Animal Health Trust

Breed Health Symposium 2018

Leading science and care for animals



Cancer in dogs

Mike Starkey PhD, Head Molecular Oncology

Talk outline

- Introduction to cancer
- Genetic predisposition to cancer
- Focus of research
- Canine cancer research at the Animal Health Trust

Cancer

- Affects 1 in 4 dogs
- No. 1 cause of death in dogs over 10 years old
- Not one disease (200 different types)

Animal Health Trust

Cancers affecting UK dogs (2002)

Tumour	Annual incidence (per 100,000 dogs)	'Severity'
Cutaneous histiocytoma (skin)	337	Benign
Lipoma (skin, soft tissue)	318	Benign
Mammary tumour (mainly carcinoma, adenocarcinoma)	191	Malignant
Adenoma (alimentary, mammary, skin)	175	Benign
Soft tissue sarcoma	142	Malignant
Mast cell tumour	129	Malignant
Lymphoma	114	Malignant
Cushing's syndrome (adrenal, pituitary gland)	96	Malignant
Osteosarcoma	57	Malignant
Epulis (oral)	47	Benign
Carcinoma (alimentary, endocrine, nasal)	29	Malignant
Haemangiosarcoma (alimentary, skin)	25	Malignant
Melanoma (oral)	13	Malignant
Seminoma (testis)	13	Benign (usually)
Adenocarcinoma (alimentary, nasal)	12	Malignant
Leukaemia	11	Malignant
Sertoli cell tumour (testis)	10	Benign (mainly)
Haemangioma (alimentary)	6	Benign
Chemodectoma (heart, carotid artery)	1	Benign (usually)
Chondrosarcoma	1	Malignant

Cancer

- Affects 1 in 4 dogs
- No. 1 cause of death in dogs over 10 years old
- Not one disease (200 different types)
  - Tumour behaviour depends upon the type of cell from which the tumour develops and the location of the cell within the body
  - Tumours of the same type behave differently if they have different 'genetic blueprints'

Cancer

- Most types of cancer affect most breeds of dog
- Some breeds have an increased risk of developing a certain type

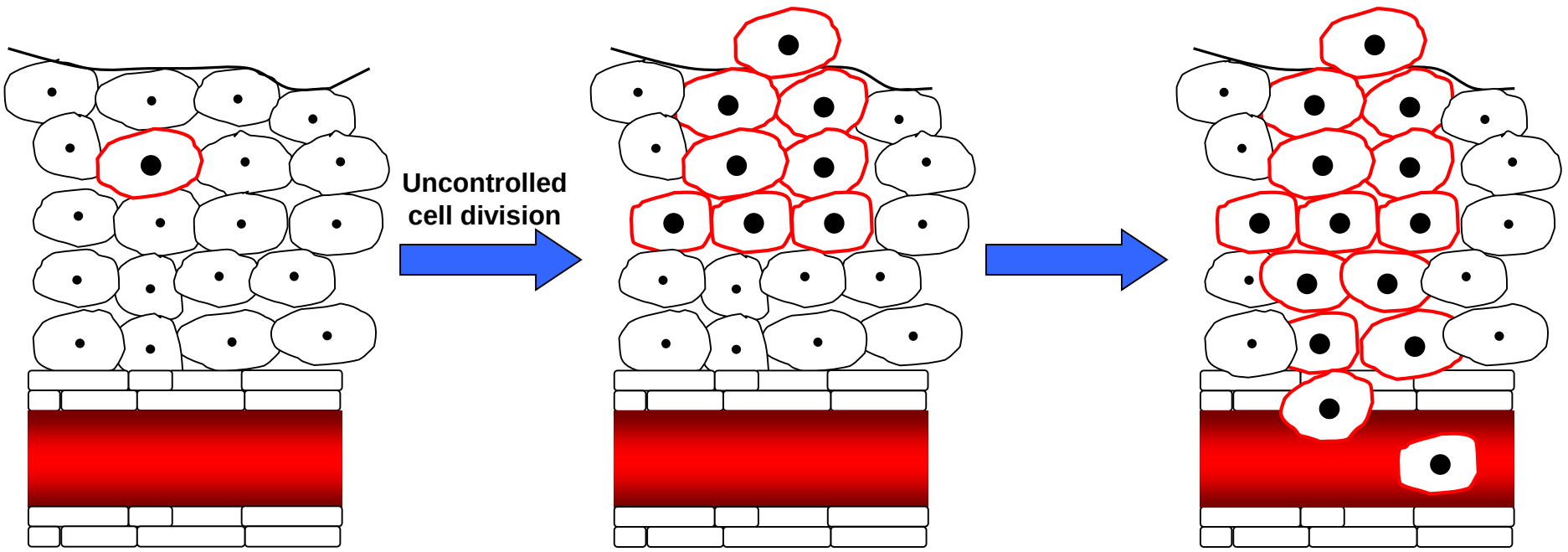
Breed susceptibilities

Breed	Cancer Type (s)
Bernese Mountain Dog	Disseminated histiocytic sarcoma
Flat-Coated Retriever	Localised histiocytic sarcoma
Scottish Terrier	Transitional cell carcinoma of the bladder
German Shepherd Dog	Haemangiosarcoma
Irish Wolfhound	Osteosarcoma
Boxer	Glioma, lymphoma, mast cell tumour
Golden Retriever	Mast cell tumour
Labrador Retriever	Mast cell tumour

Cancer

- Most types of cancer affect most breeds of dog
- Some breeds have an increased risk of developing a certain type
- Main cause of death is tumour spread (metastasis)

Tumour spread (metastasis)

