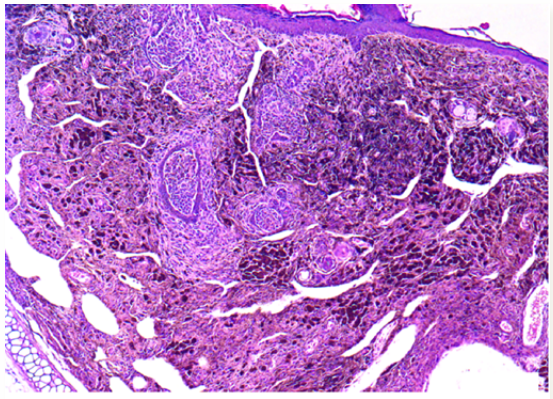
Transgenics

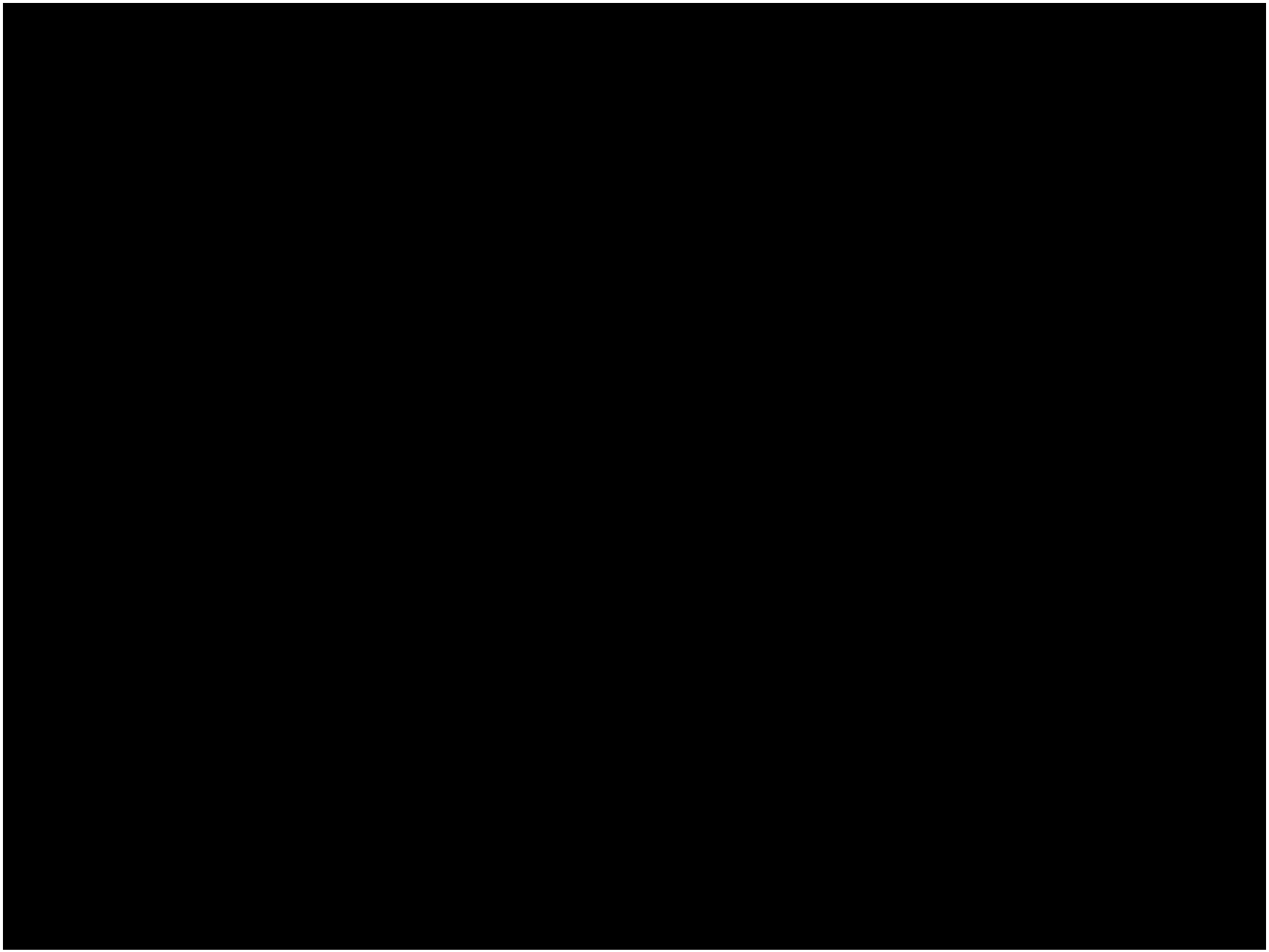
Friedrich Beermann
ISREC/SV/EPFL

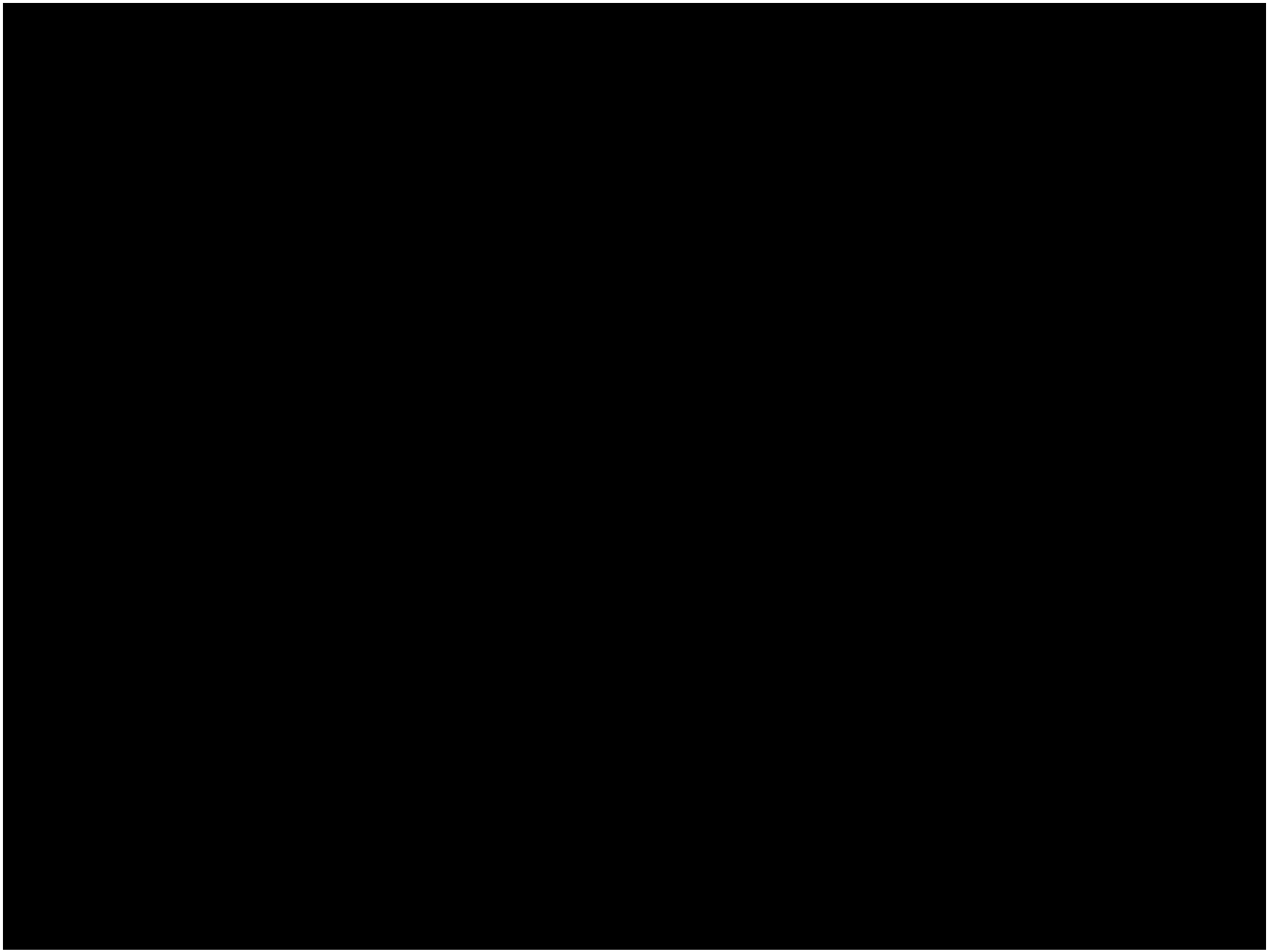


JAN 2012

Today:

- Introduction – transgenics in general
- Transgenics in cancer research
- A mouse model of melanoma
- Arguments for mouse models and models

