the pig as a biomedical model for human cutaneous melanoma : the MeLiM strain

Giorgia Egidy

The Pig as a biomedical model for human cutaneous melanoma : The MeLiM strain

Génétique Animale et Biologie Intégrative
Team : Genetics, Microbiota & Health

Giorgia Egidy Maskos Ph.D.

New challenges for animal sciences
Jouy-en-Josas

Giorgia.egidy-maskos@inra.fr

19/11/2019

- Introduction on GABI – INRA, GeMS team
- Facts on cutaneous melanoma, therapies
- Animals models of melanoma
- Malignant melanoma traits in Minipigs MeLiM
- MeLiM, a model for successful cancer regression
- Animal Welfare

GENETIQUE ANIMALE ET BIOLOGIE INTEGRATIVE

http://www6.jouy.inra.fr/gabi_eng

GABI develops research on the characterisation and exploitation of genetic variability of animals for efficient and sustainable breeding

Studies focus on:

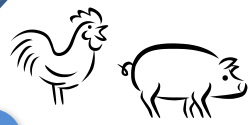
- **Fundamental research on farm animals and model animals**
- **Applied research on breeding, genetic improvement**

Genetics, Microbiota, Health team : GeMS

Swine experimental unit

@Bridge facility histology
CRB GADIE

Genetics
Genomics



Immunity

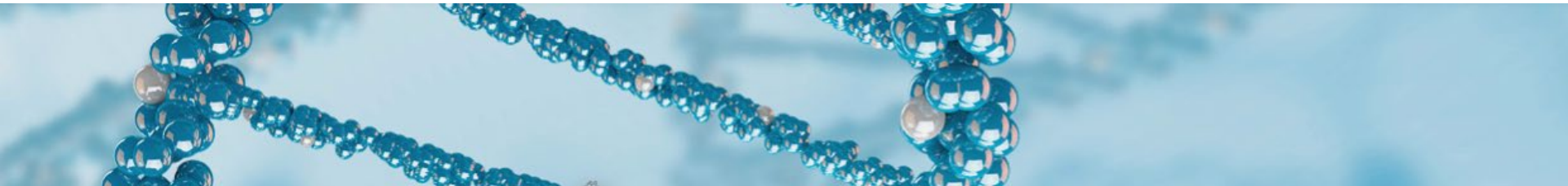
intestinal
Microbiota

The Animal: an holobiont

Animal as livestock

Health

Animal models



Genetics, Microbiota, Health team : GeMS

Collaboration INRA / BGI-Shenzen / Copenhagen University

- 7,7 millions genes, 719 MGS
- race, sex, age Influences
- Abundance of antibiotic resistance genes according to countries

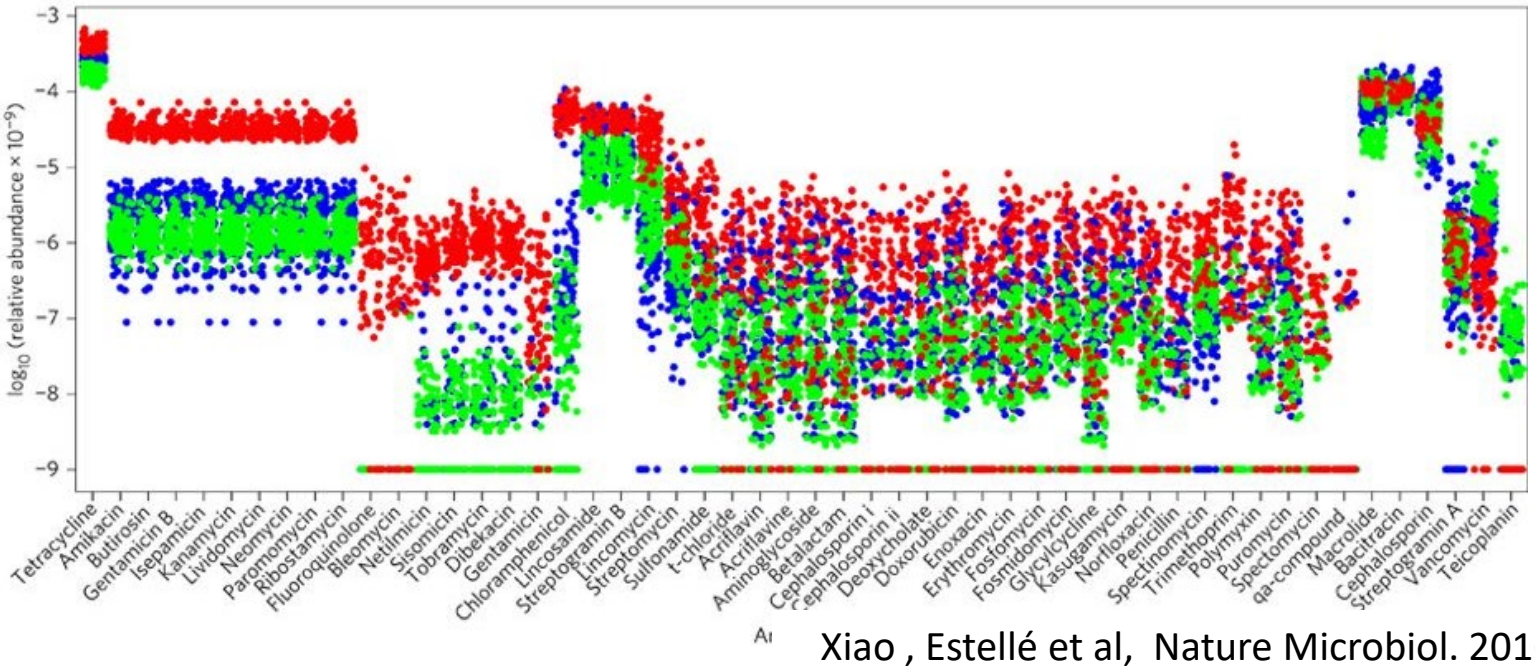
nature microbiology

LETTERS

PUBLISHED: 19 SEPTEMBER 2016 | ARTICLE NUMBER: 16161 | DOI: 10.1038/NMICROBIOL.2016.161

A reference gene catalogue of the pig gut microbiome

Liang Xiao[1†], Jordi Estellé[2†], Pia Kiilerich[3†], Yuliaxis Ramayo-Caldas[2], Zhongkui Xia[1], Qiang Feng[1‡], Suisha Liang[1], Anni Øyan Pedersen[4], Niels Jørgen Kjeldsen[4], Chuan Liu[1,5], Emmanuelle Maguin[6], Joël Doré[6,7], Nicolas Pons[7], Emmanuelle Le Chatelier[7], Edi Prifti[7‡], Junhua Li[1,8], Huijue Jia[1], Xin Liu[1], Xun Xu[1], Stanislav D. Ehrlich[7,9], Lise Madsen[1,3,10], Karsten Kristiansen[1,3*], Claire Rogel-Gaillard[2*]