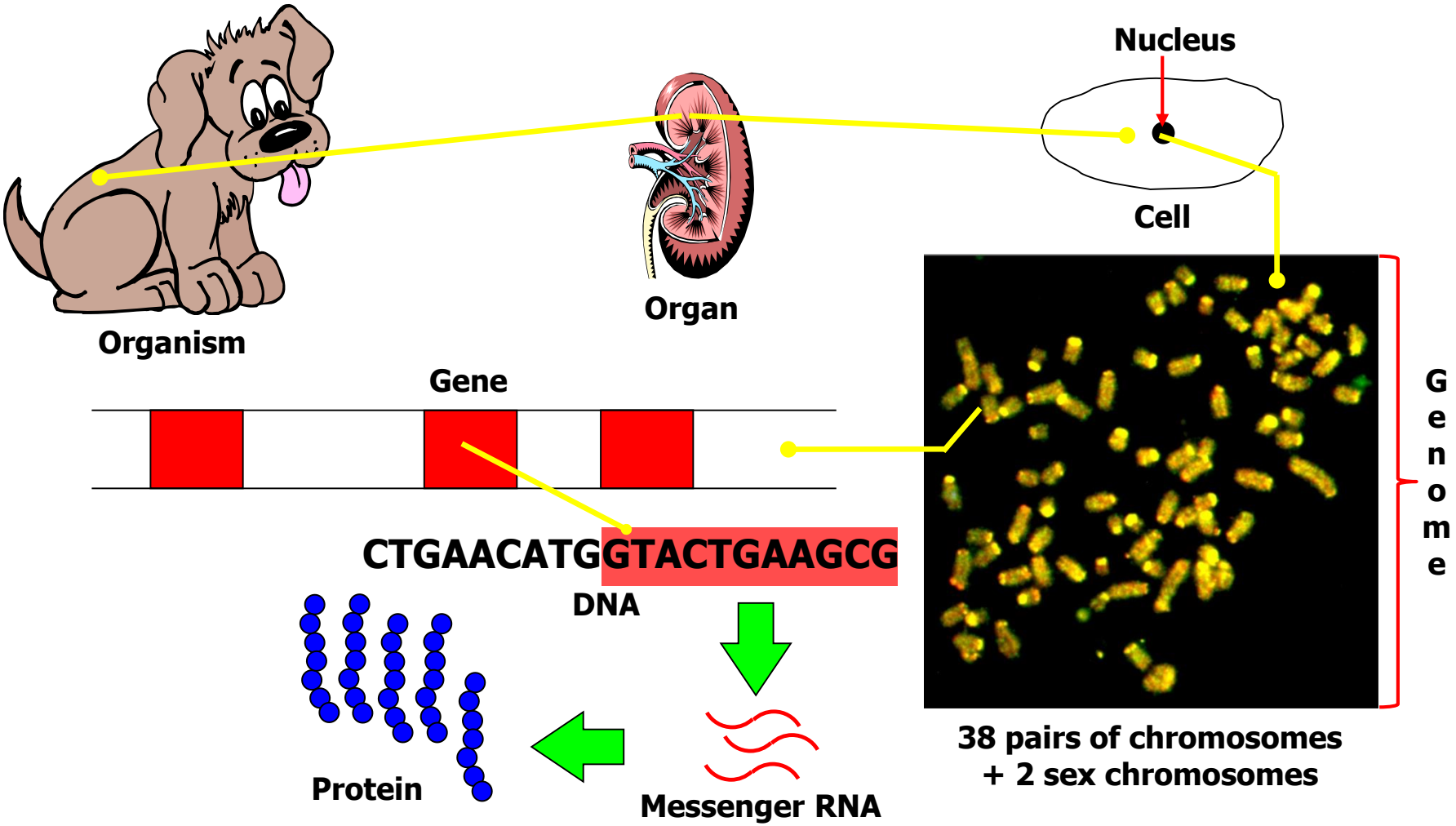


One ‘damaged cell’ develops into a tumour cell.

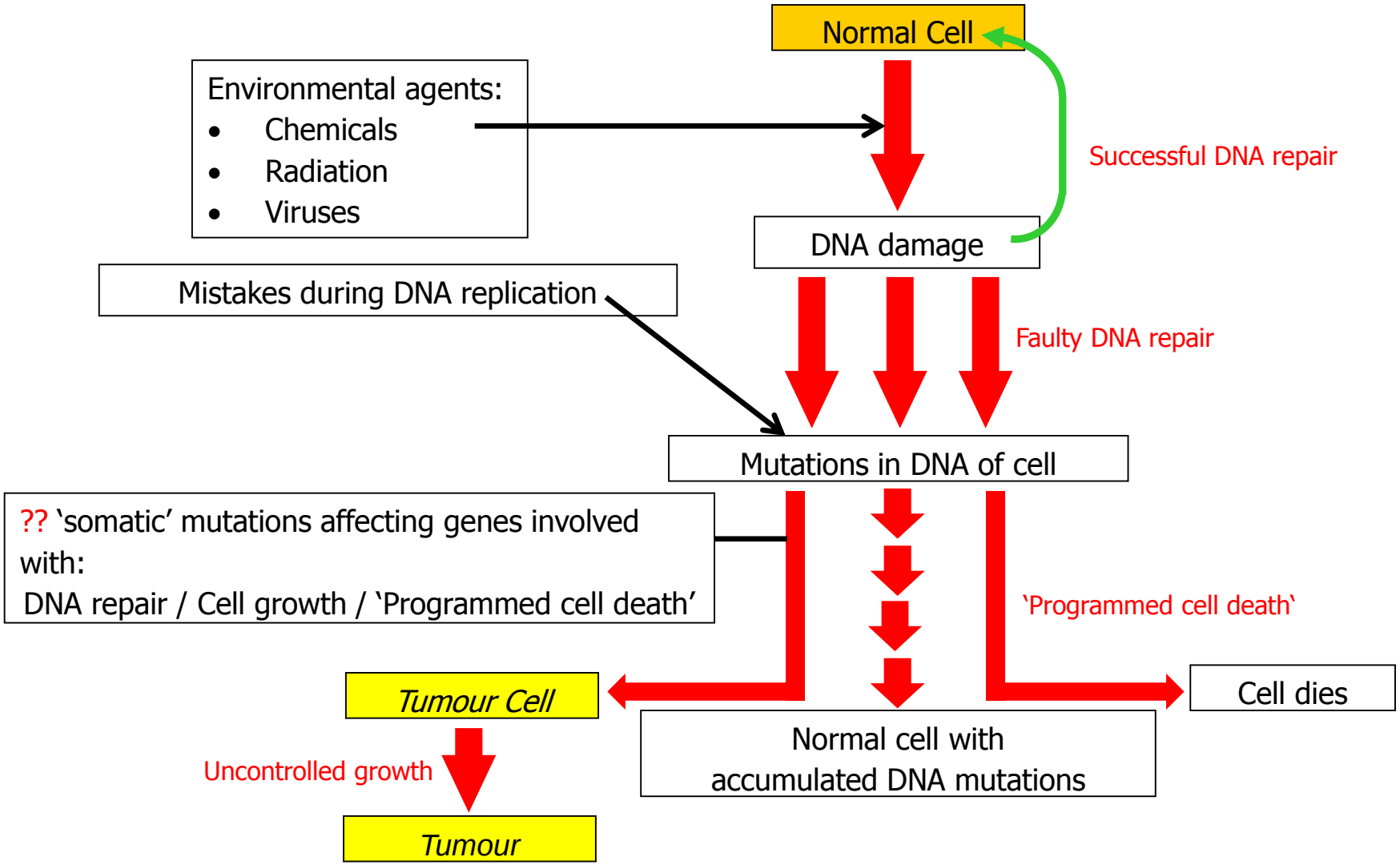
Mass of abnormal cells (*tumour*) don’t work properly, and begin to invade nearby parts of the body.

