somatic mutations : applications for veterinary medicine

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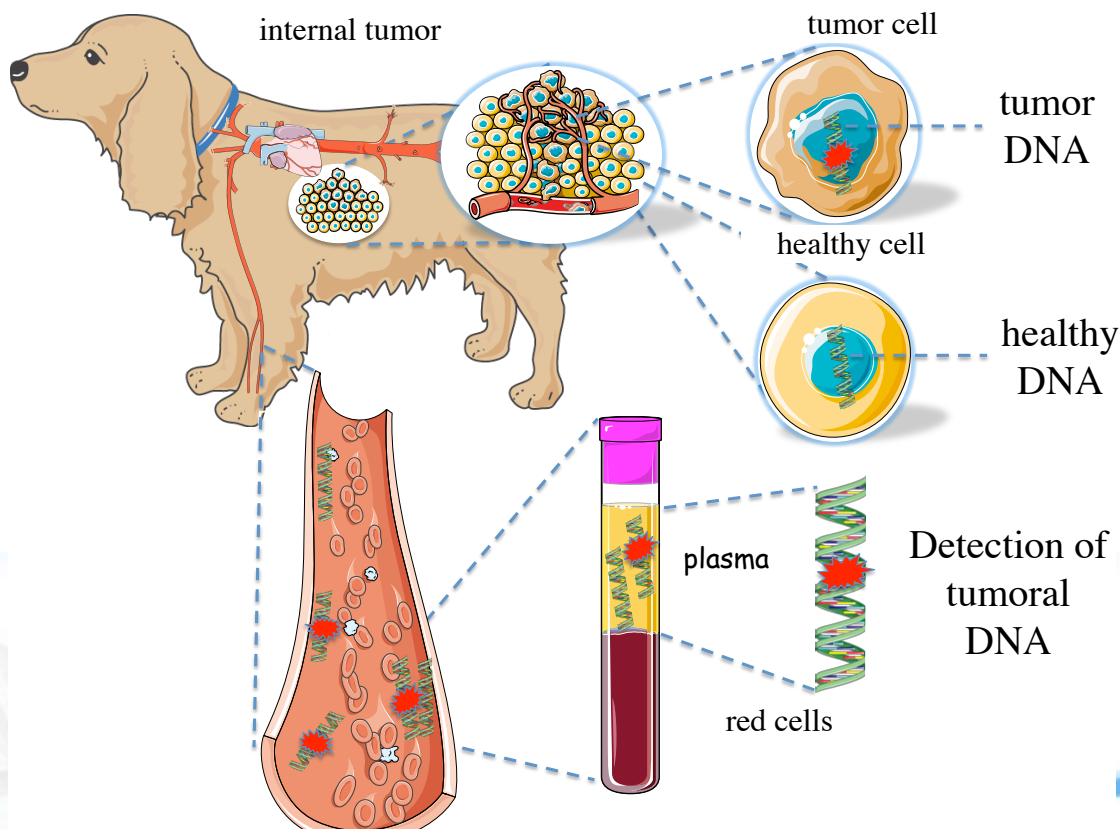
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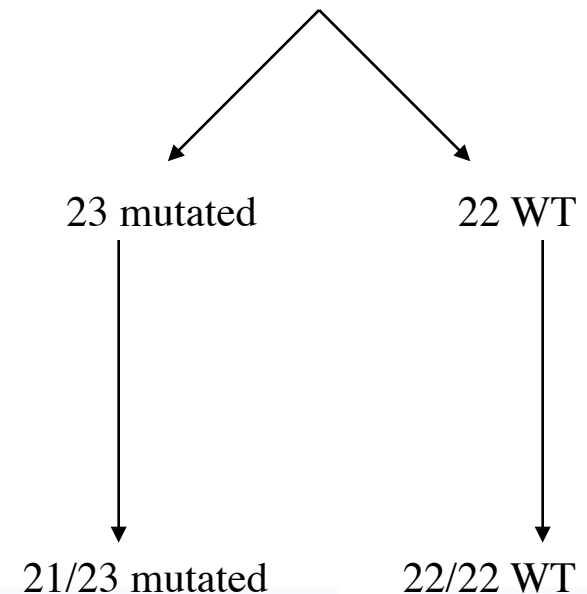
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45 histiocytic sarcomas



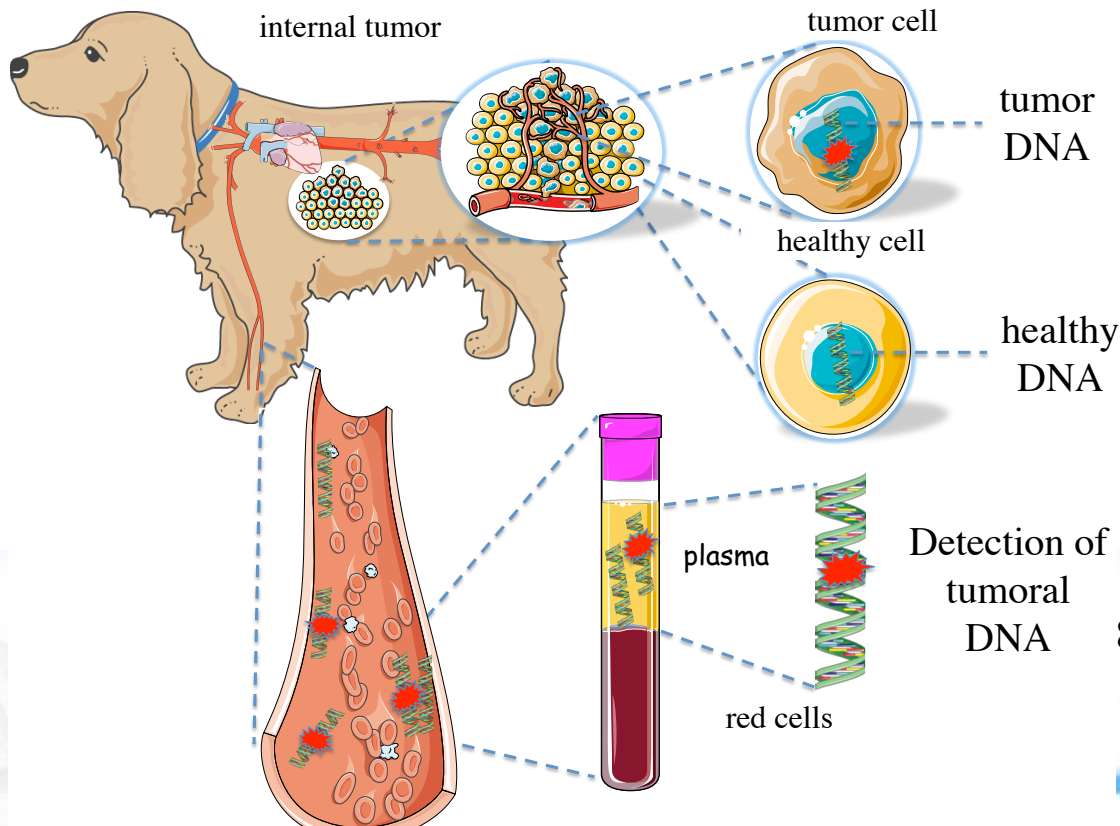
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## Somatic mutations : applications for veterinary medicine