Xiao , Estellé et al, Nature Microbiol. 2016

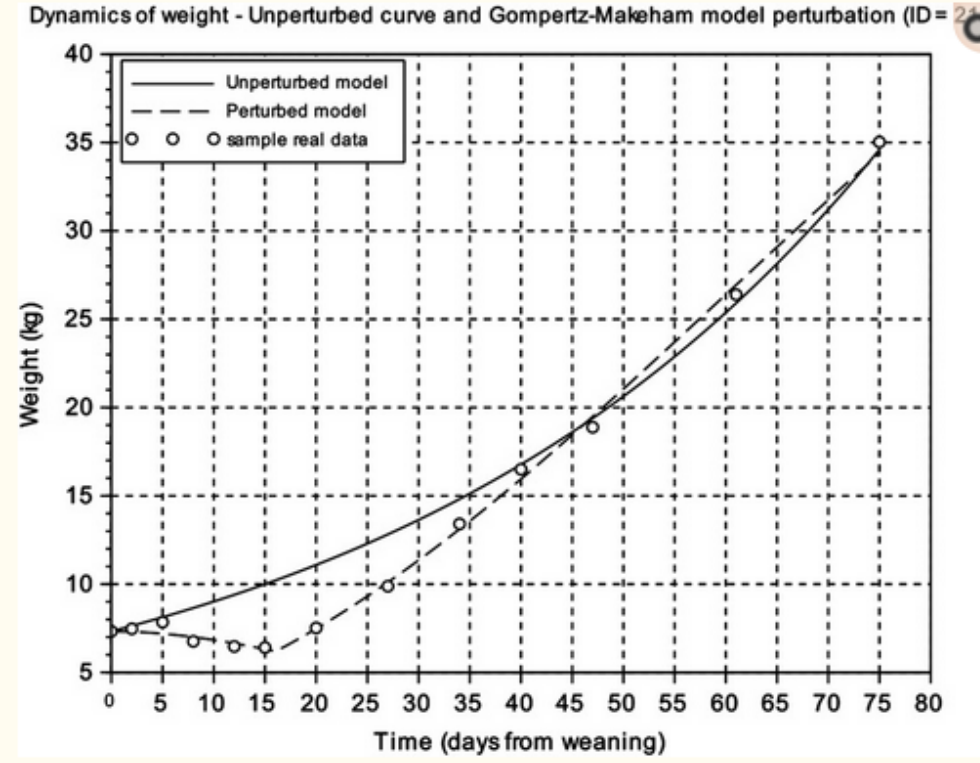
Genetics, Microbiota, Health team : GeMS

Towards the quantitative characterisation of piglets' robustness to weaning: a modelling approach

Collaboration UMR MoSAR
R Munoz-Tamayo
N Friggens

Comparison of the weight dynamics as predicted by the unperturbed and the perturbed (Gompertz–Makeham) models

o different BW measures of the individual piglet relative to days from weaning
__ the predicted response of the unperturbed growth model
--- the perturbed growth model response



Revilla *et al* Animal, 2019

Genetics, Microbiota, Health team : GeMS

FAANG
Functional Annotation of Animal Genomes

A coordinated international action to accelerate genome to phenome

About FAANG | Structure | Activities | Data and Tools | To participate | more

FAANG Aims:

- Standardize core assays and experimental protocols
- Coordinate and facilitate data sharing
- Establish an infrastructure for analysis of these data
- Provide high quality functional annotation of animal genomes

Working groups

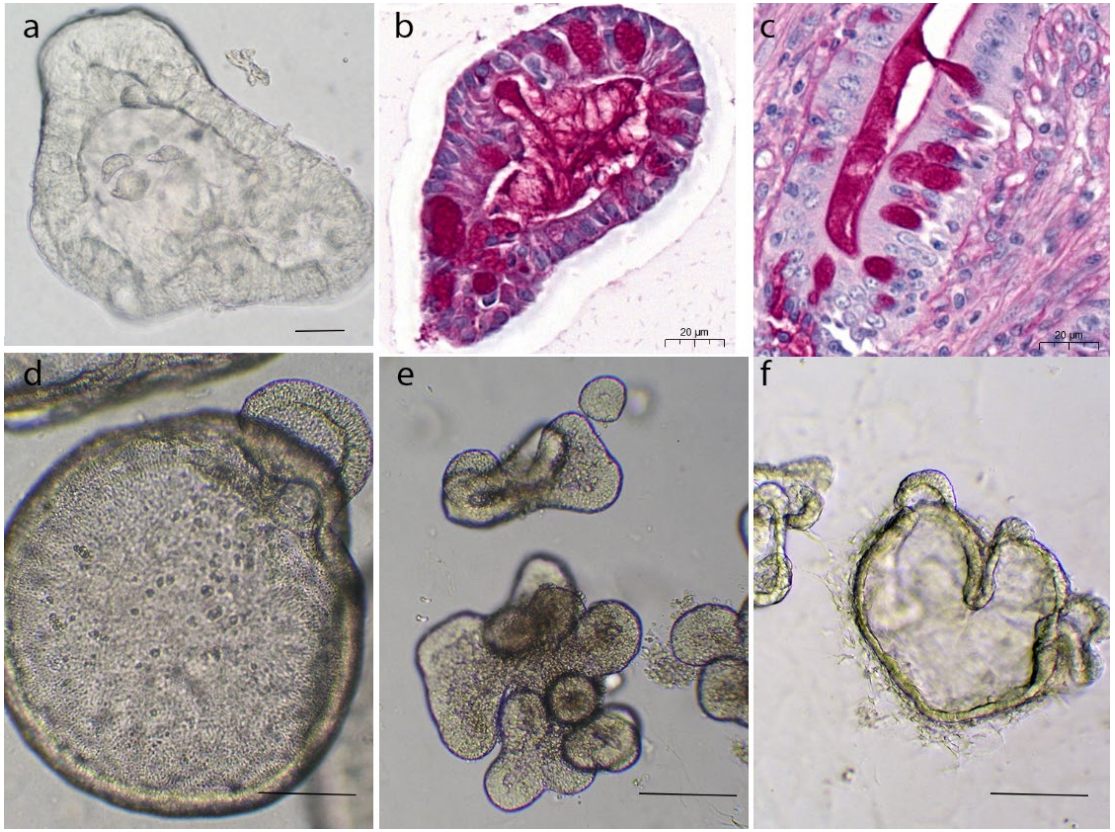
- Steering Committee
- Scientific Advisory Group
  - Animals, Samples, and Assays (ASA)
  - Bioinformatics and Data Analysis (B&DA)
  - Communication (COM)
  - Metadata and Data Sharing (M&DS)

Giuffra & Tuggle, Annu Rev Anim Biosci 2019

Genetics, Microbiota, Health team : GeMS

Gut organoids

- Obtained from embryonic cells, iPSC, and adult stem cells from any organ
- Near physiological models to dissect complex traits into molecular phenotypes in a reproducible manner
- Biobanking friendly
- Powerful ethical compliant research platform for G-P in livestock & in vivo phenotyping : 3R



Pig tissues: a, b: ileum organoid, c: original tissue staining
d: colon organoid, e: duodemun organoid, f: lung organoid

Pigs as biomedical models

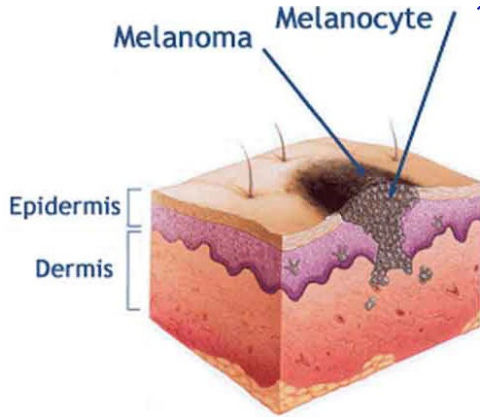
INRA
SCIENCE & IMPACT

Skin colour :

Coat colour :

Cutaneous melanoma in Man : Public health concern

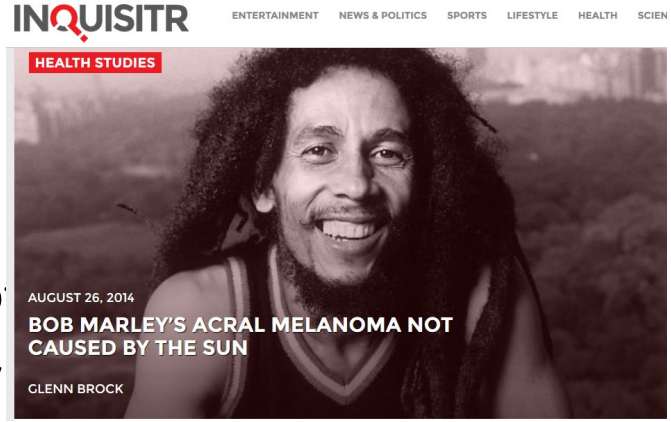
- Malignant tumour derived from melanocytes



- Highest rise in incidence rates since 1970
- 133 000 new cases/year (1500 in France)
- <5% of cutaneous cancer but 83% deaths

Intermittent exposure, chronically sun damaged skin or not!