Cancer cells break away and travel through blood stream to other parts of the body (*metastasis*).

Biology of the dog

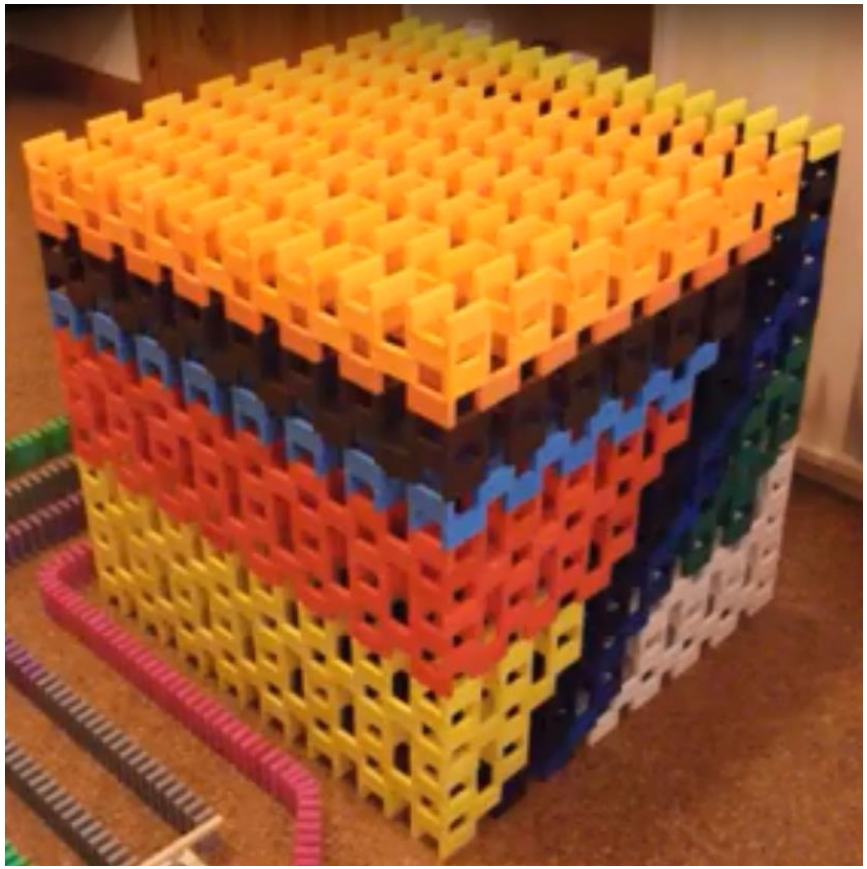


How does cancer arise?



It is difficult to identify the somatic mutations that cause cancer!

Normal cell

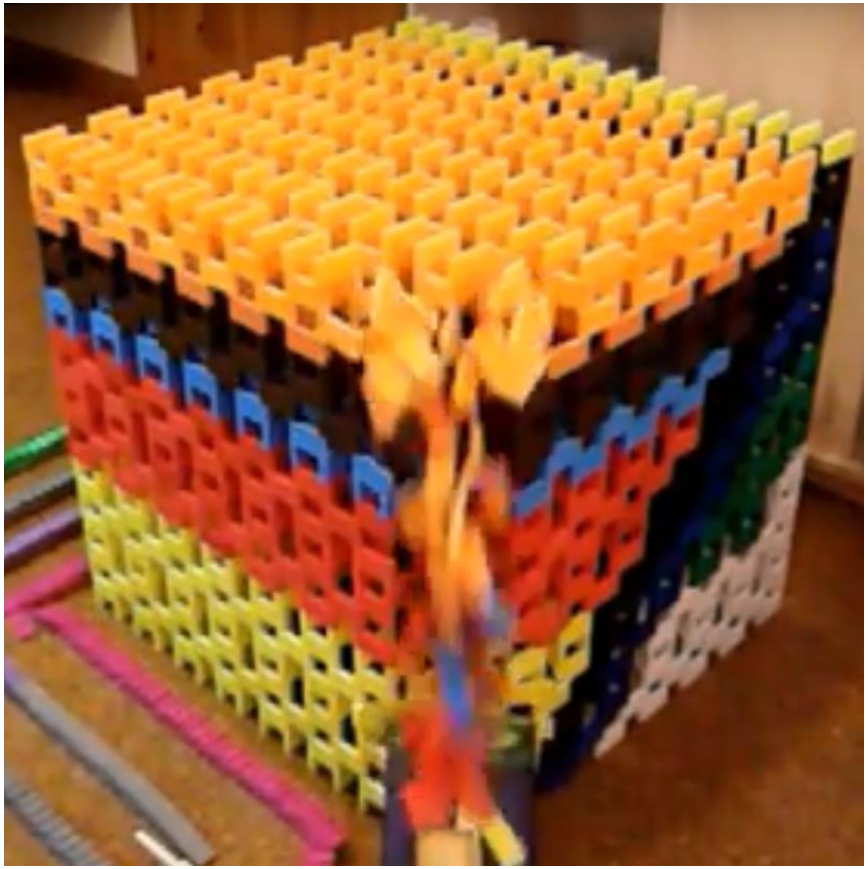


It is difficult to identify the somatic mutations that cause cancer!