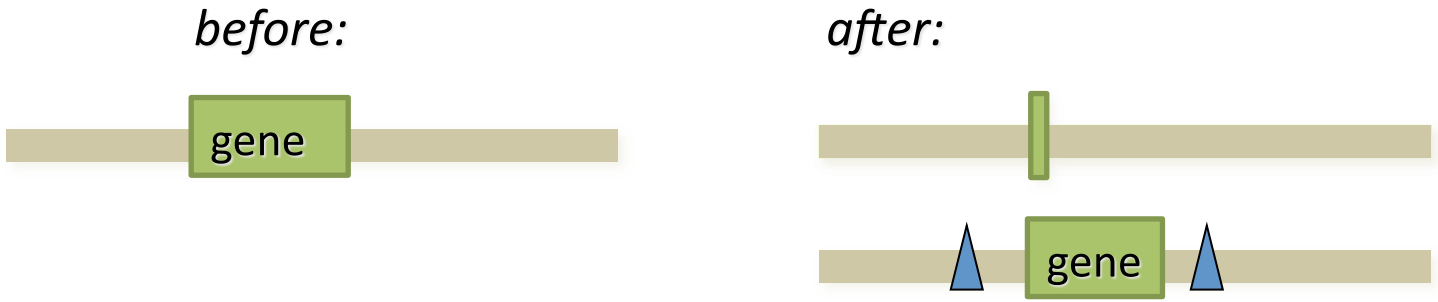




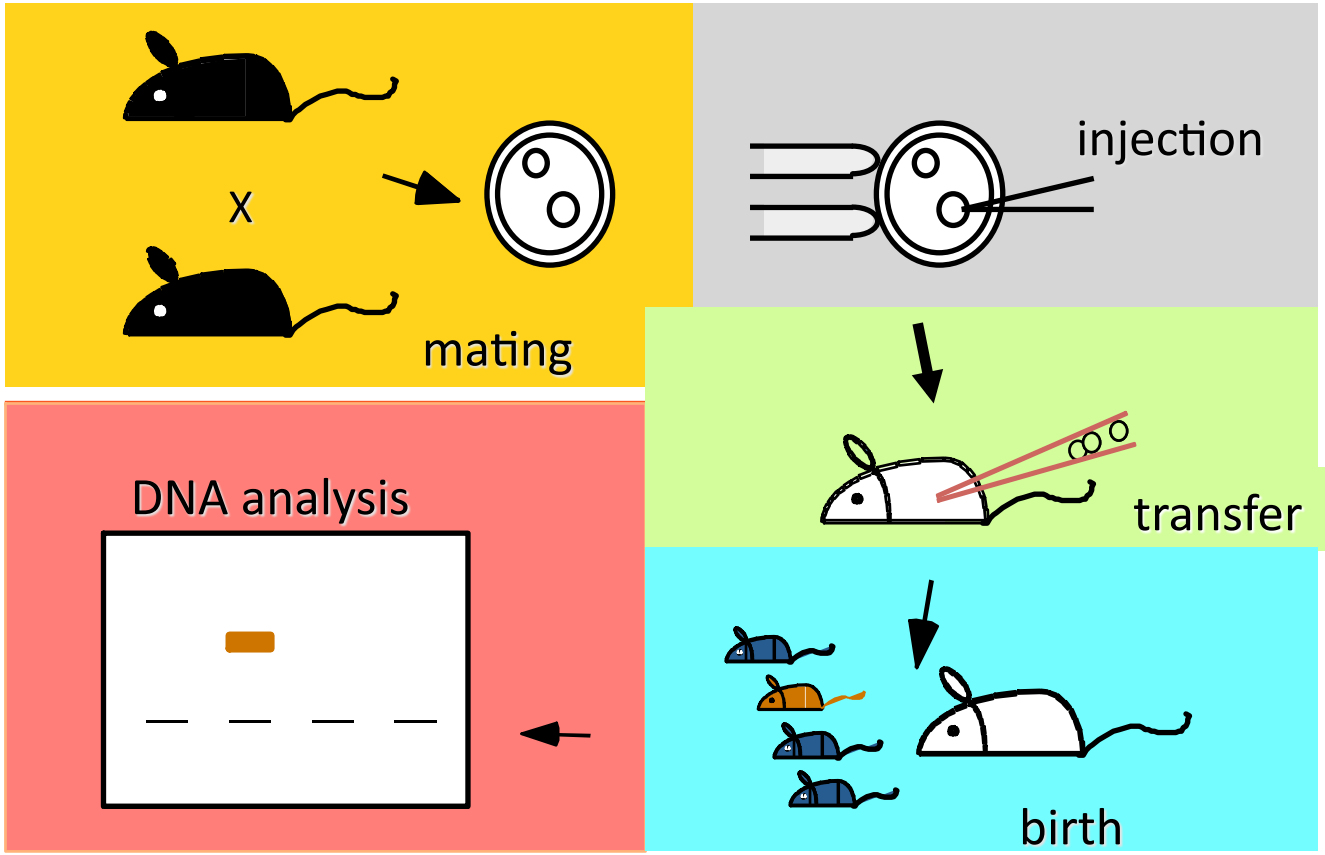
… new transgenic strains can be made by

1. Pronuclear or lentiviral injection / « gene addition »

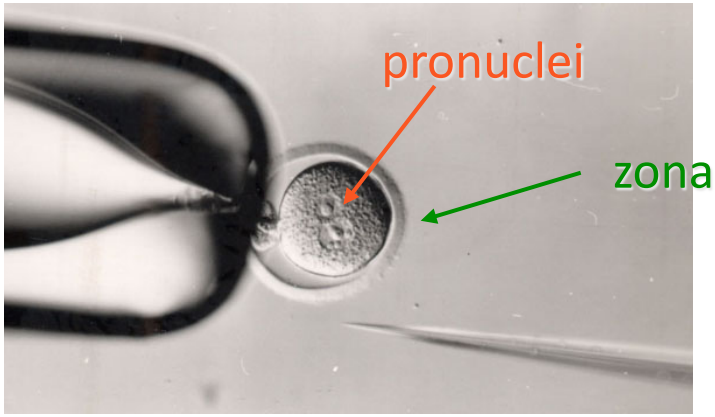
2. ES cells / blastocyst injection / “knockout” or modified allele