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 )

- detectable in plasma of affected dogs



45 histiocytic sarcomas

23 mutated

22 WT

21/23 mutated

22/22 WT

84 control dogs ( lymphoma, melanoma , mast cell tumor, healthy):

-> 1/84 false positive

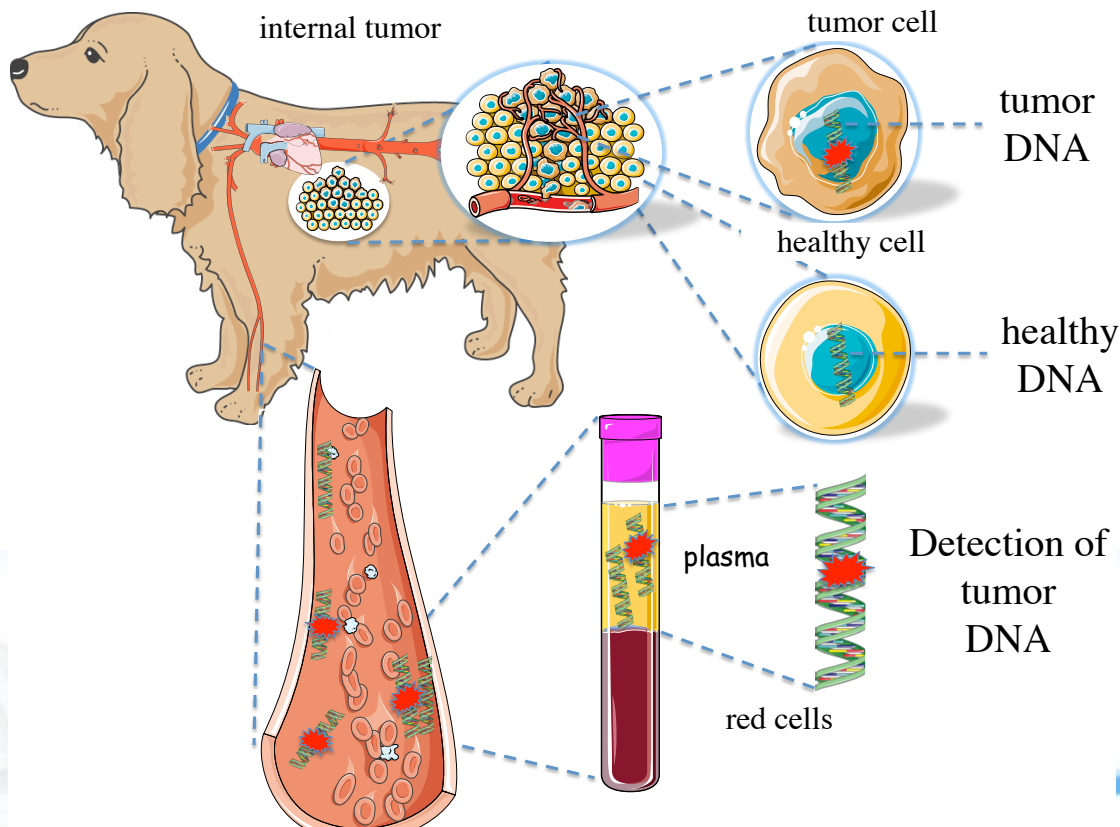
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Interest for the diagnosis:

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

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

specificity 98.8 %  
*Prouteau et al. 2021*





# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

Interest for the diagnosis:

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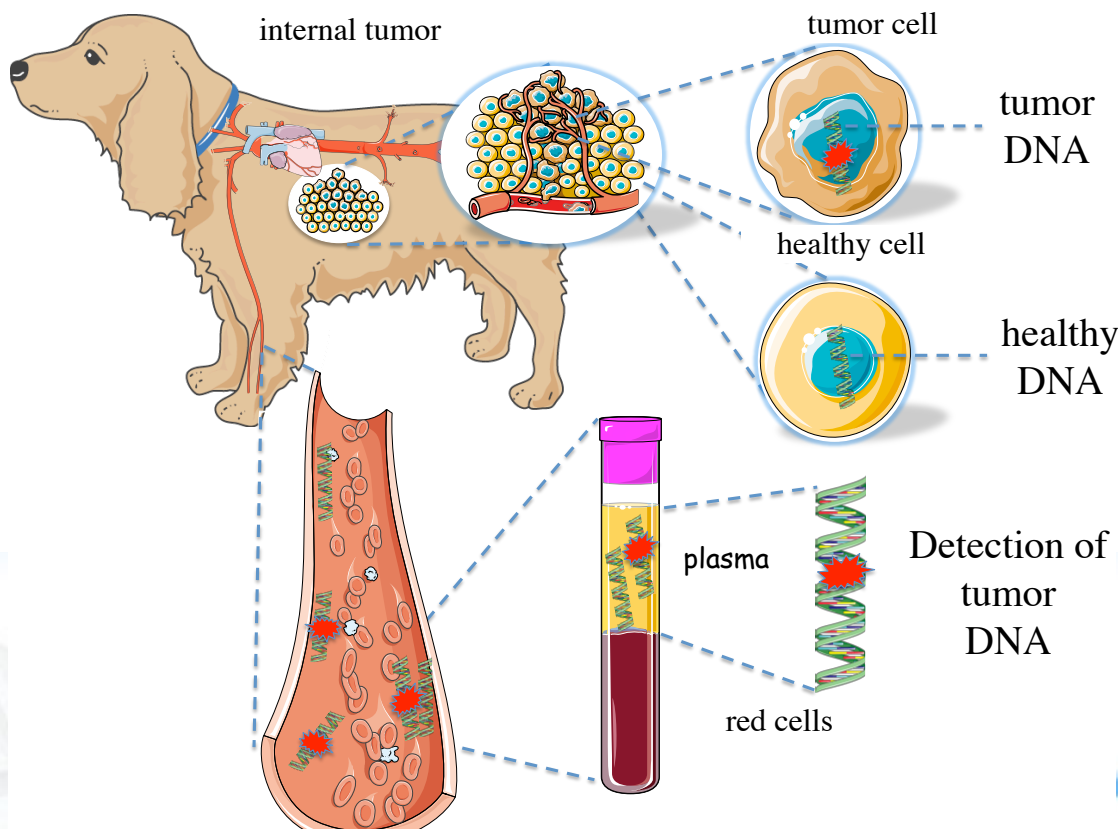
*Prouteau et al. 2021*