- 1st most common cancer in 25-30 years old group



- 90% sporadic, 10% familial occurrence

Risk factors:

Environment : exposure to UV radiation, pho

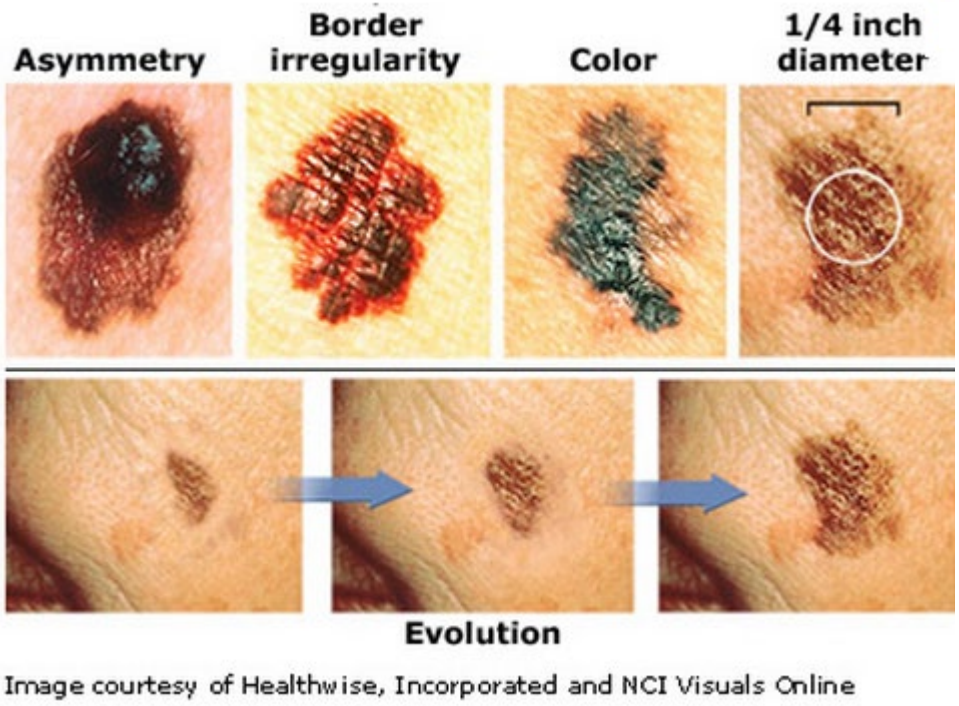
host : genetic background, nb of nevi, skin ty

CDKN2A, *CDK4* (40% familial cases), *MC1R* (low risk)

Cutaneous melanoma : diagnosis

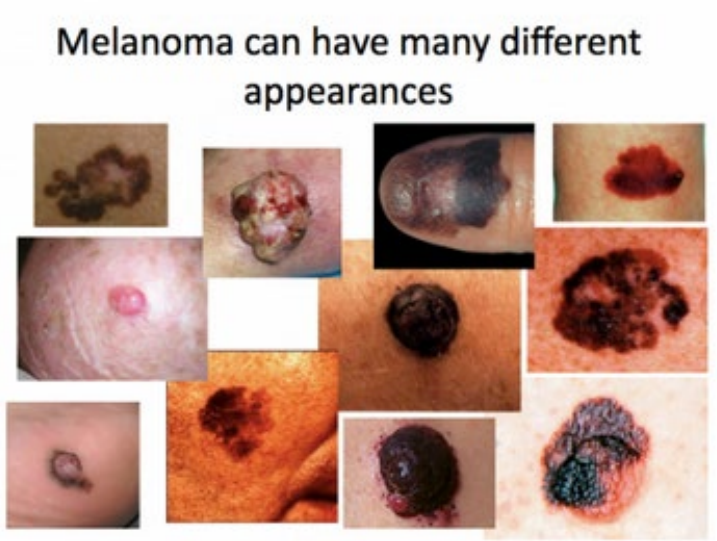
Clinical heterogeneity

ABCDE rule: JAMA (**2004**), 292:2776



Combined neoplasms are often observed

By **Donna Moncivaiz**, *March 30, 2015 at 9:16 am*



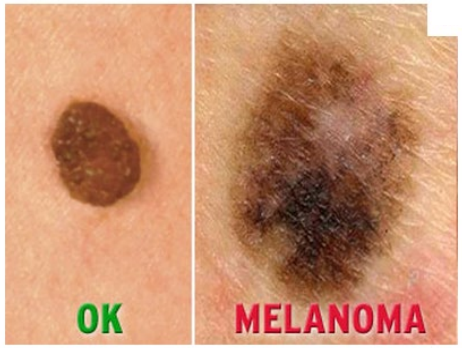
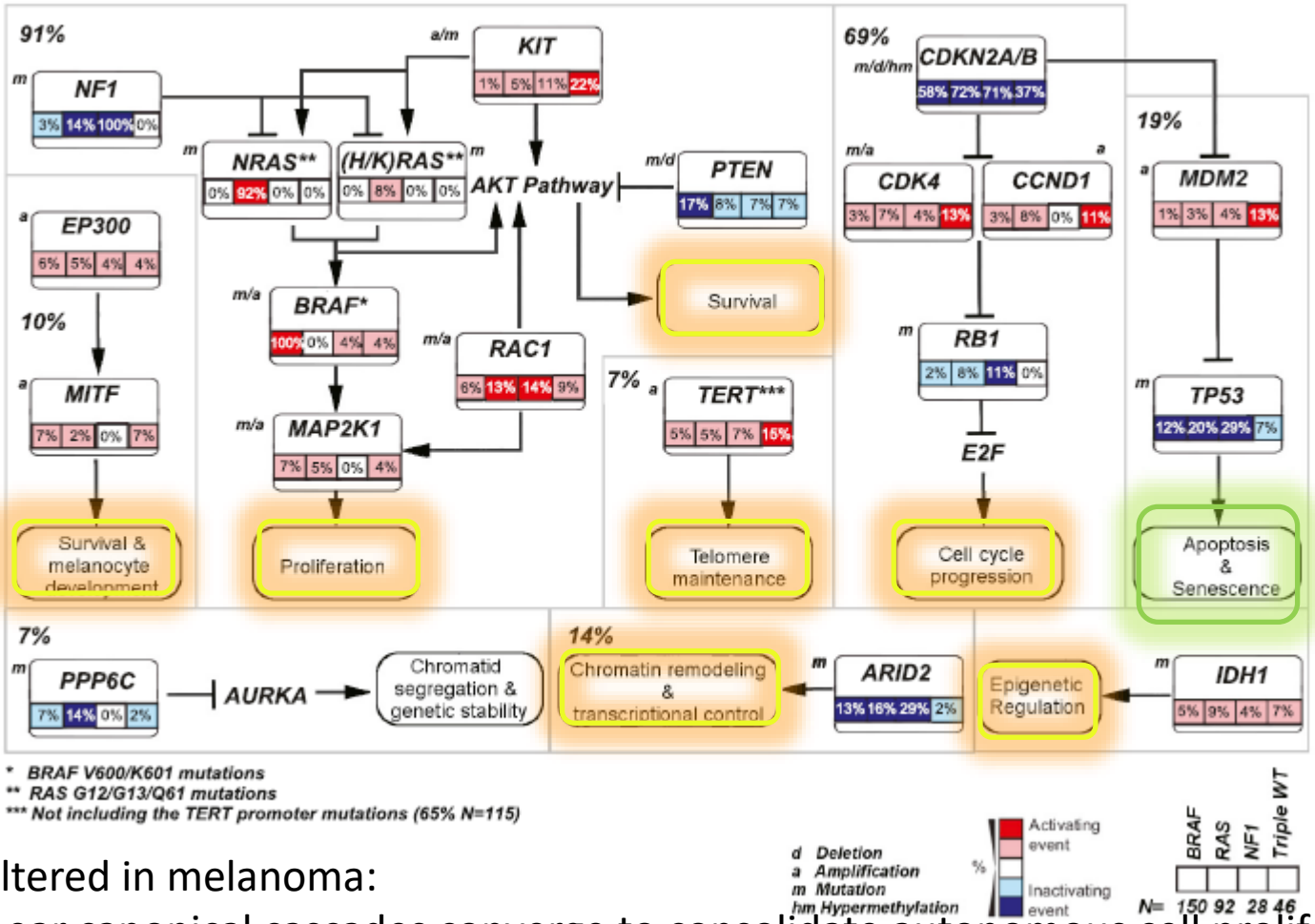