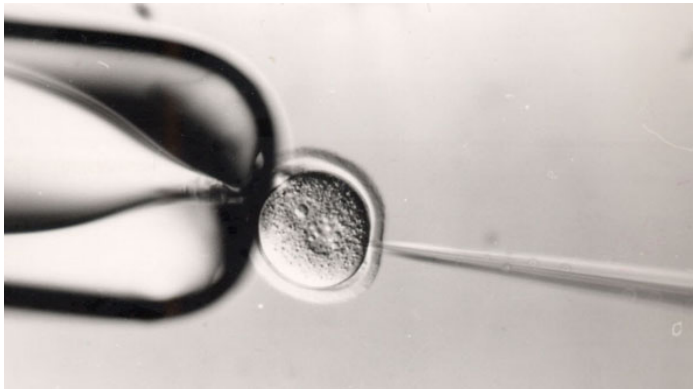


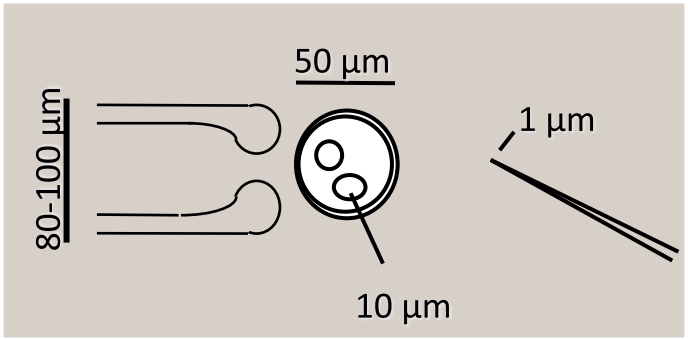
Transgenic mice by pronuclear injection



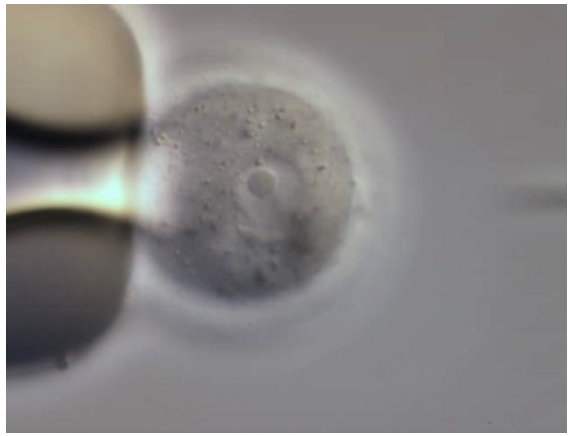
holding pipette







microinjection pipette containing DNA

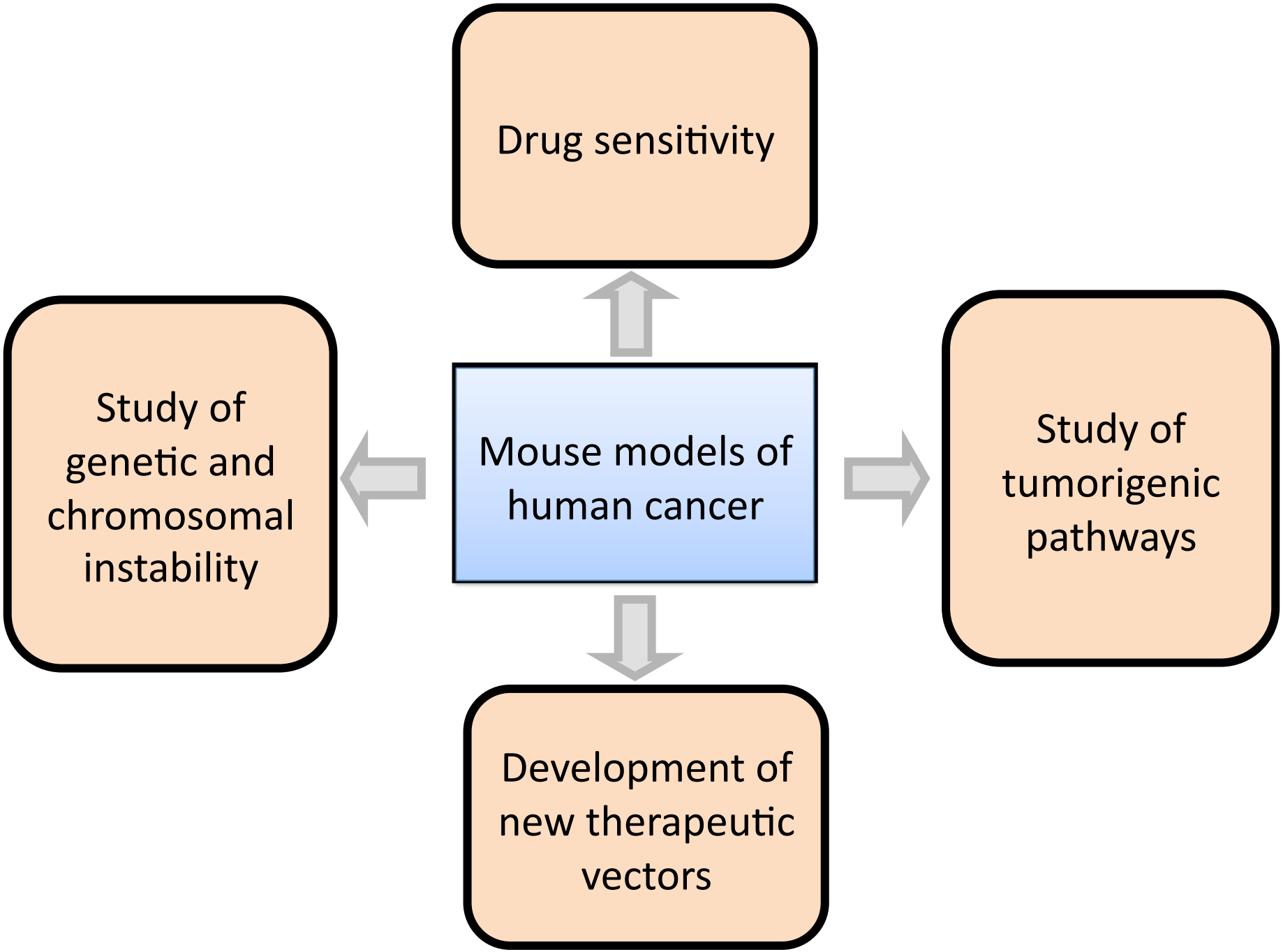


http://www.transtechsociety.org/members/videos.php
J. Wilbertz, Sweden

Mice in Cancer Research

Why mouse ?