-> a tool for diagnosis of none biopsable masses

-> a prognostic tool?

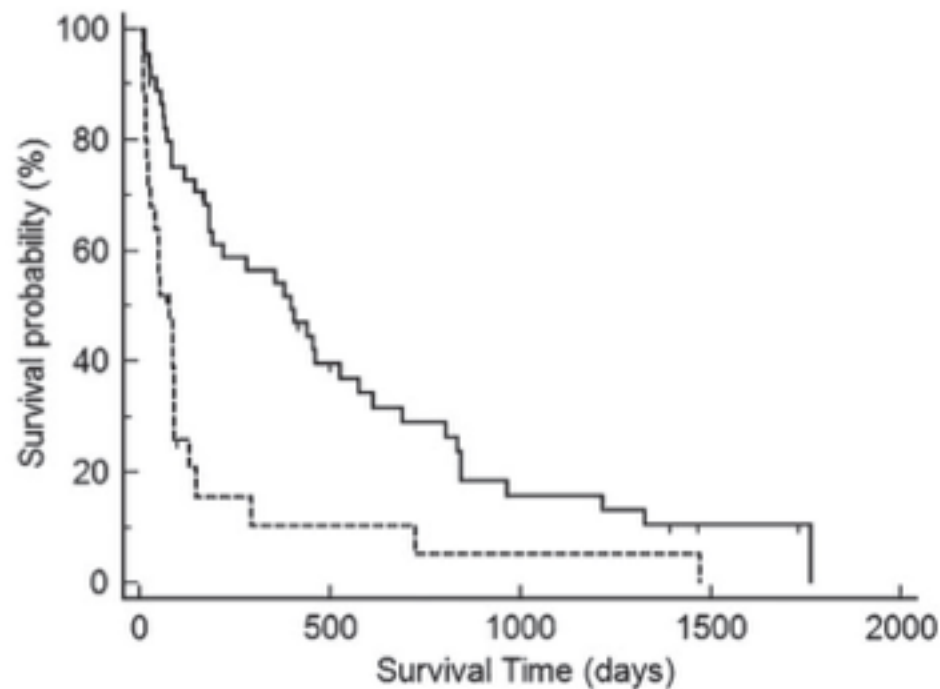
-> to follow treatment efficacy?

-> useful for earlier diagnosis?



# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

Prognosis marker ?



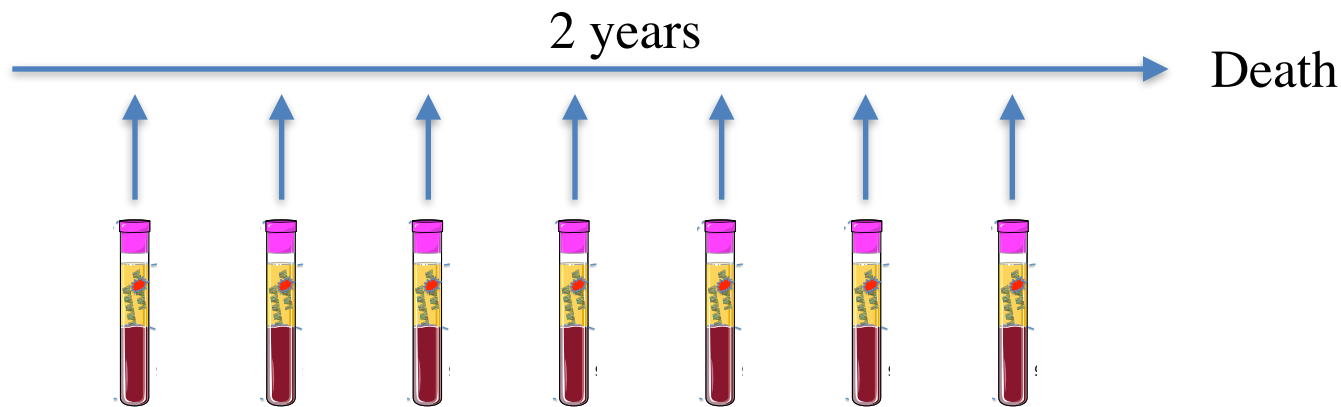
**Figure 3.** Kaplan-Meier survival curve estimating median OS for dogs diagnosed with LHS (solid line;  $n = 46$ ) compared to dogs with DHS (dashed line;  $n = 25$ ). Dogs diagnosed with LHS had significantly ( $P = 0.0002$ ) longer median OS (398 days) than dogs diagnosed with DHS (78 days). Vertical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



# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

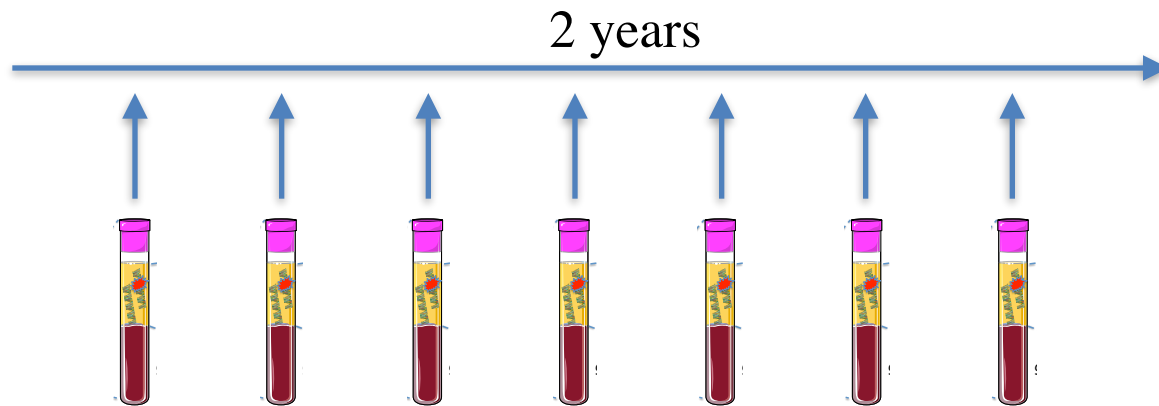
Follow up 30 dogs from two breeders : at risk (Age/ HS-test)



# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

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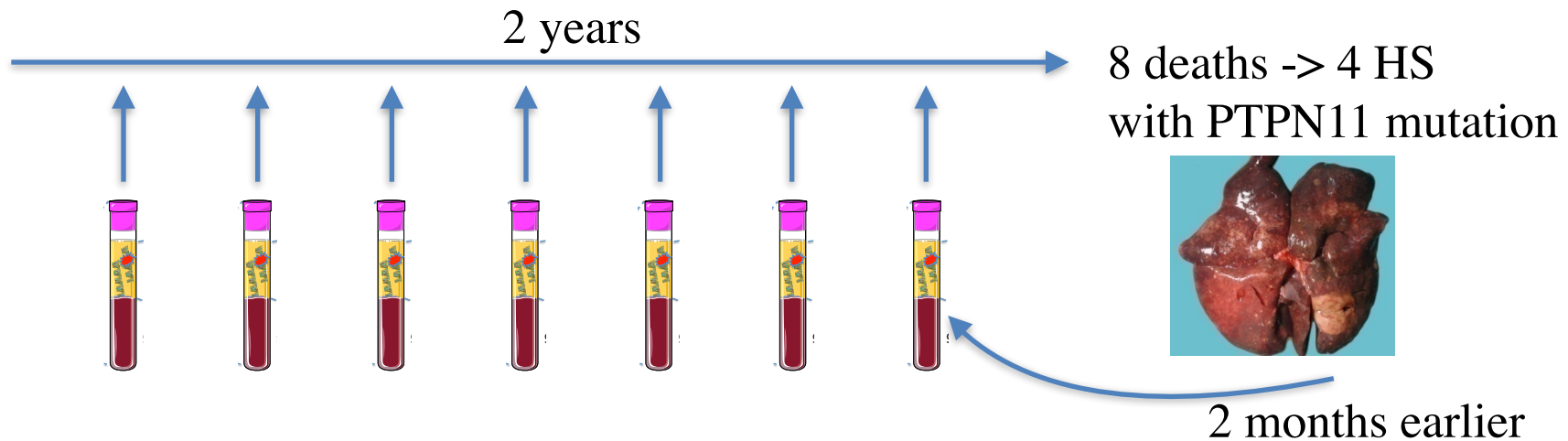
8 deaths -> 4 HS  
with PTPN11 mutation



# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

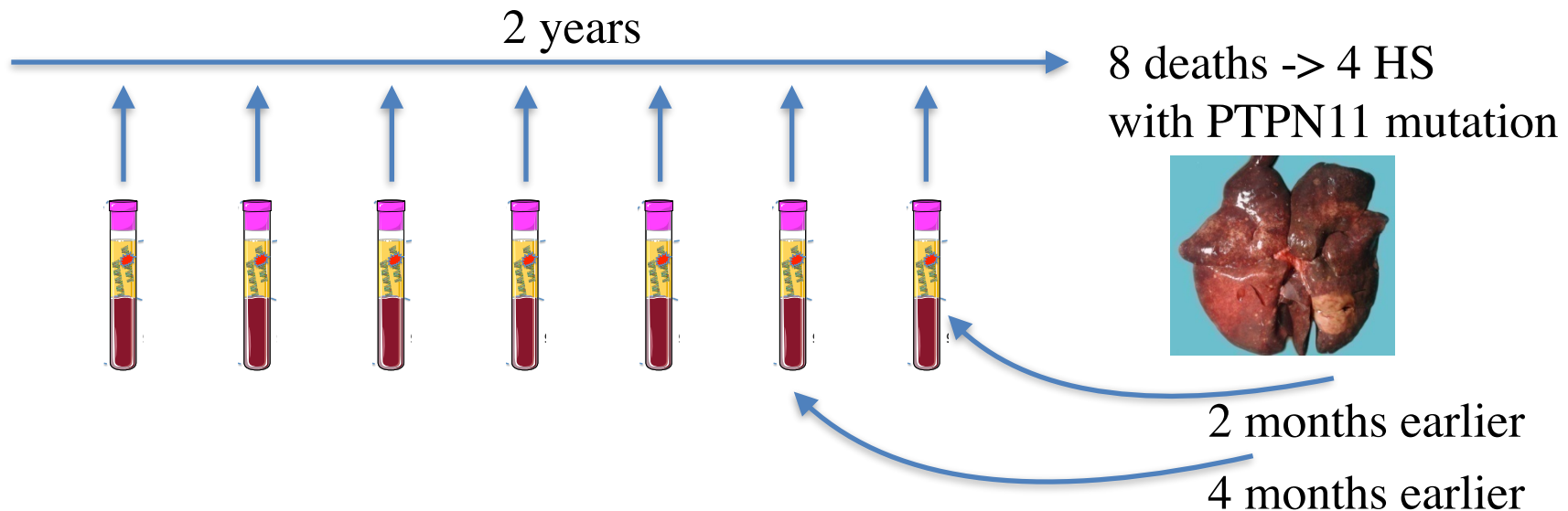
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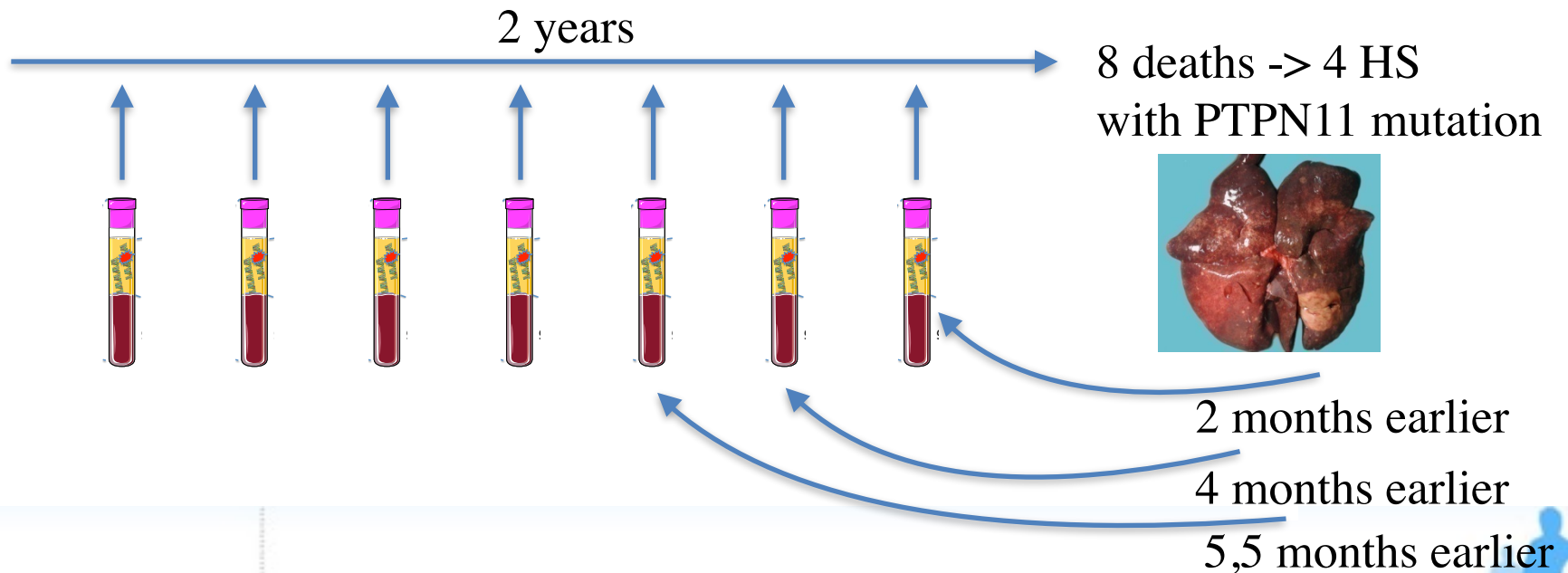




# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

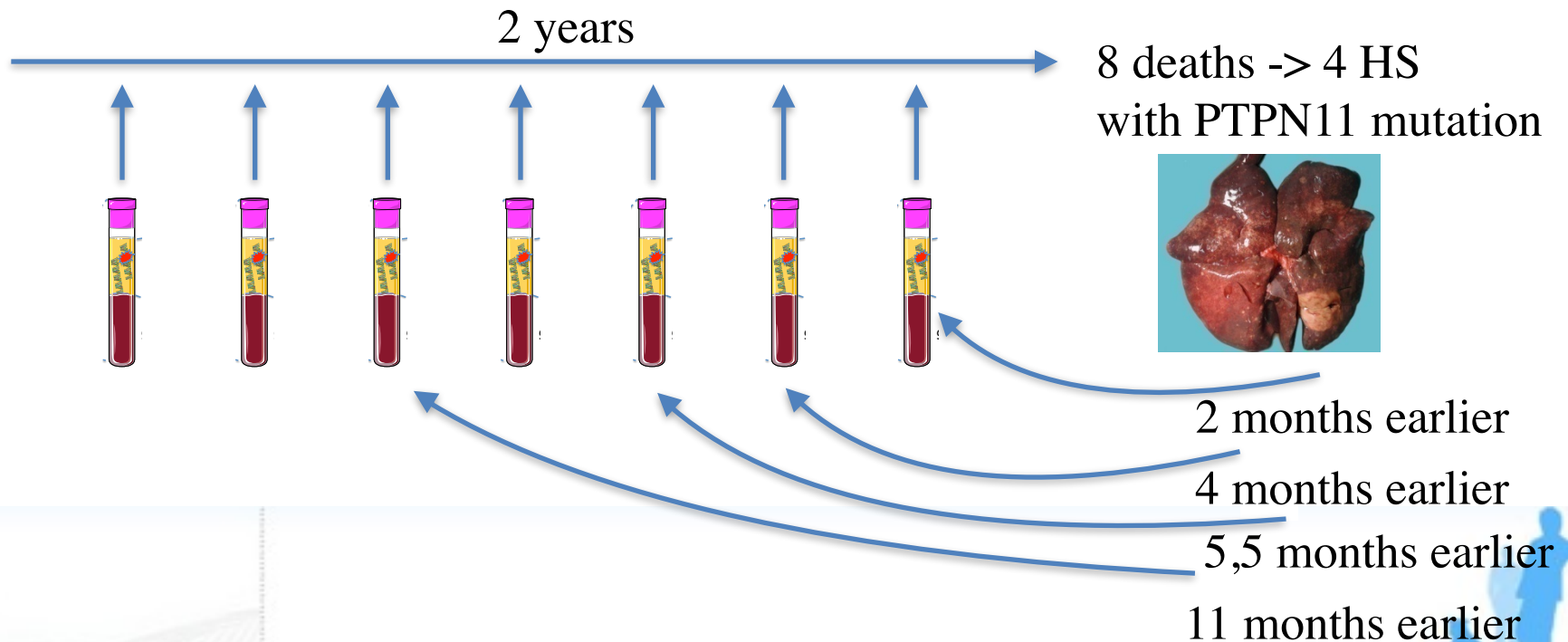
Follow up 30 dogs from two breeders : at risk (Age/ HS-test)