Photo website Utah research center

Genomic classification of cutaneous melanoma

The Cancer Genome Atlas Network, Cell 161: 1681, 2015



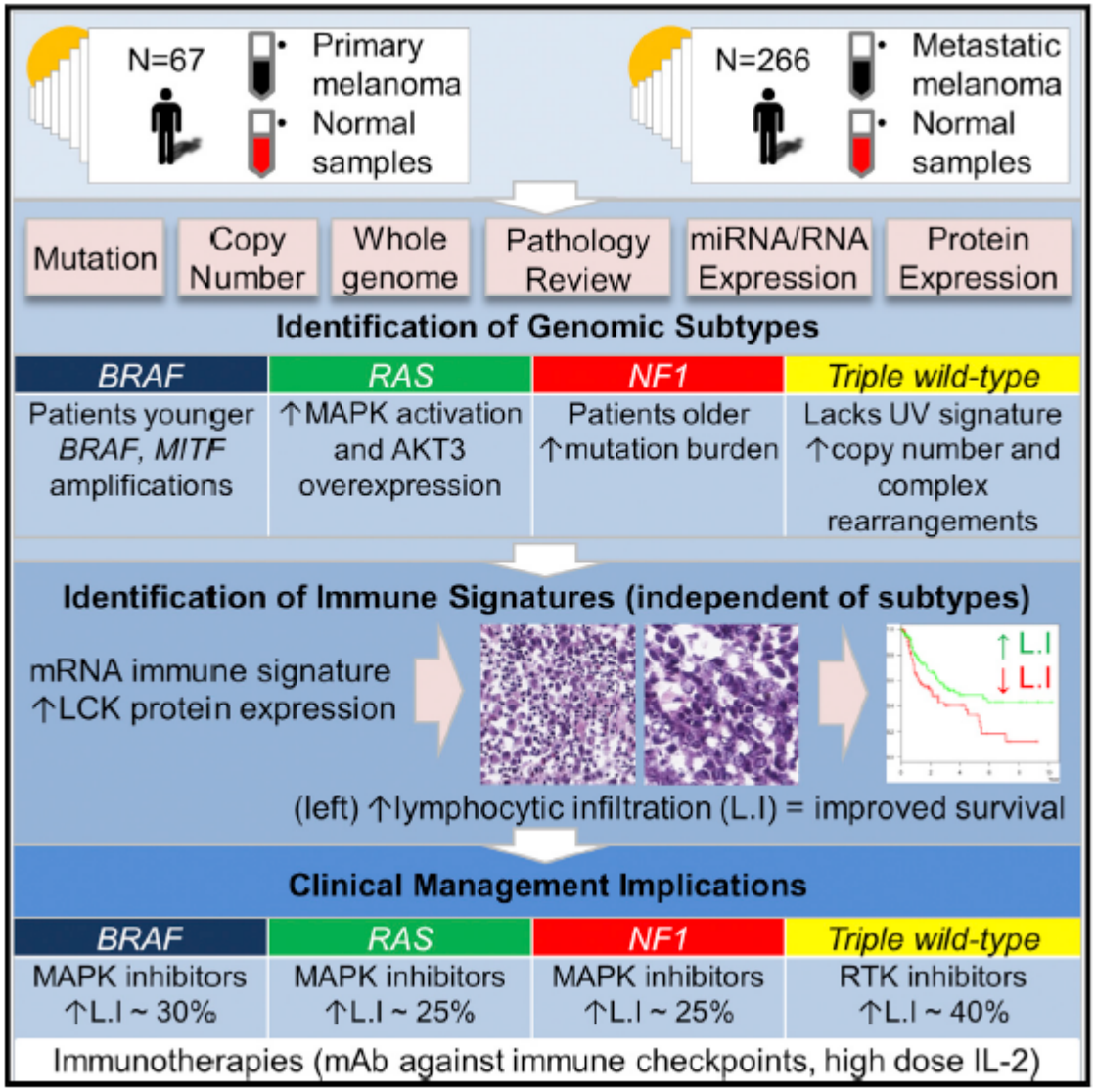
Pathways altered in melanoma:

Multiple linear canonical cascades converge to consolidate autonomous cell proliferation.

Cancer regression would gain from reactivation of apoptosis and senescence.

Genomic classification of cutaneous melanoma

The Cancer Genome Atlas Network, Cell 161: 1681, 2015



Prognosis correlated only to tissue lymphocytic infiltration

Available treatments

1. Excision surgery — 4 Stages I & II : no metastases → Excision ☺
   III & IV : metastases detected → bad prognosis
2. Chemotherapies : (dacarbazine) low response rates and low survival

3. New therapeutic strategies :

   Gene targeted therapies : Kinase inhibitors

   a. BRAF inhibitors (vemurafenib, dabrafenib, sorafenib)
   b. MEK inhibitors (trametinib)

   rapid response, last 6-9 months, need other therapies

   side effects: arthralgia, fatigue, nausea, rashes, photosensitivity, keratoacanthoma,pyrexia

   Stimulation of immune responses: Immune checkpoint blockers

   Benefit only 15% of patients but no available biomarker to identify them

   a. Anti Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (ipilimumab)
   b. Programmed-death-1 (PD1) (nivolumab) and its ligand (PD1L) (pembrolizumab)

   Slow but long term remission up to 5 years

   side effects: toxicity to the bowel, lung and liver with potential life-threatening colitis, pneumonitis, hepatitis, need high doses steroids, fatigue, skin rashes, vitiligo

Available treatments

1. Excision surgery — 4 Stages I & II : no metastases → Excision ☺
   III & IV : metastases detected → bad prognosis
2. Chemotherapies : (dacarbazine) low response rates and low survival

3. New therapeutic strategies :
   Gene targeted therapies : Kinase inhibitors
   a. BRAF inhibitors (vemurafenib, dabrafenib, sorafenib)
   b. MEK inhibitors (trametinib)

rapid response, last 6-9 months, need other therapies

side effects: arthralgia, fatigue, nausea, rashes, photosensitivity, keratoacanthoma,pyrexia

Stimulation of immune responses: Immune checkpoint blockers

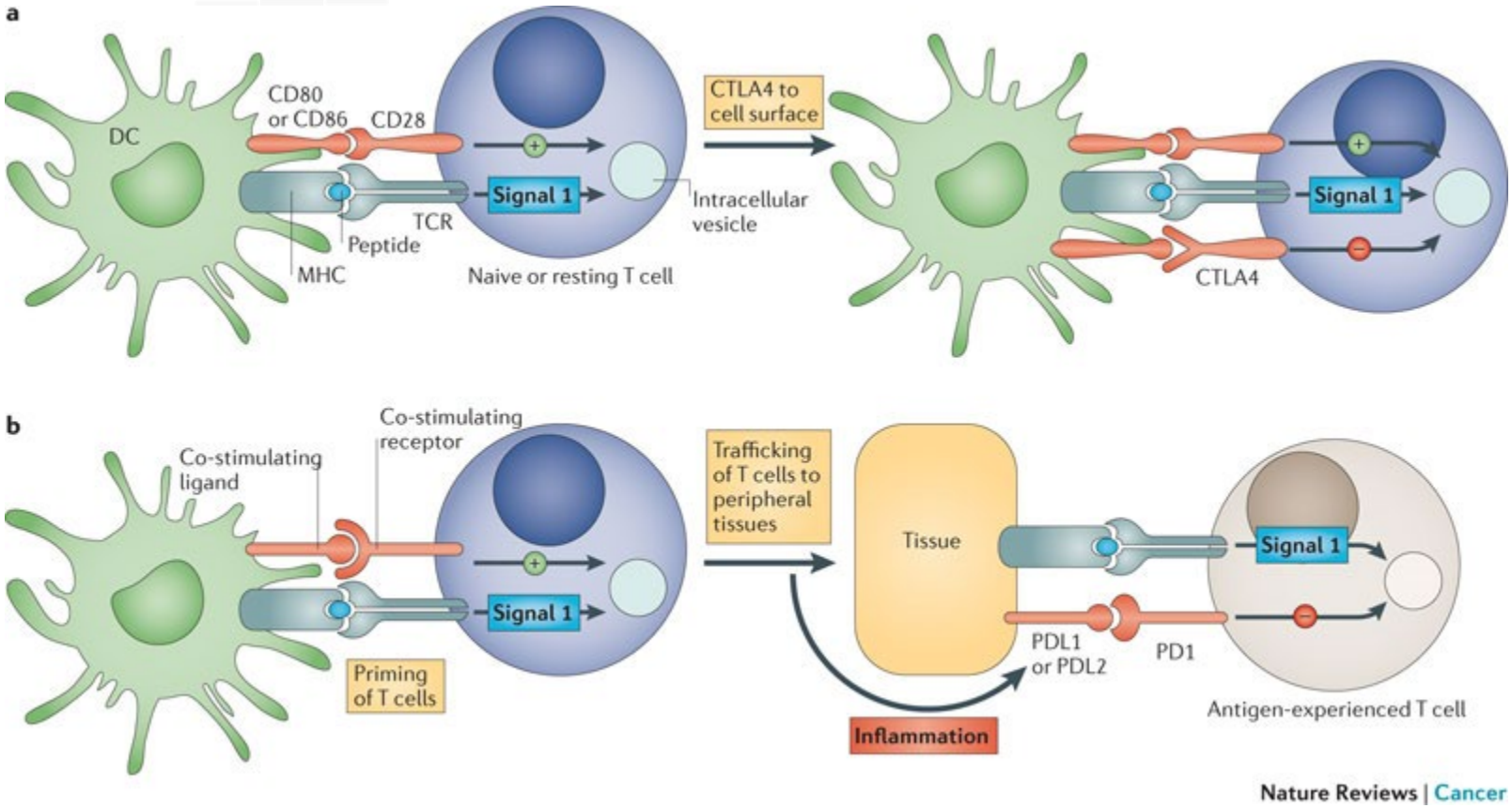
Benefit only 15% of patients but no available biomarker to identify