- Genome similar to human
- Specific mutations feasible
- Comparable organs and physiology
- Similar/identical origin of tumorigenesis


Drug sensitivity
Study of genetic and chromosomal instability
Mouse models of human cancer
Study of tumorigenic pathways
Development of new therapeutic vectors

Mice as models for cancer

Xenografts (transplants)

- Human cancer cell lines are injected into athymic nude mice
- *In vivo* and *in vitro* tumor propagation
- Various routes of introduction (SC, IV)
- Additional procedures offered (hormone supplementation, surgical alteration)

Murine Tumor Models:

- Syngeneic cancer cell lines generate tumors in mice with an intact immune system
- Serves as a model of metastatic disease

Carcinogen-induced tumors

- Carcinogen-induced animal models include the DMBA and NMU mammary tumor models in rats
- 65-85% incidence of mammary tumors in rats
- Average of 3-5 tumors per animal
- Wide spectrum of benign and highly malignant tumor types

Transgenic tumor models

- Customer-specific research protocols and tumor models are implemented

CHARLES RIVER
LABORATORIES

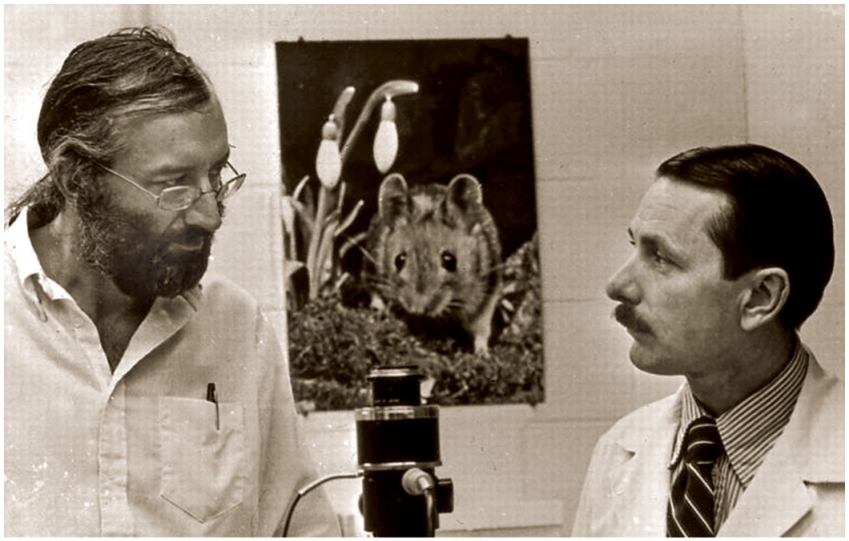
Mouse models of human cancer

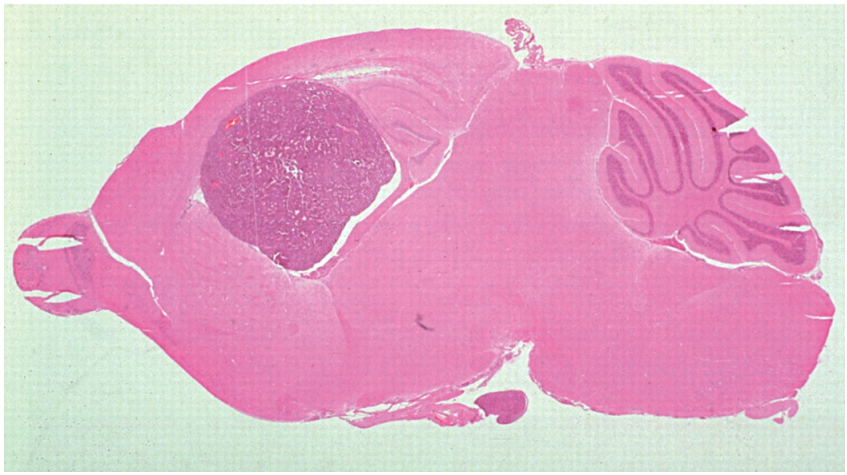
Cancer in the mouse should look and act like the human disease

- ✓ Same gene and/or pathways should be affected in tumor initiation and progression
- ✓ Same or similar histological features of human tumors and they should progress through the same stages
- ✓ The response of a given tumor to a particular therapy in the mouse should accurately reflect the response in human patients

van Dyke & Jacks, Cell, 2002

Palmiter & Brinster 1981 – brain tumors in SV-TK mice



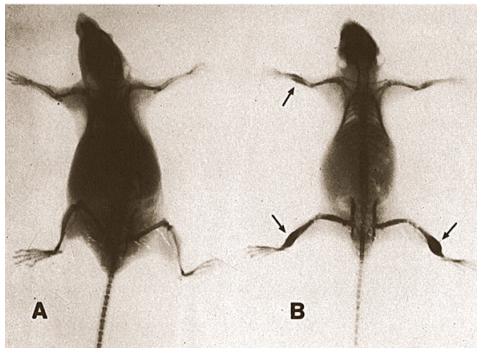


Douglas Hanahan et al. Genes Dev. 2007; 21: 2258-2270

Leder & Stewart
1983/4 –
breast tumors in
MMTV-oncomice

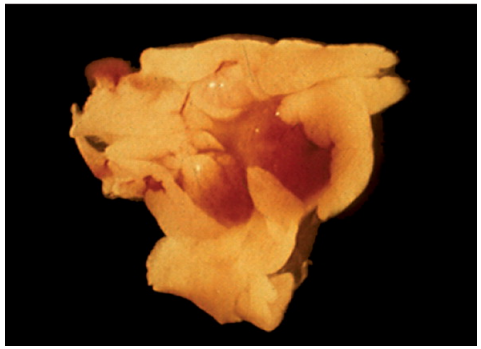
Douglas Hanahan et al. Genes Dev. 2007; 21: 2258-2270