# 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)

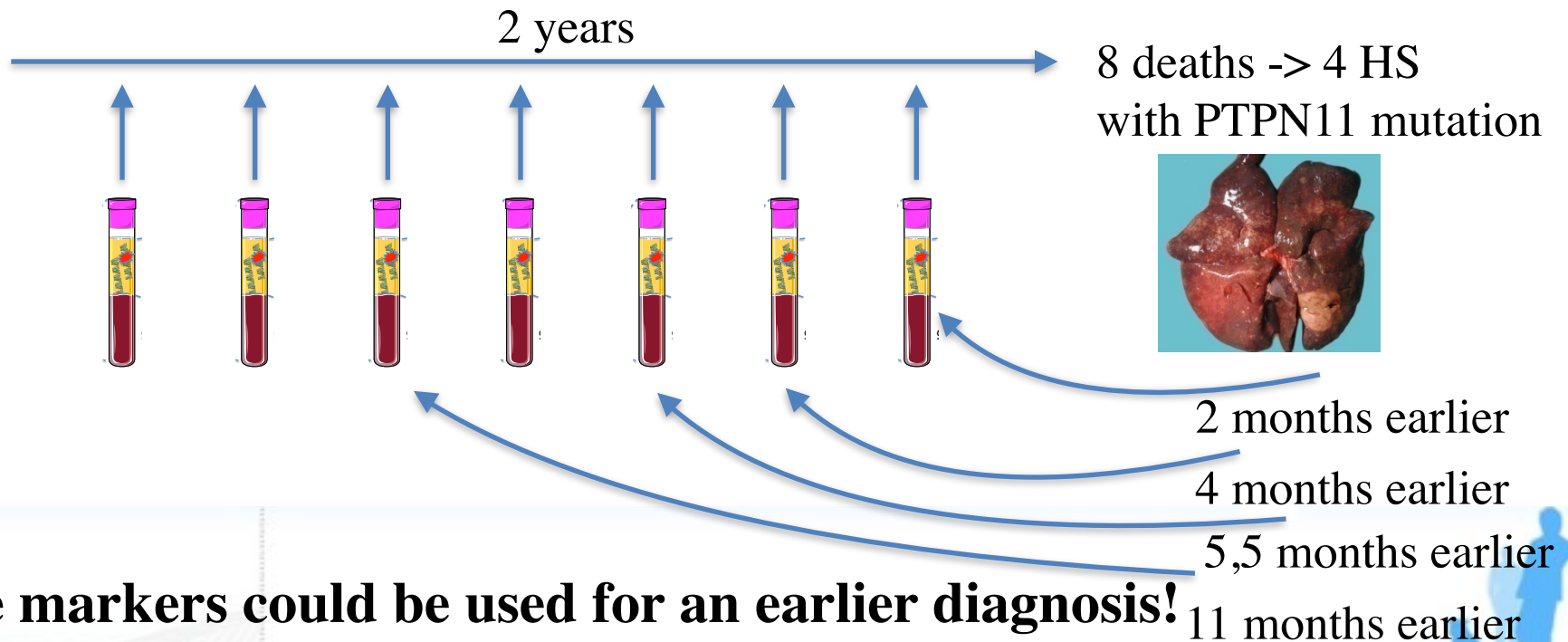


# Canine histiocytic sarcoma:

## Somatic mutations : applications for veterinary medicine

A marker for an earlier diagnosis ?

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

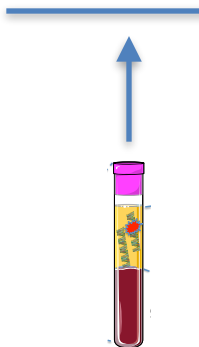


**These markers could be used for an earlier diagnosis!**



A marker for an e

Follow up 30 do



## OPEN ACCESS

**Citation:** Flory A, Kruglyak KM, Tynan JA, McLennan LM, Rafalko JM, Fiaux PC, et al. (2022) 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. PLoS ONE 17(4): e0266623. <https://doi.org/10.1371/journal.pone.0266623>

**Editor:** Joseph Alan Bauer, Bauer Research Foundation, UNITED STATES

**Received:** November 24, 2021

**Accepted:** March 23, 2022

**Published:** April 26, 2022

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**Data Availability Statement:** All relevant data are within the paper and its [Supporting Information](#) files.

**Funding:** This study received funding from PetDx. The funder had the following involvement with the study: study design, data collection and analysis, decision to publish, and preparation of the manuscript. Study sites were compensated for

## RESEARCH ARTICLE

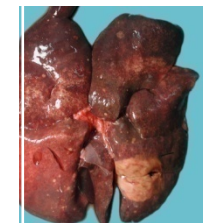
# Clinical validation of a next-generation sequencing-based multi-cancer early detection “liquid biopsy” blood test in over 1,000 dogs using an independent testing set: The CANcer Detection in Dogs (CANDiD) study

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

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\* [jrafalko@petdx.com](mailto:jrafalko@petdx.com)