Tumour cell

It is difficult to identify the somatic mutations that cause cancer!

We're missing this bit!



Solution: 'Decode' the DNA from hundreds of each type of tumour

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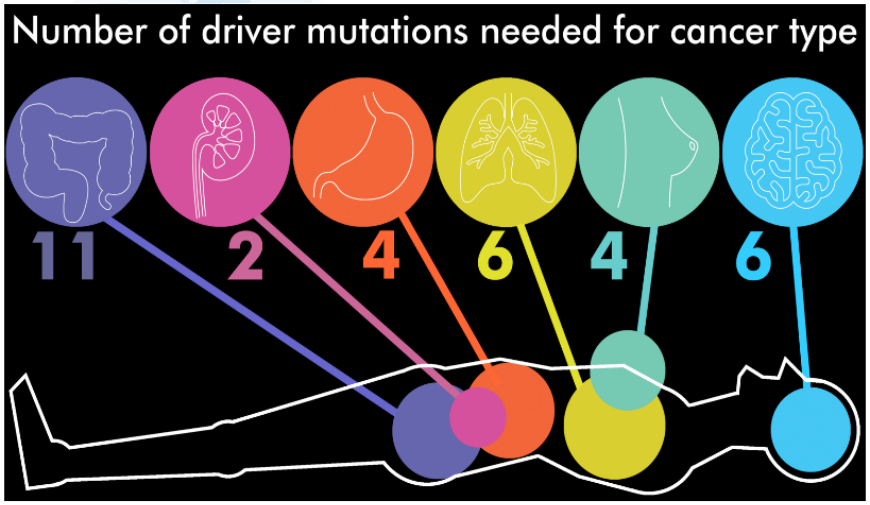
Health

'Handful of changes' make cancer

By James Gallagher
Health and science reporter, BBC News website

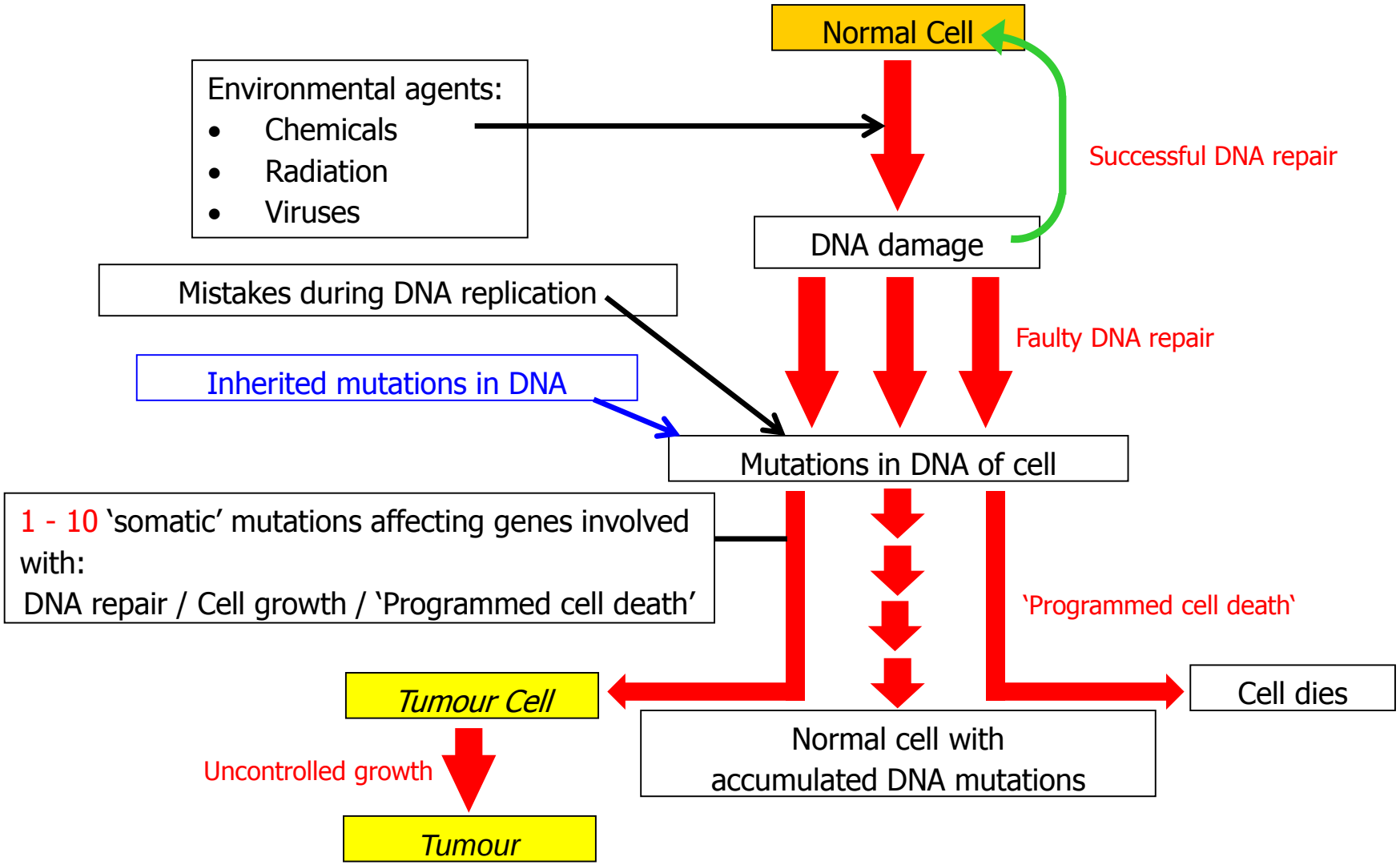
19 October 2017 | Health Share

The results show the number of mutations driving cancer varies considerably across different cancer types