Wagner – bone tumors using the Fos oncogene



Douglas Hanahan et al. Genes Dev. 2007; 21: 2258-2270

Hanahan 1986 – RIP-Tag mice and pancreatic tumor



Douglas Hanahan et al. Genes Dev. 2007; 21: 2258-2270

Organ	Histopathology	Genetics
Lung	Adenocarcinoma	*Kras*[55]
	Squamous cell carcinoma	NA
	Large cell carcinoma	NA
	Small cell carcinoma	*Rb1;Trp53* (REF. 167)
Colon	Polypoid adenocarcinoma	*Kras;Apc*[168]
	Hereditary non-polyposis carcinoma	*Msh6*[169]
Breast	Ductal carcinoma	*Brca2;Trp53* (REF. 170)
	Lobular carcinoma	*Cdh1;Trp53* (REF. 171)
Pancreas	Ductal adenocarcinoma	*Kras; Cdkn2a*[172], *Kras;Trp53* (REF. 173)
	Mucinous cystic neoplasm	*Kras;Dpc4* (REF. 96)
	Intraductal papillary mucinous neoplasia	NA
Prostate	Prostate carcinoma	*Pten*[174], *Pten;Nkx.1* (REF. 175), *Rb1;Trp53* (REF. 176)
Liver	Hepatocellular carcinoma	*Apc*[177], *Myc;Trp53* (REF. 178), *Myc;TGFA*[179]
Ovary	Endometrioid carcinoma	*Kras;Pten*[180], *Apc;Pten*[181]
	Serous carcinoma	NA
	Mucinous carcinoma	NA
Oesophagus	Squamous cell carcinoma	*Pten;Dpc4* (REF. 182); *Ccnd1;Trp53* (REF. 183)
	Adenocarcinoma	NA
Bladder	Transitional cell carcinoma	*Hras*[184]
Kidney	Renal cell carcinoma	*Apc;Trp53* (REF. 185)
Brain	Astrocytoma	*Pten;Rb1* (REF. 186)
	Glioblastoma	*Nf1;Trp53* (REF. 198)
Stomach	Gastric carcinoma	*Wnt;Ptgs2;Ptges*[188]
Skin	Melanoma	*HRAS;Ink4a*[27]
	Squamous cell carcinoma	*Xpd**[189]

* Requires exposure to UVB light. NA, none available.

Examples of genetically engineered mouse models that recapitulate human solid cancers

Example

Mouse models for melanoma research

Identification of melanoma-relevant genes

Dissection of the biological roles of genes involved in melanoma

In vivo model for UV-induction of melanoma

In vivo model to understand gene-gene interaction

Testing potential diagnostic and therapeutic approaches against melanoma

Example

- The anatomic location of melanocytes in mice overlaps with that of humans but is somewhat different

- Human melanocytes are predominantly located at the junction of epidermis and dermis and also are present within the hair follicle

- Murine melanocytes are predominantly associated with hair follicles or are present within the interfollicular dermis, and only rarely are present at the dermal/epidermal junction

- Early human melanoma is characterized by upward spread of atypical melanocytes within the epidermis (Pagetoid spread)

Choose a relevant human mutation

Example

Cancer type	*HRAS*	*KRAS*	*NRAS*	*BRAF*
Biliary tract	0%	33%	1%	14%
Bladder	11%	4%	3%	0%
Breast	0%	4%	0%	2%
Cervix	9%	9%	1%	0%
Colon	0%	32%	3%	14%
Endometrial	1%	15%	0%	1%
Kidney	0%	1%	0%	0%
Liver	0%	8%	10%	3%
Lung	1%	19%	1%	2%
Melanoma	6%	2%	18%	43%
Myeloid leukaemia	0%	5%	14%	1%
Ovarian	0%	17%	4%	15%
Pancreas	0%	60%	2%	3%
Thyroid	5%	4%	7%	27%

The mutation data was obtained from the Sanger Institute Catalogue of Somatic Mutations in Cancer web site[148].