hs -> 4 HS  
TPN11 mutation



months earlier  
months earlier  
5,5 months earlier  
1 months earlier



# Detection of cancer 5 months earlier

These marker

# 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?

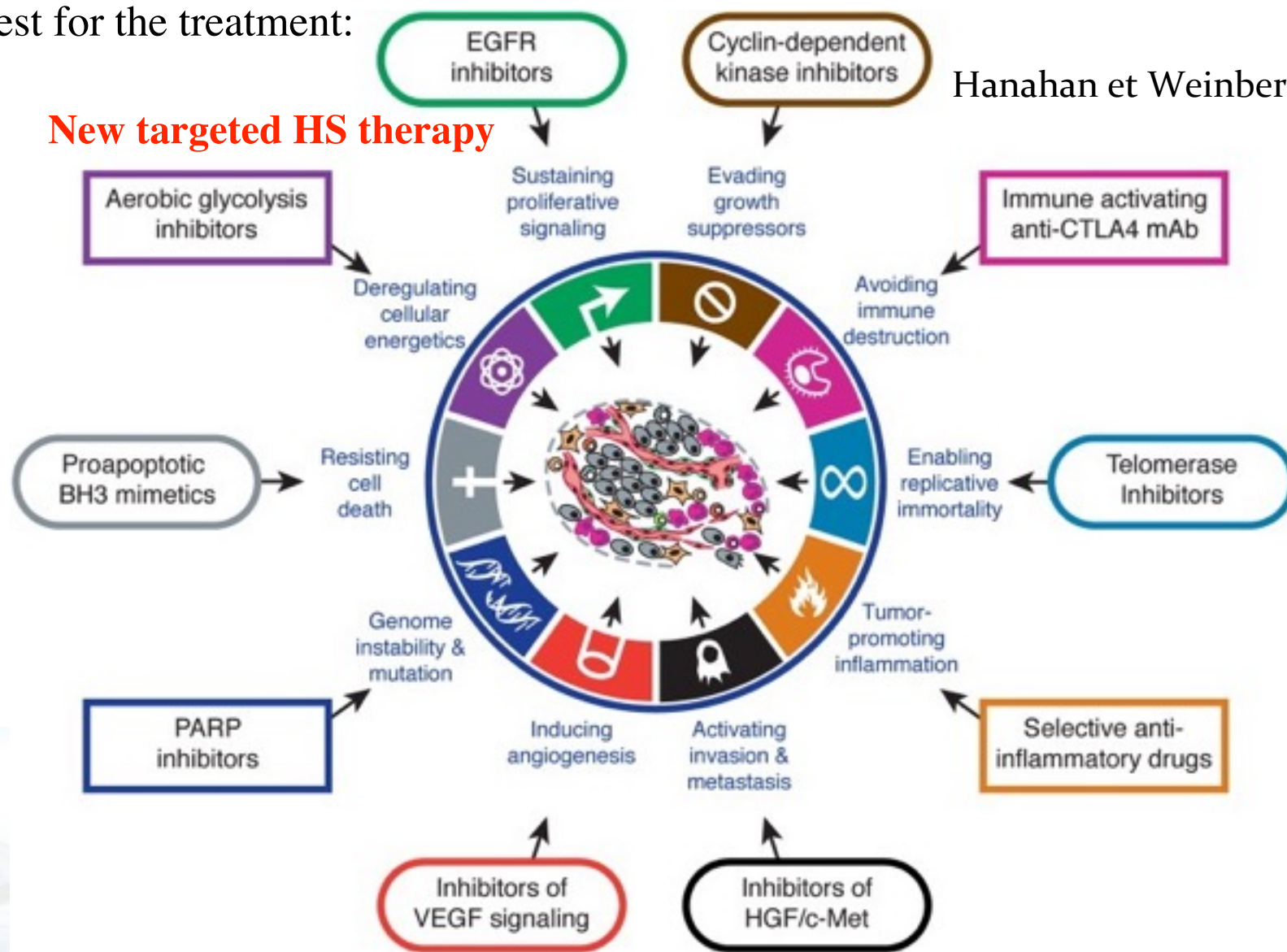


# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

Interest for the treatment:

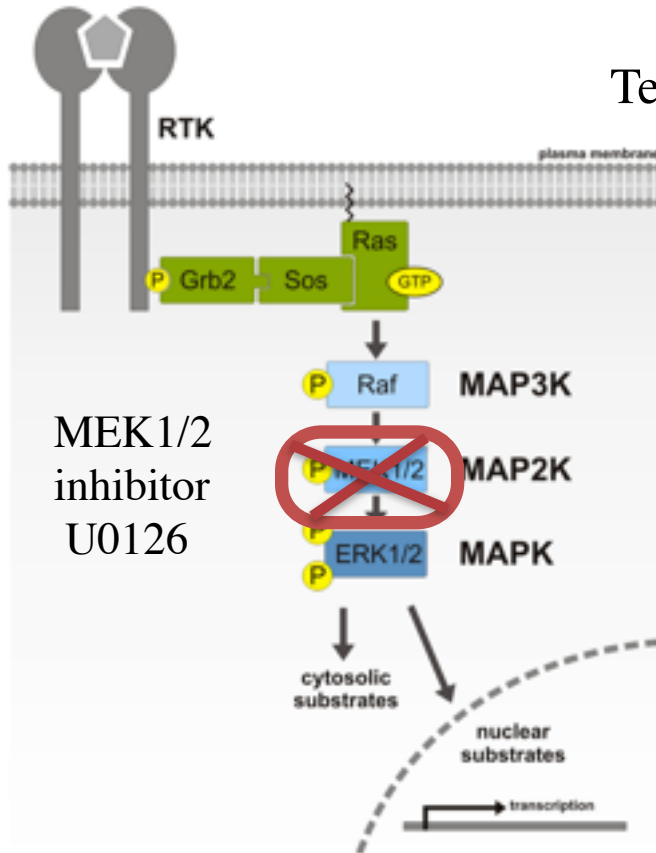
Hanahan et Weinberg. Cell. 20

**New targeted HS therapy**



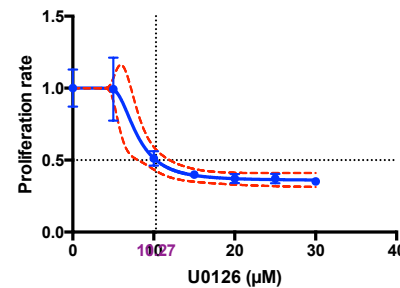


# Canine histiocytic sarcoma: Somatic mutations : applications for veterinary medicine

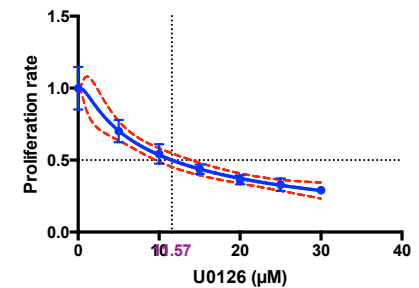


Tests of proliferation rate (72h) with MEK inhibitor

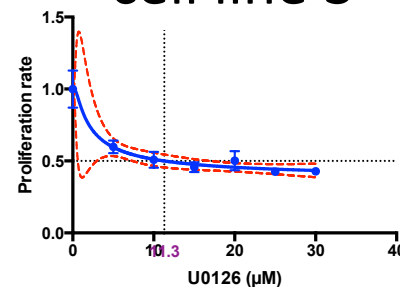
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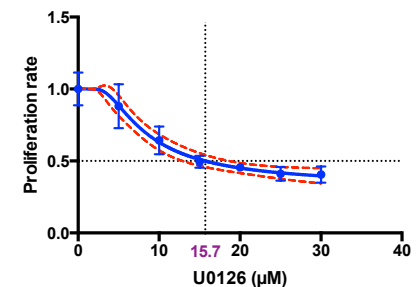
cell line 2