a. Anti Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antib
b. Programmed-death-1 (PD1) (nivolumab) and its ligand (PD1L) (

Slow but long term remission up to 5 years

side effects: toxicity to the bowel, lung and liver with potential life-threatening colitis, pneumonitis, hepatitis, need high doses steroids, fatigue, skin rashes, vitiligo

Immune checkpoints regulate different components in the evolution of an immune system



Non human spontaneous melanoma models

Induced

By UV / carcinogens

Xyphophorus, opossum, mouse, hamster, rat, guinea pig, gerbil

By transgenesis

Mouse

Spontaneous

Xyphophorus (rare)

Rats (2-4%)

Hamster

Horse (80% grey horses >15 years old)

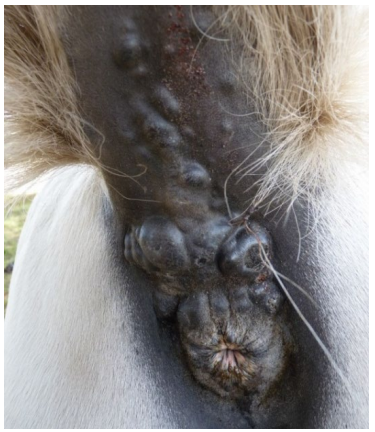
Dog (4-10%)

Angora Goat (rare)

Cattle (< 2%)

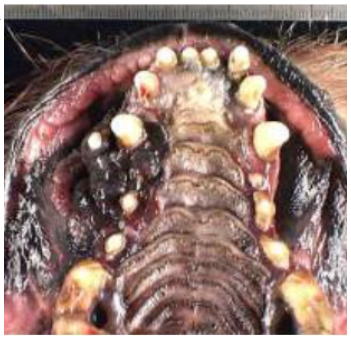
Cat (1%)

Pigs



Perineal melanoma Grey horse

Source : www.nadis.org.uk



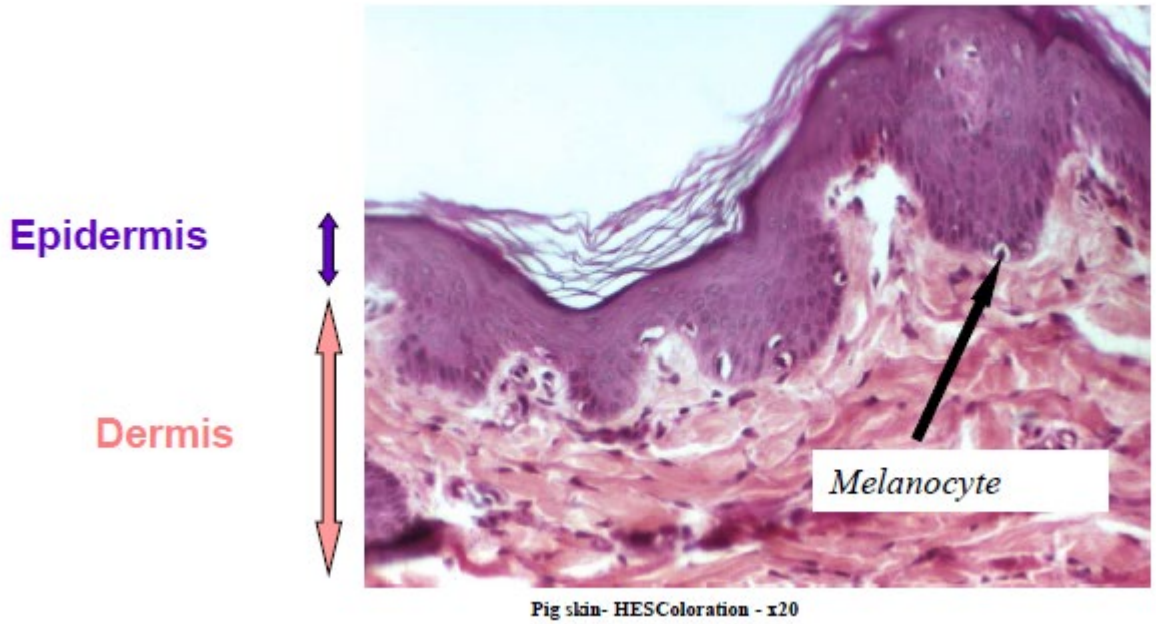
Dog mouth melanoma

Source :Unité d'histologie et d'anatomie pathologique, ENVA

Animal models are a good opportunity to study oncogenesis, for testing new therapeutic approaches

Pig skin models human skin

Similar : organisation (dermis and stratified epidermis)
position of melanocytes
amounts of hair



Pig skin offers a well-developped model for skin pathologies, including melanoma

Pig immune system models human's

Swine immunome 2013:

Pigs & humans share 42 genes not found in mice
Mice & humans share 14 genes not found in pigs

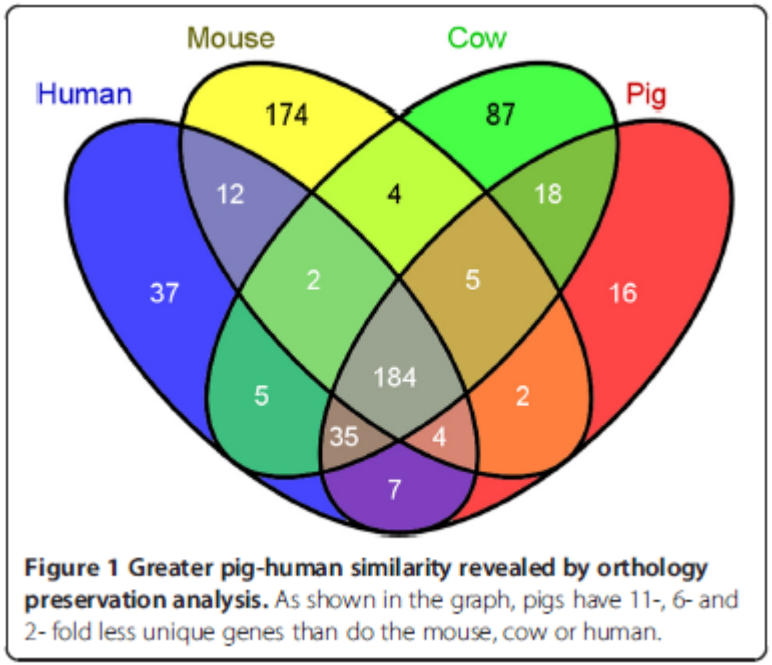


Figure 1 Greater pig-human similarity revealed by orthology preservation analysis. As shown in the graph, pigs have 11-, 6- and 2- fold less unique genes than do the mouse, cow or human.