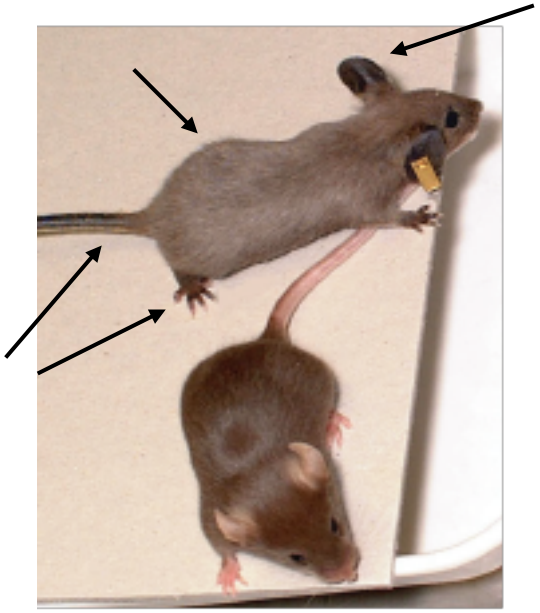
Example

A mouse model for melanoma: Expression of transgenic N-ras^{Q61K}

N-ras and B-raf are frequently mutated in melanoma

Tyr::N-ras^{Q61K}

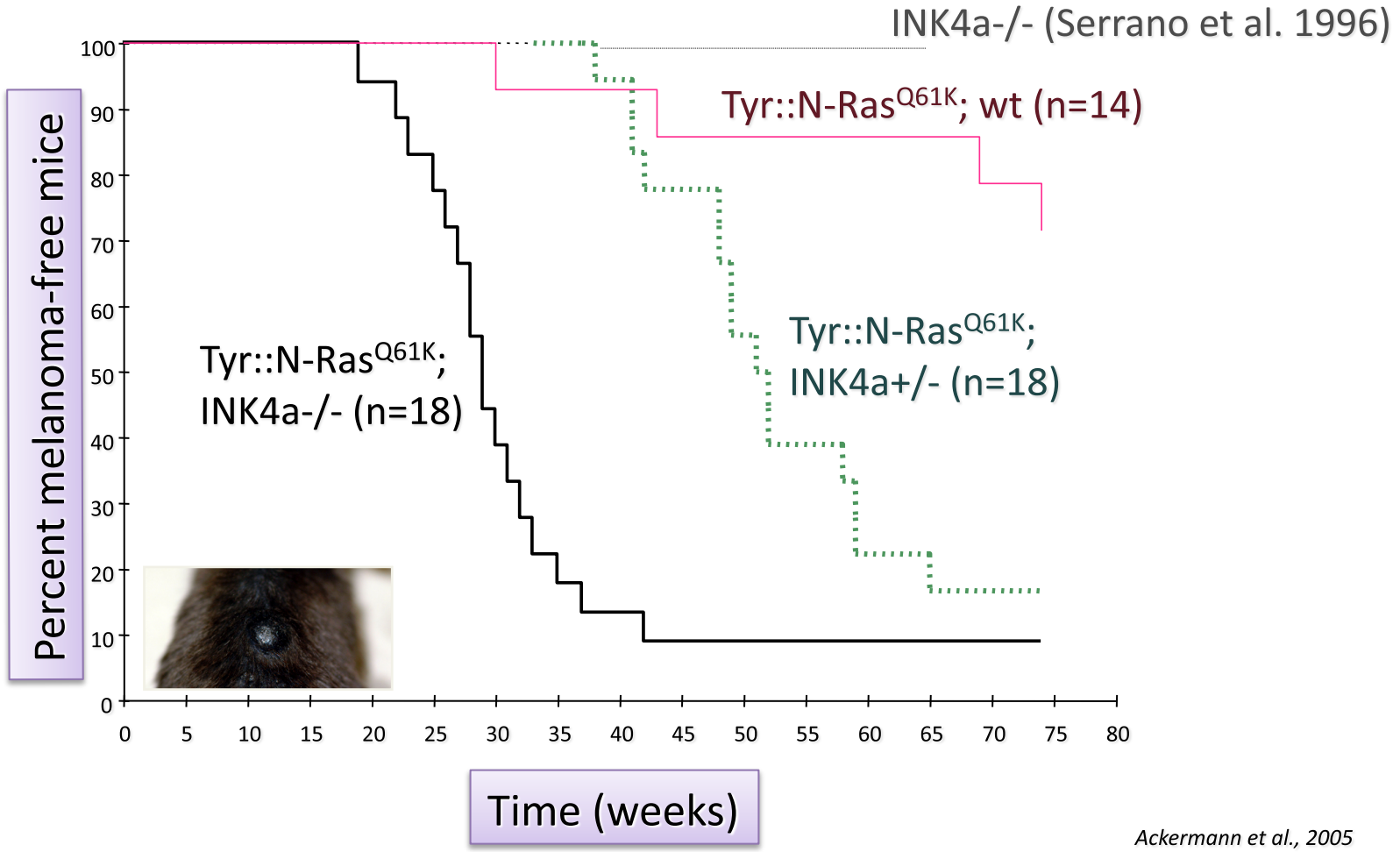
mouse tyrosinase
enhancer & promoter



Transgenic mice were generated by pronuclear injection of the construct into fertilized oocytes from B6D2F1 male x female matings

Melanoma in *Tyr::N-ras*^{Q61K} transgenic mice

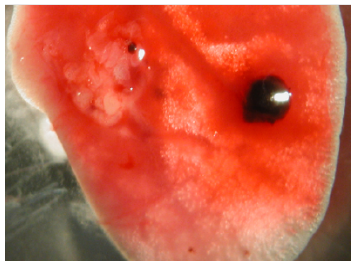
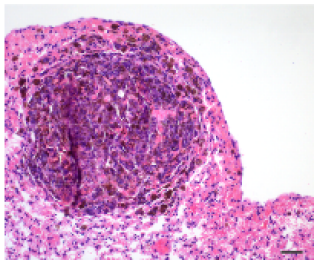
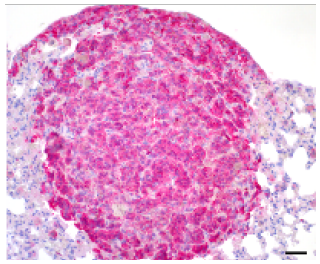
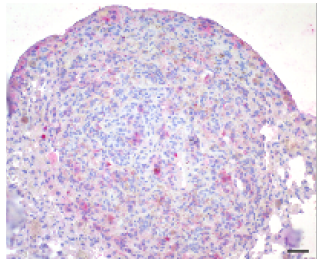
After breeding to mice lacking INK4a/ARF (p16/p19) to overcome e.g. senescence



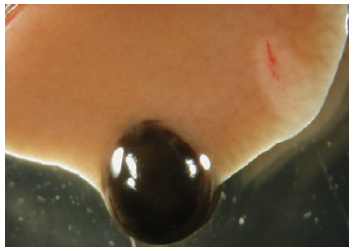
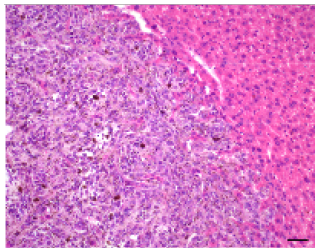
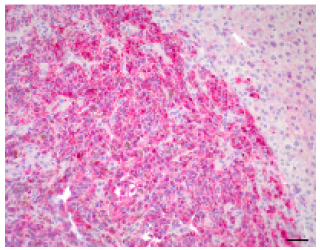
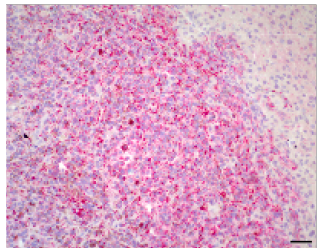
Ackermann et al., 2005