Approximate number of driver mutations needed to cause cancer by area of the body

Cancer: a disease of DNA



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- **Genetic predisposition to cancer**
- Focus of research
- Canine cancer research at the Animal Health Trust

Human cancers: Genetic risk factors

- Mutation in DNA that is inherited (present in all cells of the body) and causes an increased risk of developing disease
- Involved in 5 - 10% of cancers (usually occur at a younger age)
- Contribute to 80%+ of cancers that run in families
- 'Hereditary cancer' - Increased risk caused by many mutations
  - Rare mutations that cause a large increased risk
    - Mutations in genes that control repair of DNA, or cell growth
      e.g. BRCA1: 5 x increased risk of developing breast cancer by age 70
  - Common mutations that cause a small increase in risk (1.5 x)
    - Mutations that 'modify' effects of mutations found in tumours

Human cancers: Genetic risk factors

- Some associated with several cancers (*cancer syndrome*)
  - e.g. Gene TP53: Li-Fraumeni syndrome (breast, osteosarcoma)

- Different 'modes of inheritance'
  - Autosomal dominant (most known): 1 copy - ↑risk
  - Autosomal recessive: 2 copies - ↑risk
  - X-linked recessive: All males - ↑risk, females - 2 copies - ↑risk

- Number of inherited mutated genes identified: 50+

- Contribution of genetic risk factors identified so far to risk of developing hereditary cancers is low
  - e.g. 22 breast cancer genetic risk factors (incl. BRCA1 & BRCA2) explain 23% of increased risk of developing familial breast cancer

Genetic predisposition to cancer in dogs

- Why do we believe that a particular breed has an inherited susceptibility to developing a **certain type of cancer**?
  - Multiple dogs in different generations of multiple families affected by the same cancer
  - The incidence of the cancer in the breed is 'significantly higher' than in most other breeds ('Odds Ratio' > 1.0)

'Odds Ratio'

- Statistic that quantifies the 'relative risk' that a particular breed has for developing a specific cancer:

$$\frac{\text{Odds of a dog in the breed being affected by the cancer}}{\text{Odds of a dog in 'all other breeds' being affected}}$$

$$\text{Odds} = \frac{\text{Number of dogs in the breed affected}}{\text{Number of dogs of that breed in the 'dog population'}}$$

- Difficult to calculate as lack of definitive data from which to measure the incidence of each cancer in each breed

'Relative Risk' v 'Actual Risk'

- Every dog has a 1 in 4 chance of developing cancer
- Relative risk of a '**predisposed breed**' developing a **specific cancer** may be 'high', but typically the actual risk for an individual dog is 'low'
  - e.g. Incidence of lymphoma in UK dogs (2003)*

	Odds Ratio	Number of affected dogs (per 1000 dogs)
Bullmastiff	6.76	2.79
Boxer	4.73	1.78
Bulldog	3.26	1.74

*Edwards *et al.*, 2003

'High' actual risk

- Histiocytic sarcoma (HS)
  - 15 - 25% of Bernese Mountain Dogs affected (disseminated HS) [Odds Ratio = 600]
  - 18% of Flat-Coated Retriever deaths (localised HS) [study of 174 dogs over a 10-year period*]

*Dobson *et al.*, 2009

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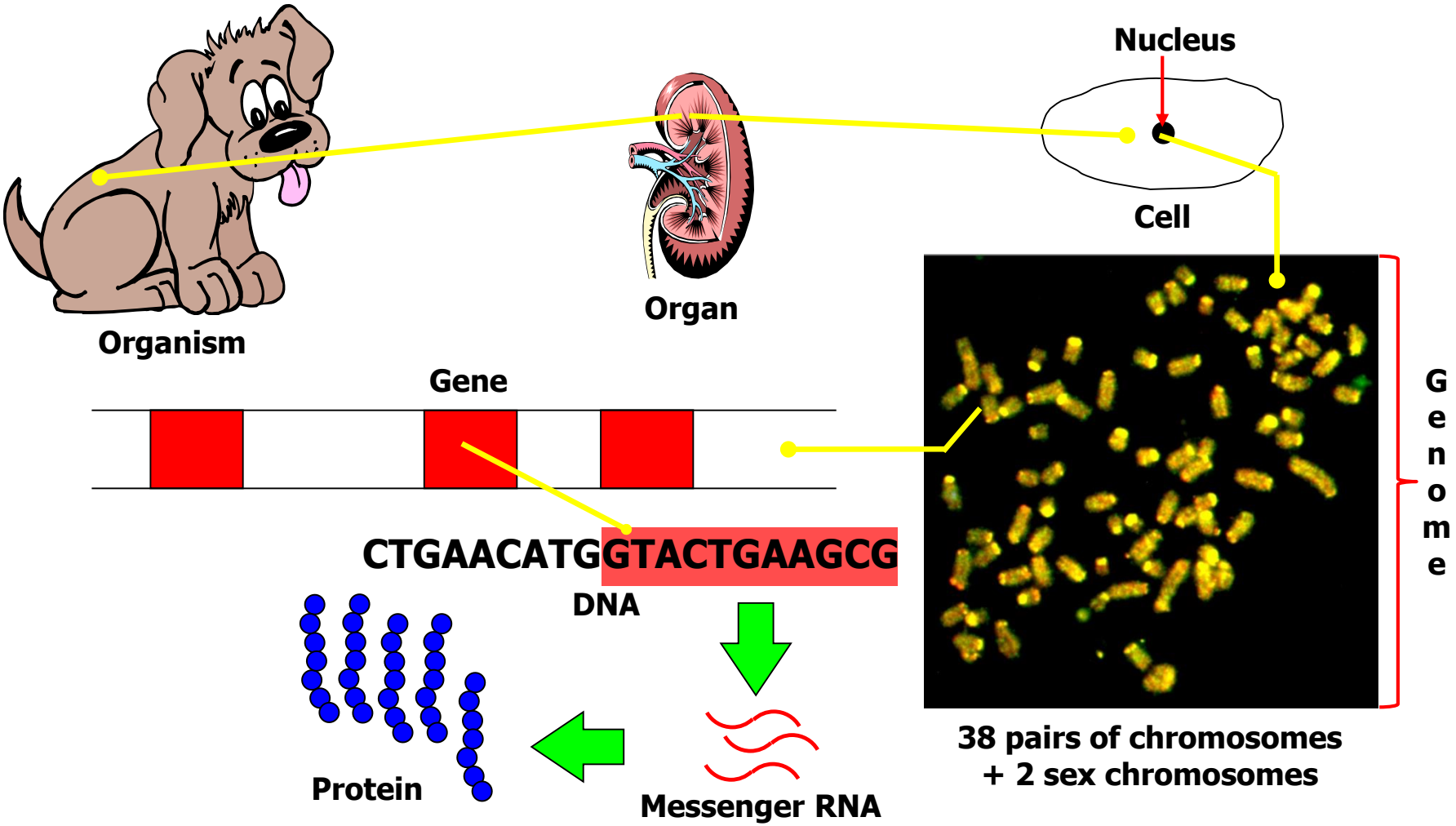
Research Aims

