Molecular biological and genetic investigations of cancers in companion animals: impact on veterinary care and comparative value for (rare) cancers in the human.

Gerard R Rutteman
Utrecht University Clinic of Companion Animals
Veterinary Specialist Center de Wagenrenk
NL

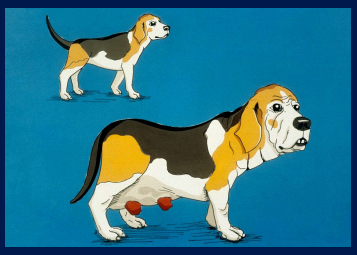
Framework for comparative research

- Standardized record keeping
- Standard pathological classification (WHO)
- Storage of DNA, plasma/serum
- Storage of tissue (rapid freezing, RNA-later, FFPE-blocks)
- Collection of DNA from healthy veterans
- Development of specific cell lines

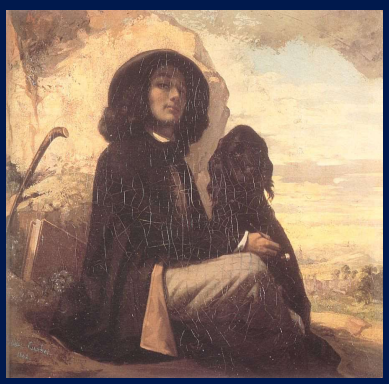
Dedicated to Professor
Wim Misdorp
1929-2009

Progestins in the Dog may lead to:

Increased Growth Hormone production in mammary tissue:
Potential role in mammary tumorigenesis



Gustave Courbet con negro perro



GH in mammary tumorigenesis

GH mRNA and protein in mammary tissue & tumors

- dog, cat, human (Mol, Rutteman, ea, 1994-1999)

Progestin-induced GH stimulates proliferation of stem cells in human breast epithelium

» (Lombardi ea, 2014)

Osteosarcoma in the dog

- Most appendicular
- Highly metastatic (>95%)
- Large breeds predisposed
  (Great Dane, Irish Wolfhound, St Bernard, Leonberger, Rottweiler, etc)

Growth / GH permissive factor?

Recognition of risk for MH (HS) in BMD

- Moore & Rosin, Vet Pathol, 1984
- NL: Schmidt, Rutteman, Van Niel, Wolvekamp, Vet Q, 1994

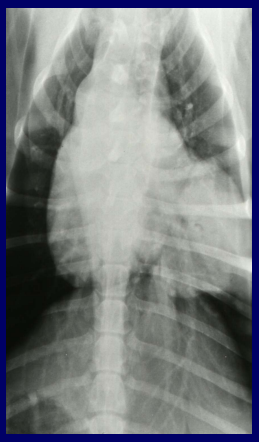
Value of comparative research in dog/cat

- May be of use to the human
- Chance to increase financial support. . .

- For HS research in BMD, my estimate of funding: 10% veterinary resources, 90% human
  (at present >> 10 million Euro)

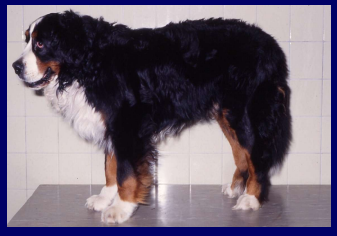
Visceral HS

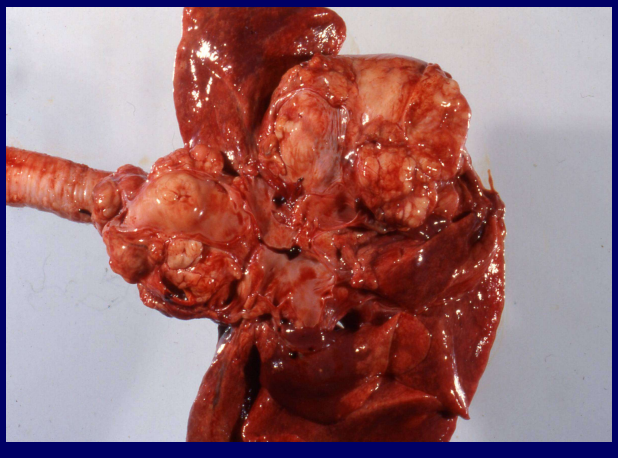
- Often multifocal
lung, liver, spleen



Cancer Predisposition in Bernese Mountain Dog

- Life time risk for malignant histiocytic tumours may be 15-20% (Erich, e.a., 2013)
- Excess >500x (compared to other breeds)





Visceral HS (earlier "MH")

- Spleen, liver, lung, BM
- Rapid multifocal development

Review initial tumor diagnosis in FCR & BMD

- Selection of records from 4 labs in NL + Cambridge in UK
- Pathology (n=894) and cytology (n=396)
- Revision including IHC

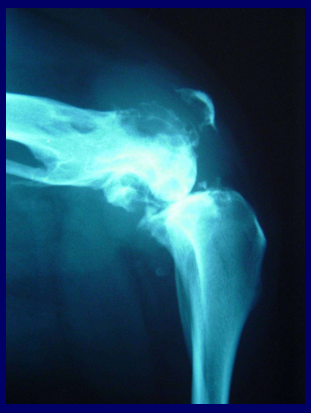
➢ Of cases with HS (1st diagnosis) 75% confirmed.