Metastasis to lung and liver

lung

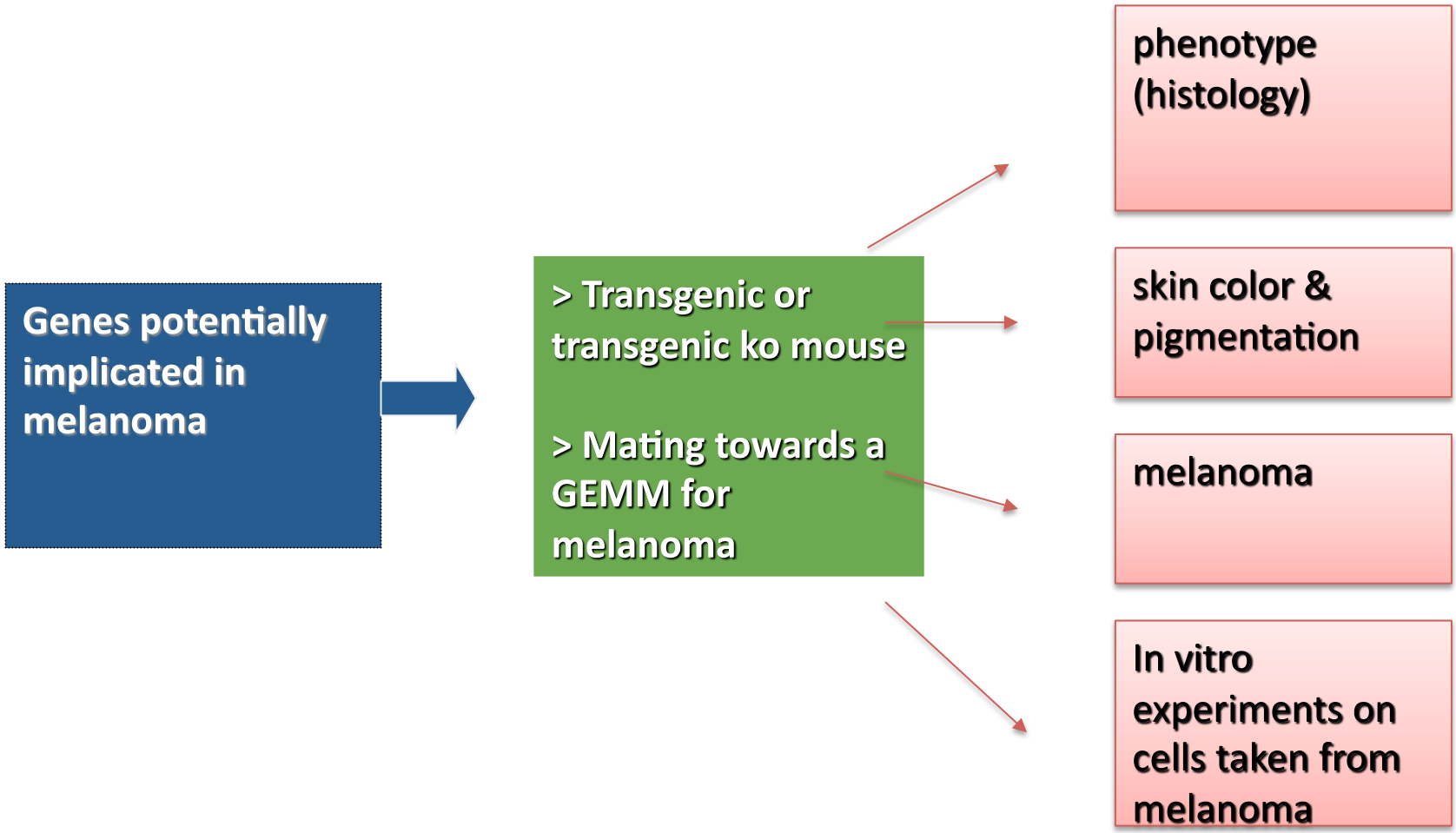
liver

Tyrosinase S100

Ackermann et al., 2005

Testing candidate genes in melanoma



Mouse models for melanoma research

Identification of melanoma-relevant genes

Dissection of the biological roles of genes involved in melanoma

In vivo model for UV-induction of melanoma

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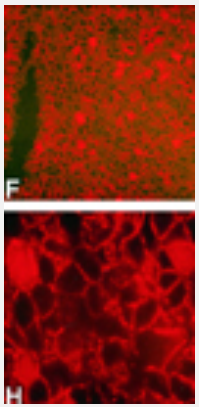
Testing potential diagnostic and therapeutic approaches against melanoma

Role of ß-catenin in melanoma

In tumors:
Mutations in exon 3 of the ß-catenin gene are rare
but nuclear and/or cytoplasmic localization is observed
focally within about 30% of the primary tumor.

ß-catenin is frequently membranous in primary tumors.

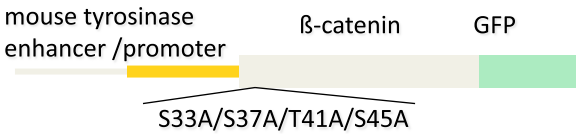
Metastases show membranous, cytoplasmic/nuclear or
negative immunostaining for ß-catenin.



From Rimm et al., 1999

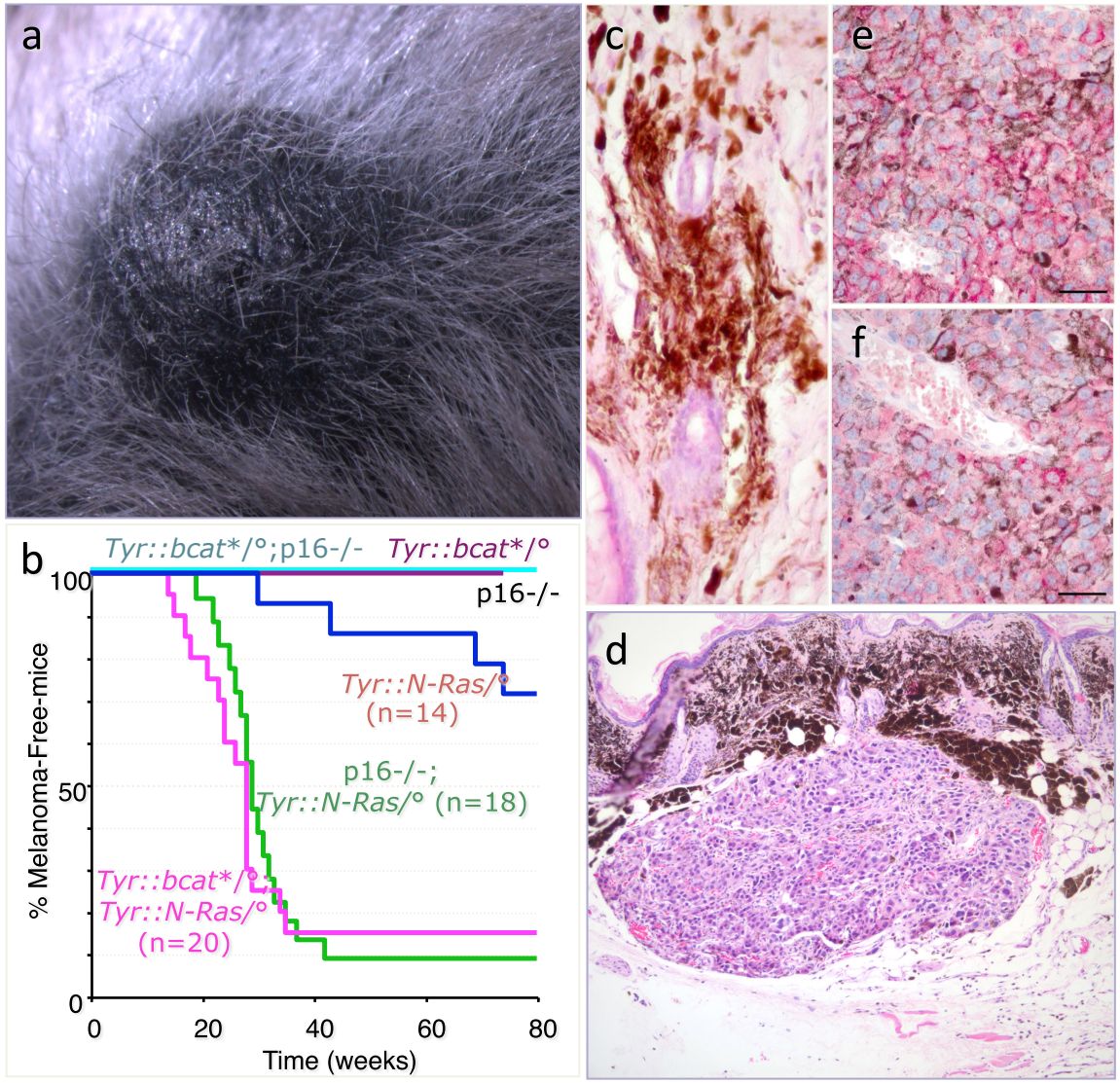
⇨ **Mice expressing active ß-catenin in melanocytes (V.Delmas/L.Larue, Orsay)**

Tyr::ßcat^{act}



⇨ **Breeding to *Tyr::N-ras^{Q61K}* melanoma model**

Melanoma in *Tyr::N-Ras^{Q61K} Tyr-ß-cat^{act}* mice



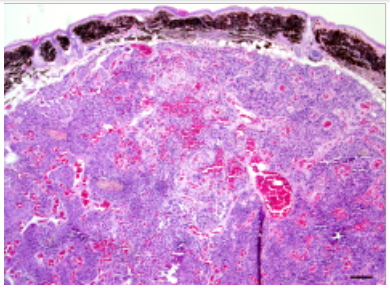
Delmas et al.