- Identify genetic risk factors for a specific cancer in a predisposed breed
  - How realistic is it to be able to reduce the incidence of a **specific cancer** in a **predisposed breed**?
    - Depends upon the number of genetic risk factors, how common they are (and the numerical size of the breed), and the extent of the increased risk that each confers
    - Genetic risk factors for cancer can be difficult to recognise
      - If they are common (and confer a small increase in risk)
      - If they do not clearly affect messenger RNA or protein

Biology of the dog



Tests for cancer genetic risk factors

- Human cancers: 14+
  - Mainly rare mutations that cause a large increase in risk
    - e.g. Hereditary breast cancer and ovarian cancer syndrome

- Canine cancers:
  - Bernese Mountain Dog Histiocytic Sarcoma Test ('Antagene')*
    - Based on 9 'genetic markers'
    - Possible results:

Index	Meaning
A	The individual tested has four times the chance of NOT developing HS
B	'Neutral index'
C	The individual tested has four times the risk of developing HS

*http://www.antagene.com/en/commander/histiocytic-sarcoma-test

Research Aims

- Identify genetic risk factors for a specific cancer in a predisposed breed
- Develop tests capable of detecting cancer/cancer recurrence earlier
- Develop less invasive ways of diagnosing cancer
- Develop more accurate ways to predict if a tumour is going to spread to other parts of the body

Research Aims

- Develop ways of predicting whether a tumour will respond to a potential treatment

- Increase understanding of how tumours develop and progress, to assist the development of 'tailored' new treatments

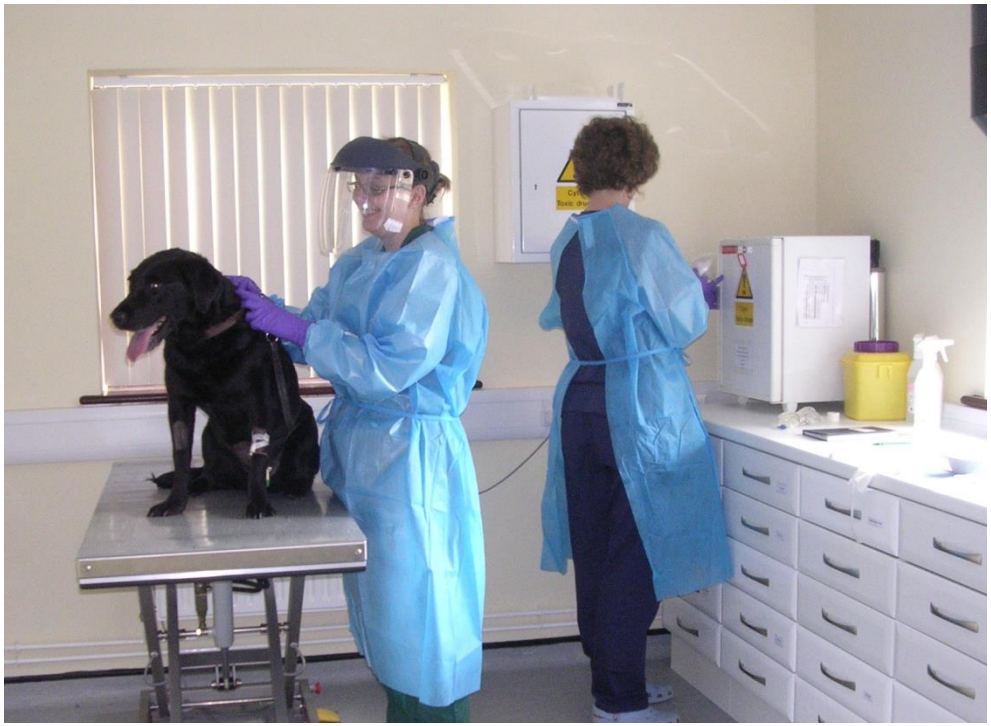
  - Identify the mutations that cause tumours (**or even a tumour**) to develop (and metastasise)

  - Identify differences between tumours that respond to a treatment and tumours that do not

  - Identify proteins produced by tumour cells that are not produced by healthy cells

Treatment of cancer patients

- Local disease
  - Surgery alone
  - Radiotherapy alone
  - Surgery and radiotherapy



- Disease likely to spread
  - Chemotherapy either before after treatment of local disease

'Targeted cancer therapies'

- Drugs that target specific cancer-promoting DNA mutations that are present in some tumours
  - e.g. Receptor tyrosine kinase inhibitors: Palladia, Masitinib (mast cell tumours)

- Immunotherapy
  - Vaccinate with a protein uniquely present on surface of tumour cells to encourage an 'immune response'; e.g. Oncept vaccine (melanoma), ADXS31-164 (osteosarcoma - clinical trial)