(Erich et al, 2018)

Tumor often near joint:

Non-visceral HS

- Destructive
- Highly metastatic



Follow up of Veterans (BMD / FCR)

- Data and DNA collected at 8 years or older
- 164 BMD
- 6 dogs developed HS
- 34 developed cancer (in 1/3 suspicion of HS)
- Thus: ¼ eliminated from genetic analysis
- 40 lost to follow up.

HS in other organs

- Kidney,
- Stomach, intestine
- Skin (differentiate form SH)
- Head & Neck
- Bladder
- CNS
- Lymph nodes (metastatic?)

Potential association of HS risk with other cancers

- Hemangiosarcoma (concurrent in some families)
- Mal. Lymphoma (concurrent in individual dogs)

Risk of mammary cancer

"The population-based incidence rates (for all ages) of malignant mammary tumours per 1000 female dogs per year were 35.47 in boxers, 3.87 in Bernese mountain dog"

L. Moe, J Reprod Fertil, 57 (suppl) 2001

What protects BMD from mammary cancer?

Gene expression in histiocytic sarcomas

STHS: primary tumor soft tissue often near joints / tendons; metastasis in most dogs < 1 year

VHS: primary tumor visceral organs, often multiple and generalized at 1st presentation; metastasis in most dogs < 4 months

(Dobson J, 2009; Erich SA et al, 2013)

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Potential factors influencing mammary cancer (MC) risk

- Function endocrine system?
- Function immune system

What if factors predisposing for HS protect from MC ?

Gene expression in histiocytic sarcomas

- Fresh-frozen tissue from FCR with HS (STHS and VHS; CD18 confirmed)
- Normal canine spleens → RNA extraction

- Microarray analysis and pathway analyses

- Confirmation using quantitative real-time PCR (qPCR) analyses

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Flatcoated Retriever: also predisposed for histiocytic sarcoma

Comparison of HS - Spleen

319 Probes differentially expressed ($P<0.05$) when 4-fold changes or larger were taking into account.

Altered expression of nine genes confirmed by qPCR.

- Down-regulated : *PPBP, SpiC, VCAM1, ENPEP, ITGAD*
- Up-regulated: *GTSF1, Col3a1, CD90, LUM*

(Boerkamp et al, 2014)

PPBP also downregulated in human myeloma

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Comparing HS/ Spleen

DAVID pathway analyses revealed various pathways that were significantly involved in the development of HS in general, most of which were involved in the DNA repair and replication process.

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(Dys)function of disease genes

- Knowledge of function of genes involved in diseases such as HS, improves the chance of just intervention (prevention / treatment)

Comparing VHS/ STHS

191 Probes were significantly differentially expressed

QPCR confirmed the significantly altered expression of three genes.

- Up-regulated: *C6*
- Down-regulated: *CLEC12A*, *CCL5*

CLEC12A: cell adhesion, negative regulator of granulocyte and monocyte function

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Thanks

- To all dog owners that contributed
- And to collaborating breed societies
- And funding organizations:

  KWF, St DOG, EC, DRC, FRC, VBSH, NBSV, NLRV, SSV, RCS

  Albert Heim Stiftung

Translation of expression studies in cancer

- Immune-histochemistry / proteomics
- Functional assays
- Tumor – stroma (ECM) interactions

- Comparison with human cancers

Exposition Art Drager Meurtant

Kunstwerk! Liemers Museum, Zevenaar, NL,
June 16 – September 9 2016

HS in Bernese Mountain Dog

MTAP-CDKN2A disease allele:

Cases: homozygote 65%, heterozygote 31%
Controls: homozygote 24%, heterozygote 60%
Major difference, not yet sufficient to use for selection

Also at CFA14 there is significance, but less so in USA:
European BMDs must provide the answer

Histiocytic sarcomas in FCR

Preliminary results of GWAS (NIH)

- Locus MH/HS in BMD: not relevant
- Two genomic regions highly significant variation between cases and controls: ongoing.