Conclusion melanoma model

⇨ Establishment of a mouse model for melanoma (melanotic, metastatic)

⇨ Impact of candidate genes (ß-catenin) for melanoma

+ ß-catenin



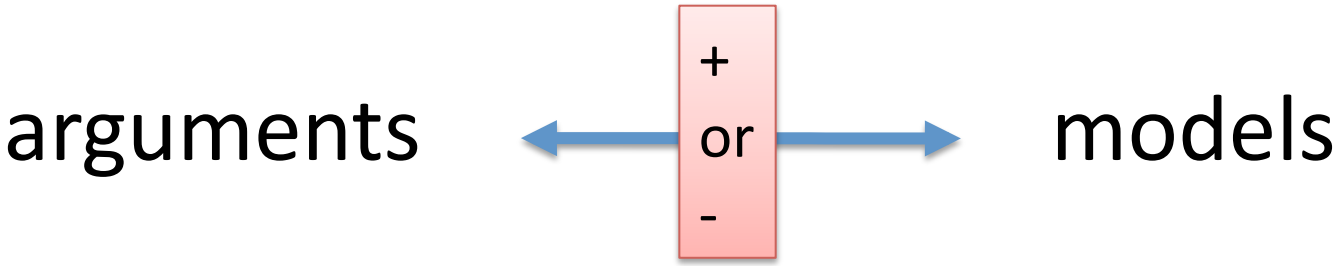
⇨ Primary melanoma are often amelanotic (hypopigmented)

⇨ Metastasis rare

⇨ Latency of 6 months

⇨ Loss of $p16^{INK4a}$ not required for melanoma formation

Beta-catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma developmen (G&D, 2007)


arguments
+
or
-
models

Mouse Models of Human Disease: Utility (I)

A. Physiologically similar to humans.

B. Large genetic reservoir of potential models has been generated through identification of >1000 spontaneous, radiation- or chemically-induced mutant loci.

C. Recent technological advances have dramatically increased our ability to create mouse models of human disease.

1. Development of high resolution genetic and physical linkage maps of the mouse genome – facilitates identification and cloning of mouse disease loci.

2. Transgenic technologies that allow one to ectopically express or make germline mutations in virtually any gene in the mouse genome; i.e., transgenic mice, ES cell knockouts.

3. Methods for analyzing complex genetic diseases.

Mouse Models of Human Disease: Utility (II)

D. >100 mouse models of human disease where the homologous gene has been shown to be mutated in both human and mouse.

    1. Mouse mutant phenotype very closely resembles the human disease phenotypes.

    2. Provide valuable resources to understand how the diseases develop and test ways to prevent or treat these diseases.

E. Allow study of disease on uniform genetic background.

F. Will aid in identifying modifier genes and are well poised to lead us into the new era of polygenic disease research.

Challenges for a novel genetic disease:

- Disease symptoms: family history
- Biochemical analysis
  Histological analysis
  Cell biology analysis
- Treatment
- Genetic basis (positional cloning or similarity to a model)
- Model systems (*in vitro*, *in vivo*)
- Establish causality
  Molecular mechanisms
- Treatment
- Replacement therapy (cell-based or gene therapy)

in vivo Models:

Similarity in biochemical and developmental features

- Physiology/behavior: rabbit, dog, monkey, ape, bird, rat, mouse
- Development: mouse, frog, chick, fish, sea urchin, fruit fly, nematode (*C. elegans)*
- Genetics: mouse, rat, zebrafish, fruit fly, nematode, yeast, bacteria
- Mutations: spontaneous vs. induced

Polydactyly in man, mouse, and chick

Basic animal research on the rise while pharma looks to new options

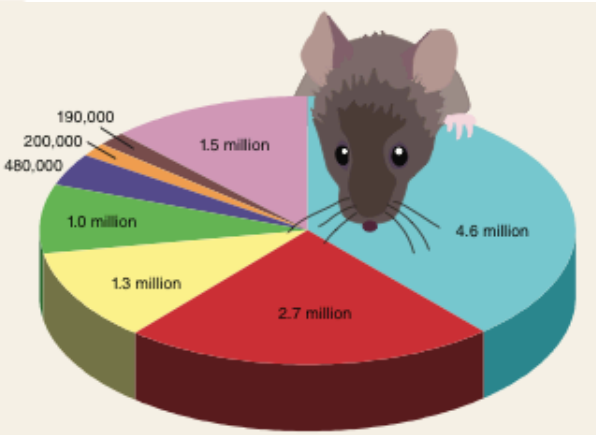
Drug companies in the EU are increasingly turning to nonanimal strategies to test medicines, but the number of animals used for basic research is on the rise, according to statistics published 30 September by the European Commission.

Although the total number of animals used for scientific purposes in the EU's 27 member states has held steady at around 12 million per year, this overall figure masks shifting trends in animal experimentation. The European Commission report, which documents data submitted for 2008, shows that studies investigating basic biological principles used approximately 4.5 million research animals—up by more than half a million from 2005. In contrast, the number of animals used in the drug discovery pipeline for human and veterinary medicines dropped by more than a million to 2.7 million animals over the same period. Toxicology testing remained constant at about 1 million animals.

"What we're seeing at the moment is a steady increase in the number of animals that are genetically modified" for basic investigations, says Simon Festing, chief executive of Understanding Animal Research, a proresearch advocacy group in London. But he adds that at the same time "there is continued pressure, particularly in safety testing, to reduce the number of animals used. This can be achieved by new technologies, from computer simulations to stem cells."

Thomas Hartung, director of the Center for Alternatives to Animal Testing at Johns Hopkins University in Baltimore and former director of the European Centre for the Validation of Alternative Methods in Ispra, Italy, notes that pharma's move to alternative testing strategies has proven to be a boon for the industry. "This has helped the drug industry enormously to bring down their attrition rates" for investigational compounds put into clinical trials, he says.

Here's where Europe's 12 million animals are being used:



The future of model organisms to understand human disease

- Direct discovery of disease genes and variants in human by genome-wide association studies (GWAS) and by whole-genome sequencing (NGS-technologies)
- Genetic architecture is hard to replicate in model organisms
- Disease modeling replaced by in vitro experiments (human cell lines) and computer simulations

Nature Review Genetics 12, 575-582, 2011

Technical plus of model organisms

- Wealth of literature for mouse, rats, fly, worm, yeast for the past 100 years
- The pace of genome resources
- The development of in vivo phenotyping and analysis (mainly true for rodents)
- The opportunities for manipulating genomes

Why do we need model organisms ?

- GWAS reflect a small effect and relatively low proportion / increase the rate of mutation discovery (mutagenic screens)
- environmental variations and heterogenous genetic background difficult to study
- Experimental interventions to get causal mechanisms
- Studies of harmful interventions only feasible in model organisms
- Achieve cellular and subcellular resolution of important events relevant to disease (GFP)

Why do we still need model organisms to understand human disease ?

- Only a fraction of human genes is functionally understood
- Linkage to a human disease does not mean understanding – it makes it just “interesting”

Model organisms allow to put disease genes in a biological context

USE MODEL ORGANISMS TO UNDERSTAND LIFE