Frequency of gross protein structural preservation between human and pig for immune related genes is nearly twine that of mouse to human and pig to mouse (Dawson et al, 2016)

Dawson et al., BMC Genomics 2013

Melanoma development in MeLiM

Inherited

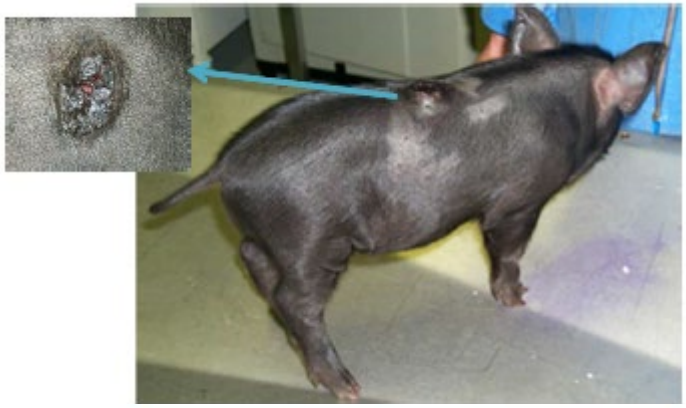
3 types of pigmented cutaneous lesions: flat, raised and small , exophytic melanoma

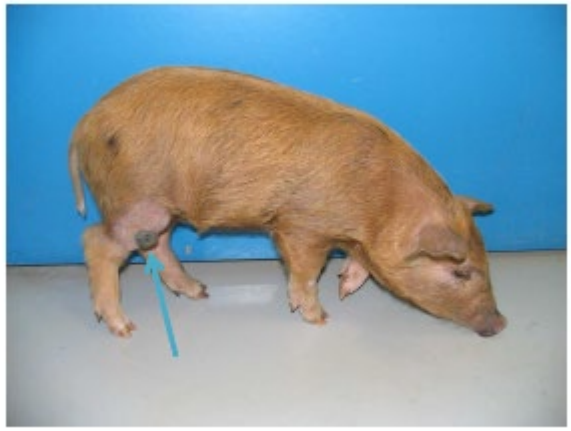
Outbreak: *in utero* or during the first 3 months

Localisation : variable

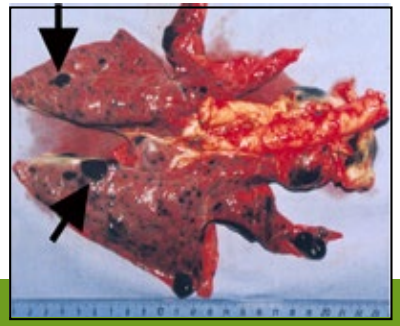
Multiple lesions : frequent
Ulceration : frequent
Metastases : draining lymph nodes, lungs, spleen, liver, digestive tract only on melanoma affected pigs

Mortality: 4%





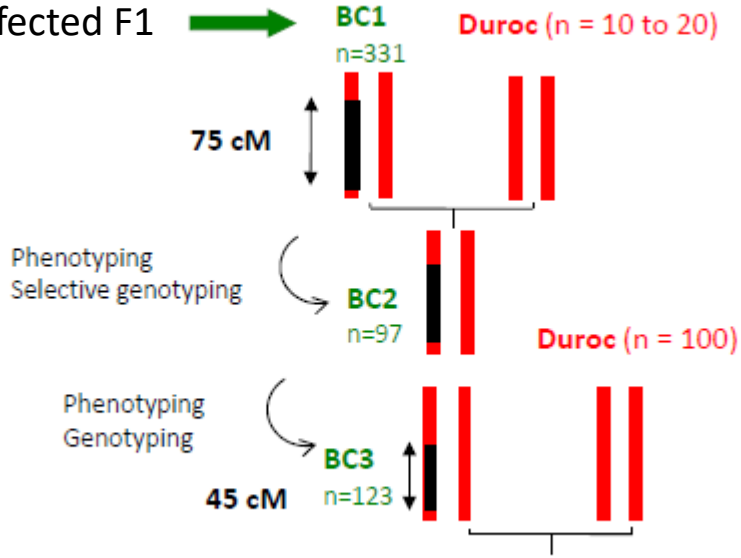
Lung melanoma metastases in a young MeLiM



Vincent-Naulleau *et al* PCR 2004

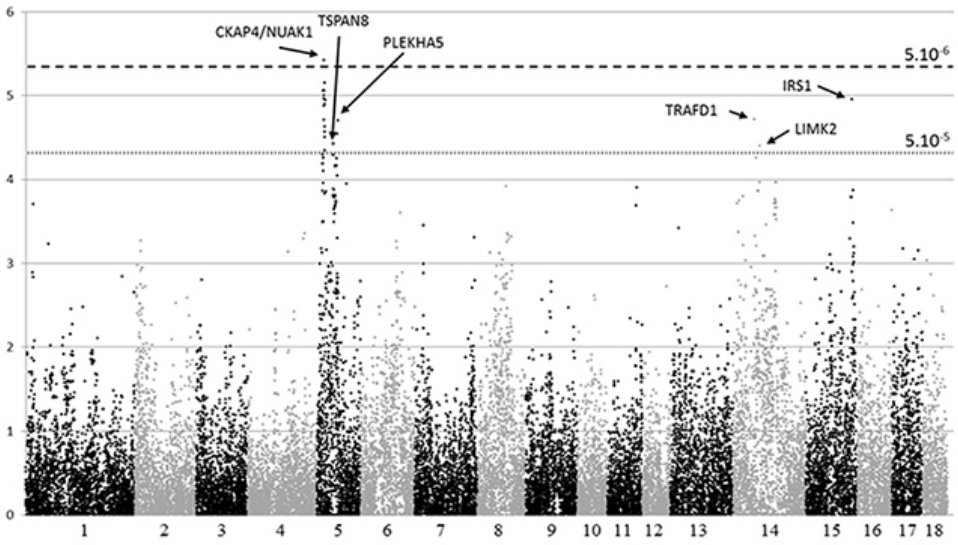
Genome wide association study in MeLiM

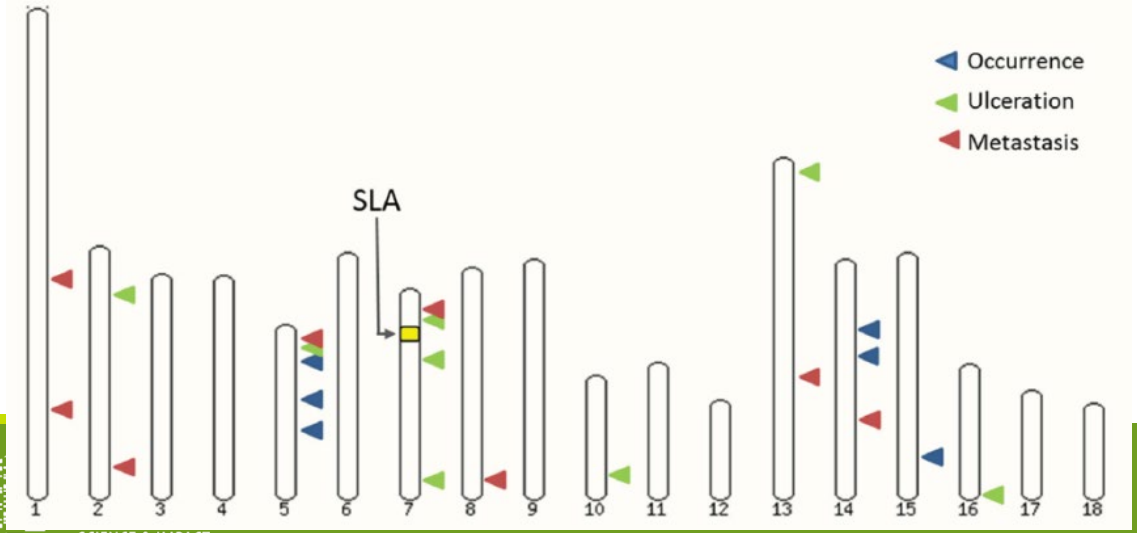
4 MeLiM x 5 Duroc
9 affected F1



BC4 : n=1200 animals

GWAS / 200 BC4 healthy *vs* melanoma





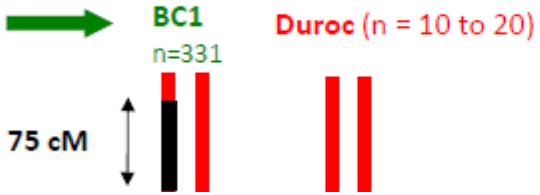
Bourneuf *et al*, Oncotarget 2018

Genetic studies in MeLiM Candidate genes:

Human candidate gene sequencing on QTL detected by linkage analysis did not reveal causative mutations on MeLiM melanoma development: *CDKN2A, CDK4, MITF, KIT*
(Le Chalony et al 2003, Bourneuf *et al* 2011, Fernandez-Rodriguez et al, 2014)

Genome wide association study in MeLiM

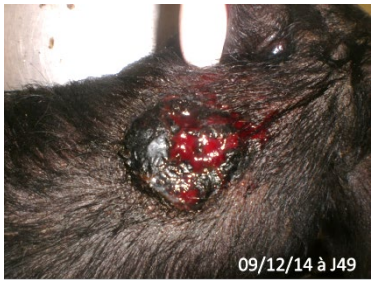
4 MeLiM x 5 Duroc
9 affected F1



60 K (pig SNP consortium)

Loci associated with phenotypes rare or difficult to measure in patients

Example: **Clinical ulceration** of tumors for which lack of clinical data, small cohorts
In pig, association of ulceration with BPAG1-e gene (p corrected=2,75.10^{-5})



BPAG1-e Restricts Keratinocyte Migration through Control of Adhesion Stability

Magdalene Michael[1,2,3], Rumena Begum[1,2], Kenneth Fong[2], Celine Pourreyrone[4], Andrew P. South[4], John A. McGrath[2] and Maddy Parsons[1]

Journal of Investigative Dermatology (2014) **134,** 773–782; doi:10.1038/jid.2013.382; published online 17 October 2013

Bourneuf *et al*,
Oncotarget 2018

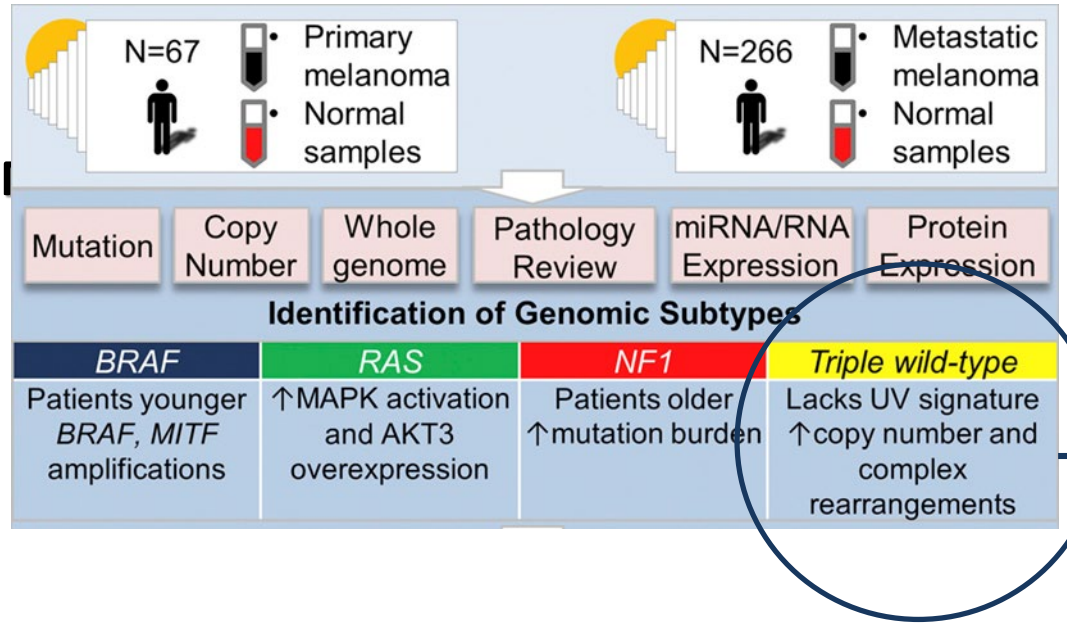
Survey of driver mutations in Melim tumors

Targeted approach on driver mutations found in human melanoma

BRAF, NRAS, HRAS, KRAS, NF1, KIT, PTEN, p53 : **no driver mutation identified**

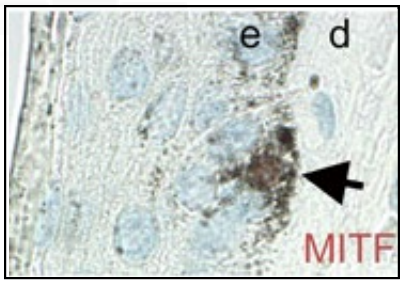
Global approach using exome sequencing of tumor DNA

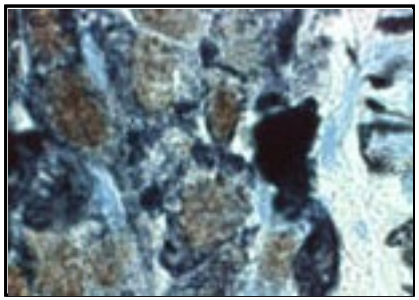
High mutation rate

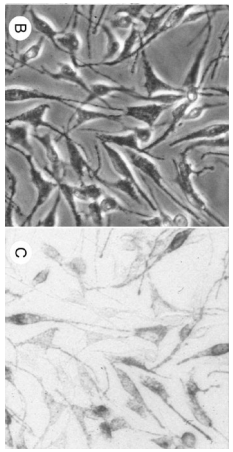


→ Polyploïdy detected in Tumors (Apiou *et al.*, 2004)