- Oncolytic viruses
  - Herpes viruses are 'engineered' so that they are safe and can specifically infect and kill tumour cells (osteosarcoma - research)

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Aims of current research

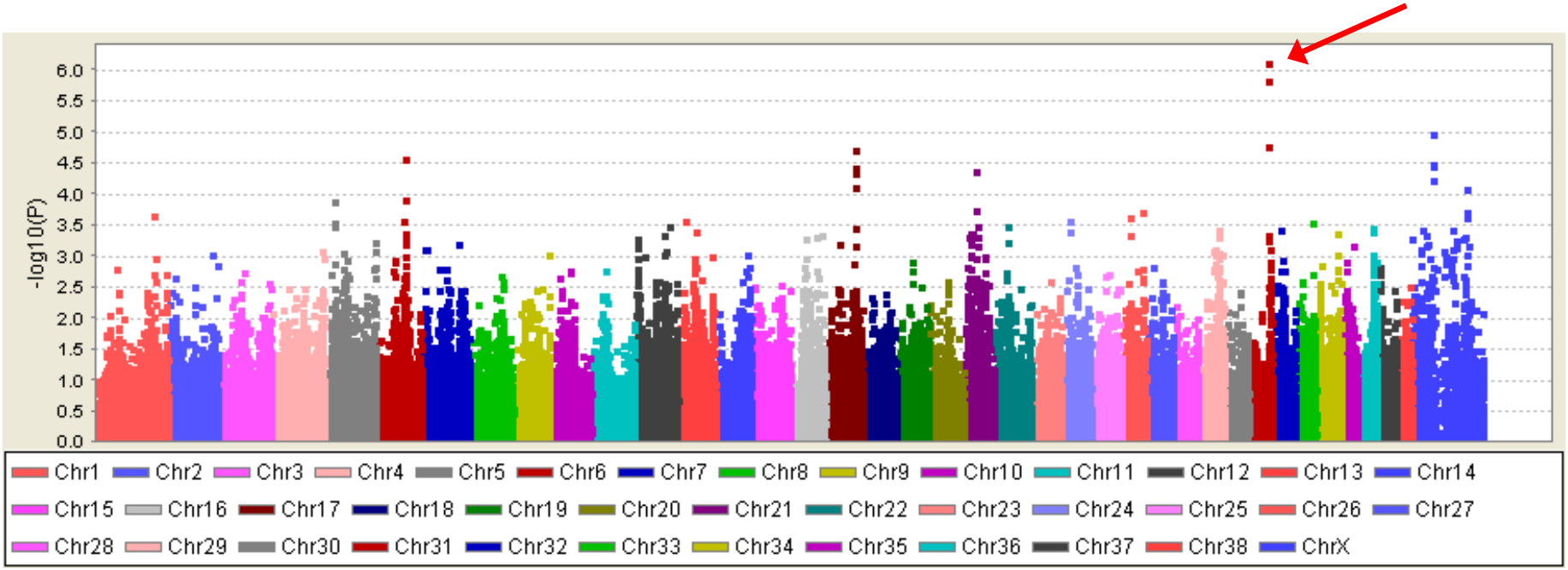
- Identify genetic risk factors for a specific common cancer in a predisposed breed
- Develop more accurate ways to predict if a tumour is going to spread to other parts of the body, and identify what alterations to the DNA in a tumour cell enable the cell to metastasise

Identification of inherited genetic risk factors that cause Labrador Retrievers to have an increased risk of developing mast cell tumours

- Most common skin cancer in dogs
- Prevalence of mast cell tumours (**MCTs**) in dogs diagnosed at AHT between 1997-1999
  - 280 dogs, 45 breeds
  - Odds Ratios
    - Boxer: 15.11
    - Golden Retriever: 6.93
    - Labrador Retriever: 4.63

'Genome wide association study': 'Mapping' of genetic risk factors for MCTs

- Compared genetic markers ('SNPs') at 173,000 positions across the 'genome' of 105 Labradors with a MCT and 85 unaffected dogs



Identification of changes to DNA on chromosome 31 that may be a genetic risk factor for MCTs

- Decode ('sequence') 2.9 million nucleotide-long region in DNA from 6 Labradors with a MCT and 6 unaffected Labradors, and then compare sequences against 'reference' Boxer genome

→ 19,930 mutations ('letter changes') compared to reference genome

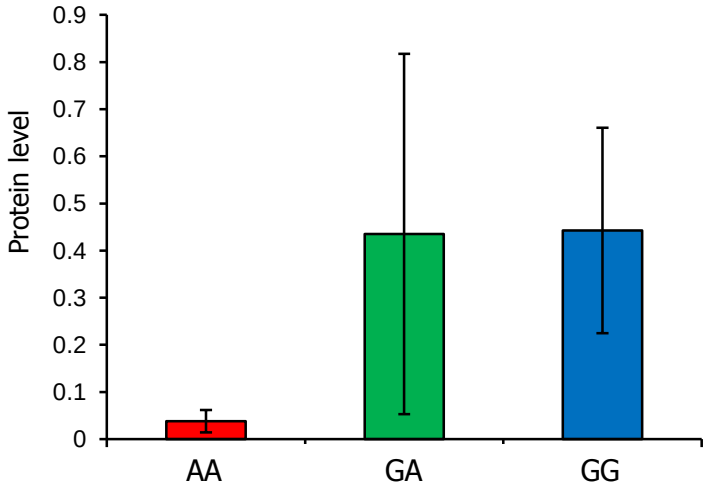
→ 4,028 mutations not present in the unaffected dogs

→ 24 mutations within genes (**3 predicted to have a 'negative effect'** on messenger RNA and/or protein) selected for further investigation