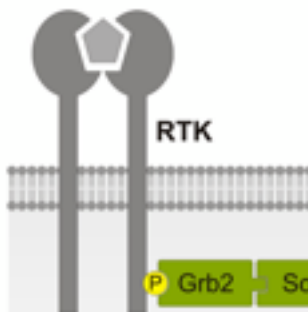
cell line 3



cell line 4



**New therapy opportunities?**



MEK1/2  
inhibitor  
U0126

## Targeting MEK in a Translational Model of Histiocytic Sarcoma

Marilia Takada<sup>1</sup>, Jeremy M.L. Hix<sup>2</sup>, Sarah Corner<sup>1</sup>, Peter Z. Schall<sup>1</sup>, Matti Klupel<sup>3</sup>, and Vilma Yuzbasian-Gurkan<sup>1</sup>

### 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 intras-

plenic 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 was validated as 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, N485\_P493del), MAP2K1, and ALK gene fusion (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., cutaneous 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. 25). Other breeds that also present with HS include flat-coated retrievers, golden retrievers, and Boxers. 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

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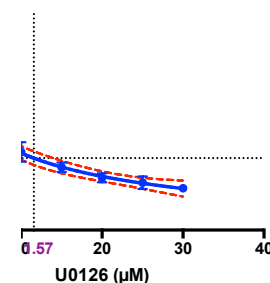
**Note:** Supplementary data for this article are available at Molecular Cancer Therapeutics Online (<http://mct.aacrjournals.org/>).

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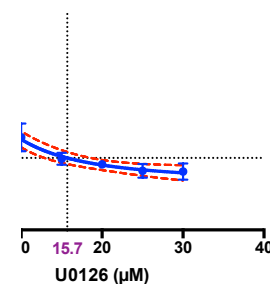
DOI: 10.1158/1535-7163.MCT-17-0273

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### CHRYSTAL rate



### COGNAC nl

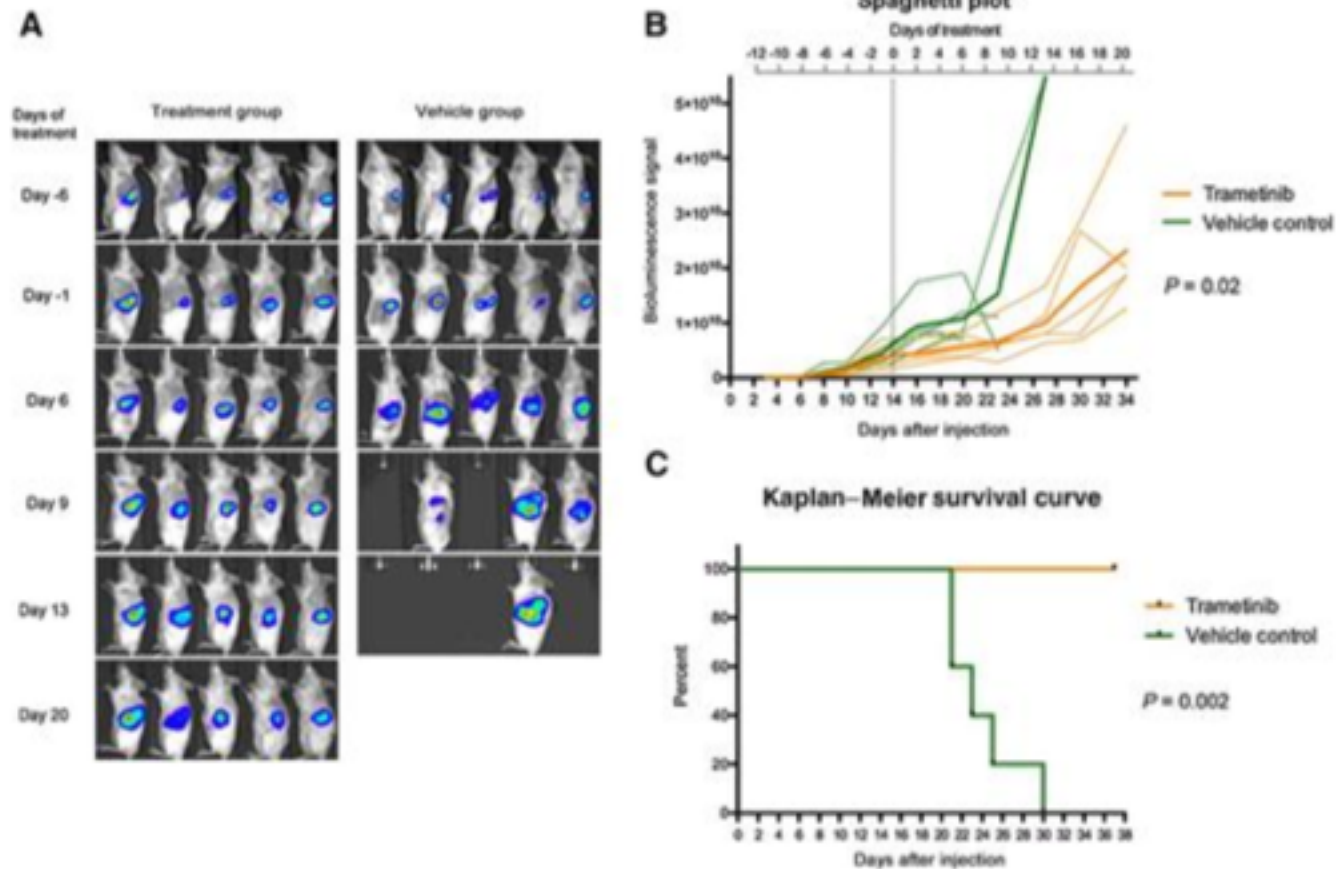


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Figure 3.



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# Pilot study of a MEK inhibitor in dogs with histiocytic sarcoma

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



## 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 ?

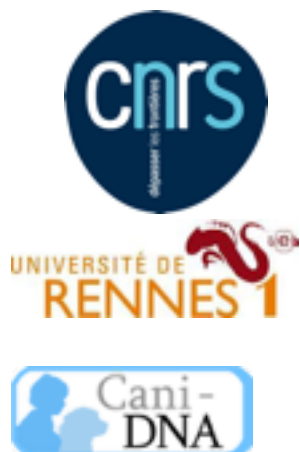
**Double benefit for vet and human medicine**

**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](mailto:benoit.hedan@univ-rennes1.fr)





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

**Fundings** CNRS, AKC, INCa,  
BMD clubs :