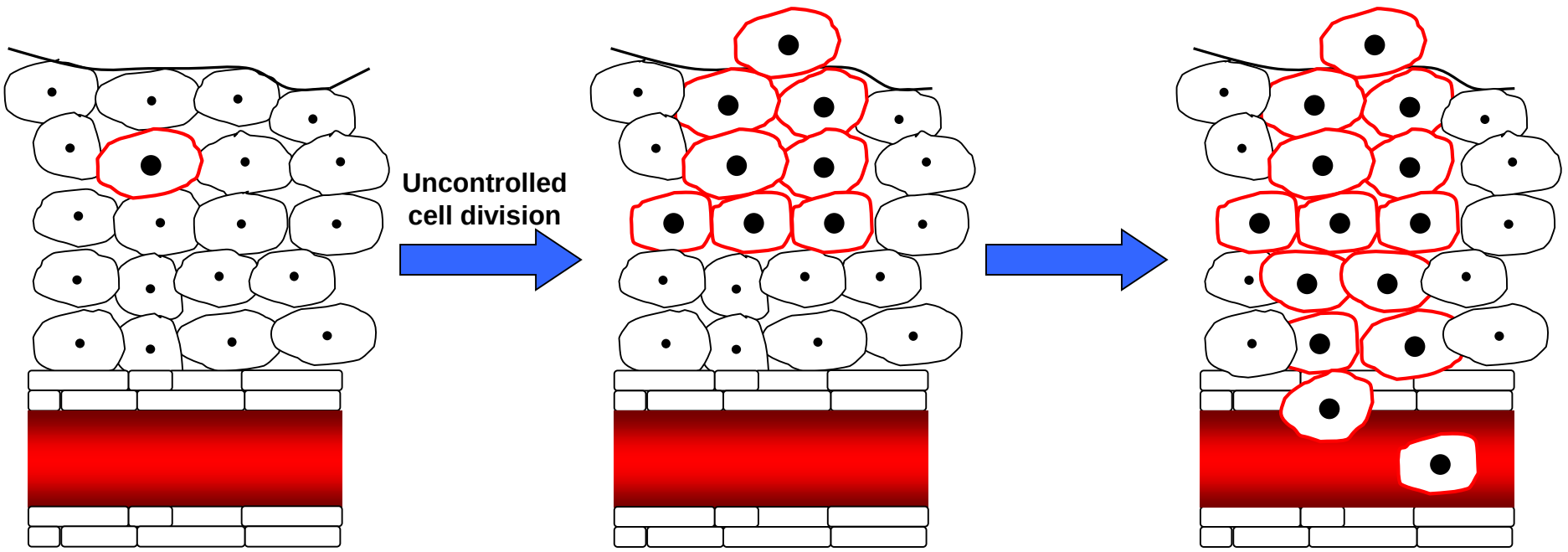
Screen for 24 mutations in 191 Labradors with a MCT and 216 unaffected Labradors

- Results:
  - 1 mutation present much more often in DNA (than would be expected by chance) in the DNA from dogs with a MCT
  - Mutation (change from 'G' to 'A') **predicted to have no effect** on messenger RNA or protein
  - Mutation actually had big effect on the amount of protein produced by the gene containing the mutation

Level of specific protein in the skin of Labradors



What does the protein do?



One ‘damaged cell’ develops into a tumour cell.

Mass of abnormal cells (*tumour*) don’t work properly, and begin to invade nearby parts of the body.

Cancer cells break away and travel through blood stream to other parts of the body (*metastasis*).

The mutation is common

- 72% of 407 Labradors (including 67% of unaffected Labradors) carry 1 copy of the mutation
- 25% of 407 Labradors (including 20% of unaffected Labradors) carry 2 copies of the mutation
- 2 copies confers a 1.7 x increased risk of developing a MCT (1 copy: 1.3 x increased risk)

The mutation is also common in Golden Retrievers

- 70% of test group of 90 dogs (including 62% of 53 unaffected Golden Retrievers) carry 1 copy of the mutation
- 26% of test group of 90 dogs (including 17% of 37 unaffected Golden Retrievers) carry 2 copies of the mutation
- 2 copies confers a 2.4 x increased risk of developing a MCT (1 copy: 1.5 x increased risk)

What next?

- Screen for mutation in a larger number of Labradors (500 with a MCT, 500 aged >8 years unaffected)
- Search for genetic risk factors for MCTs in other parts of the Labrador genome highlighted by Genome wide association study
- Promote discussions (e.g. with Kennel Club) as to 'what to do' about common, low risk genetic risk factors for 'complex diseases' such as cancer

Obstacles to progress in canine cancer research

- Complexity of the 'disease' (No quick answers!)
- Shortage of samples (particularly tumour biopsies)
- Money
  - No government funding for research on cancer in companion animals
  - Limited number of organisations that provide grants for research on cancer in dogs (Kennel Clubs, Dogs Trust, British Small Animal Veterinary Association, Morris Animal Foundation)
  - AHT is the only UK charity engaged in research on cancer in dogs (and cats and horses)

PLEASE HELP RESEARCH TO FIGHT CANCER IN DOGS

To carry out our vital research we need samples from dogs that have cancer. To collect the most important samples, tumour biopsies, we need dog owners to contact us quickly, before a dog starts treatment. Please contact us if you can help us to collect any of the samples listed below.

Animal *Health* Trust

THE KENNEL CLUB CANCER CENTRE AT THE ANIMAL HEALTH TRUST

• oncologyres@aht.org.uk • 01638 751000 ext. 1214 • www.aht.org.uk/cancer_research

If you would like to support our work with a donation, please visit www.aht.org.uk/donate

SAMPLES NEEDED FOR ANIMAL HEALTH TRUST CANCER RESEARCH

- **A biopsy of the following tumours from ANY breed of dog:**
  Glioma, lymphoma, mast cell tumour, oral melanoma, osteosarcoma, uveal melanoma
- **A surplus blood sample from ANY breed of dog affected by a mast cell tumour or an oral melanoma**
- **A cheek swab OR surplus blood sample from one of the affected dogs shown below:**

Glioma

Boxer

Lymphoma

Bullmastiff

Boxer

Mast Cell Tumour

Labrador Retriever

Boxer

Weimaraner

Shar Pei

Golden Retriever

Osteosarcoma

Irish Wolfhound

Leonberger

Great Dane

Greyhound

Uveal Melanoma

Labrador Retriever

Golden Retriever

German Shepherd

Thank you for your attention!