Transcriptome analysis in MeLiM uncovers RACK1 mRNA







Julé *et al*
PCR 2003

Cell line of epidermal melanocytes : *PigMel*

1^{ary} Lung metastases cells from MeLiM melanoma

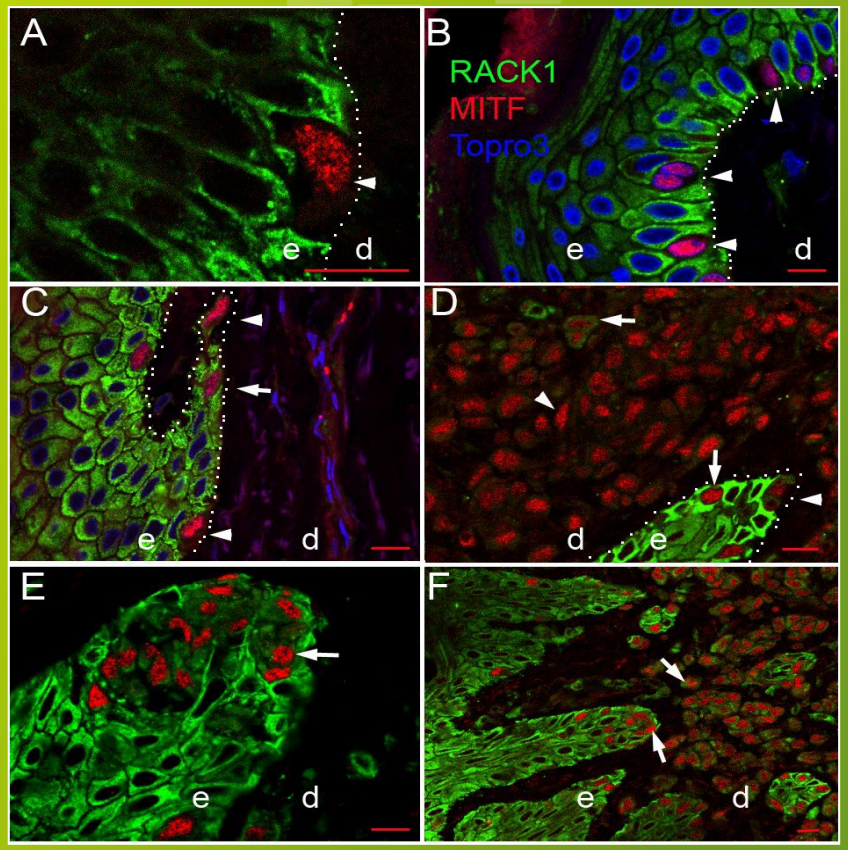
Serial analysis of gene expression

55 genes differentially expressed ($p<0,05$):
G-beta like protein GNB2L1

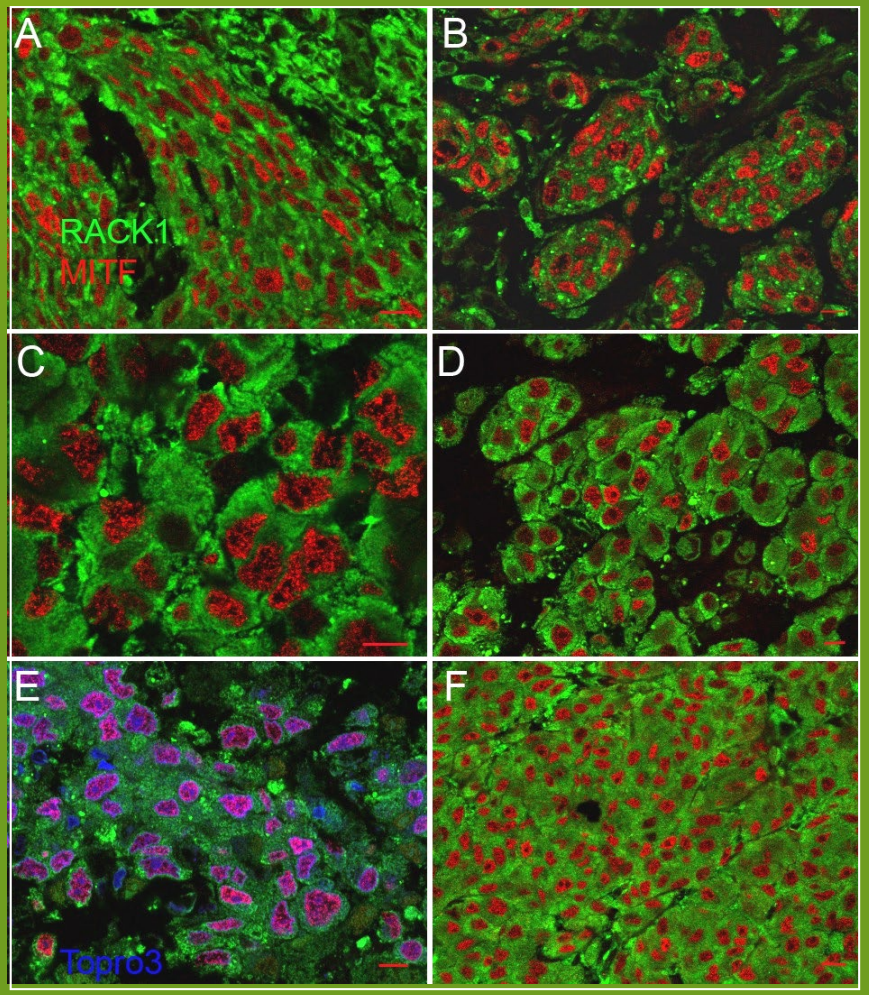
RACK1 overexpressed in MeLiM melanomas

Egidy *et al*, Molec Cancer 2008

RACK1 in human nevi and cutaneous melanomas



Egidy *et al*, Molec Cancer 2008



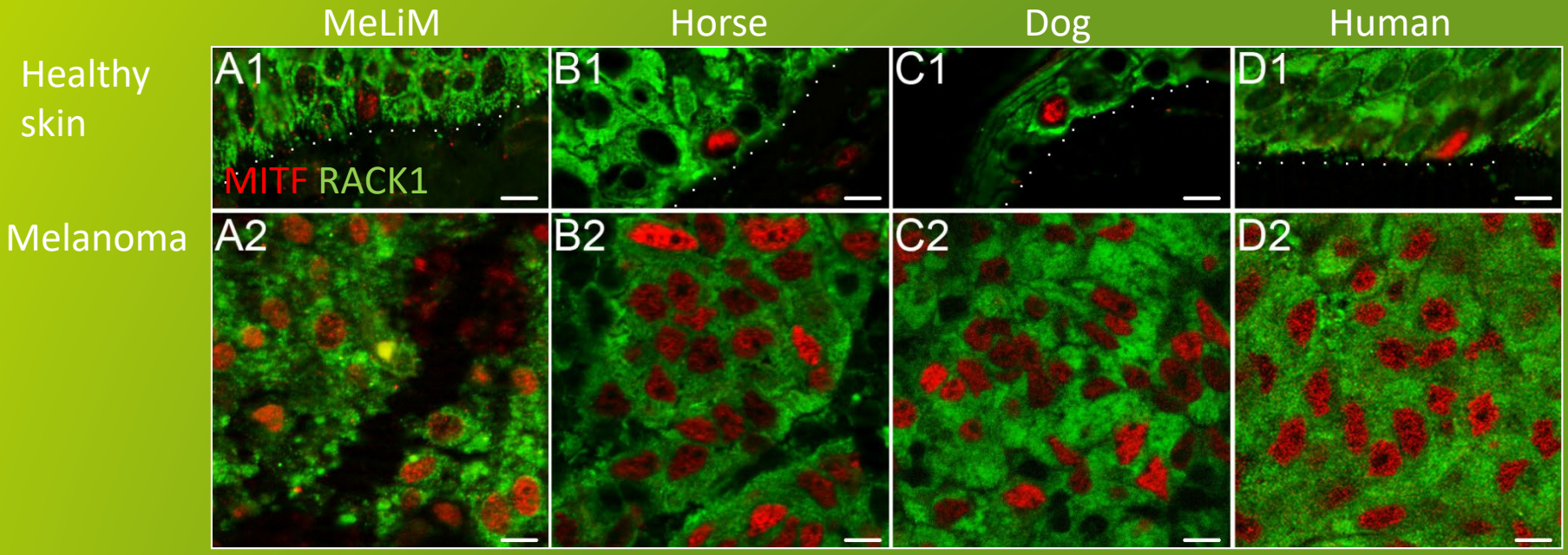
- RACK1 signal is increased in cutaneous and metastatic melanomas
- RACK1 distribution is homogeneous over malignant lesions

INRA SCIENCE & IMPACT

EnvA École nationale vétérinaire d'Alfort

RACK1, diagnostic marker of cutaneous melanomas

- Conserved distribution pattern in melanomas from 4 species



Campagne *et al* BMC Res Vet 2012
Campagne et al, Vet Pathol, 2013

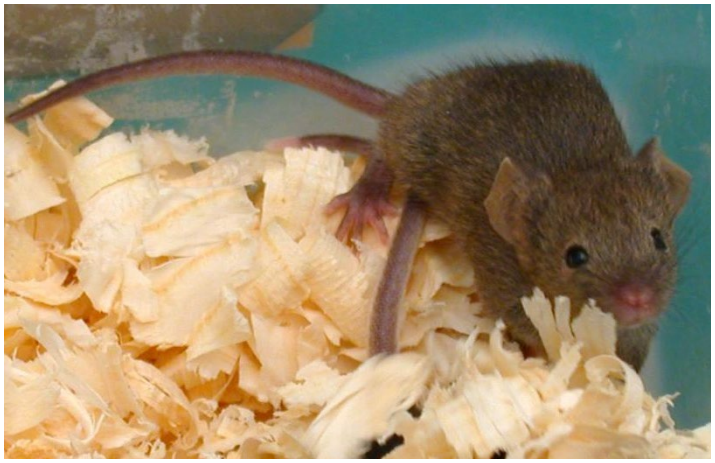
Gene expression profiling in MeLiM led to the identification of RACK1 as potential marker of malignancy for human and veterinary melanocytic proliferations

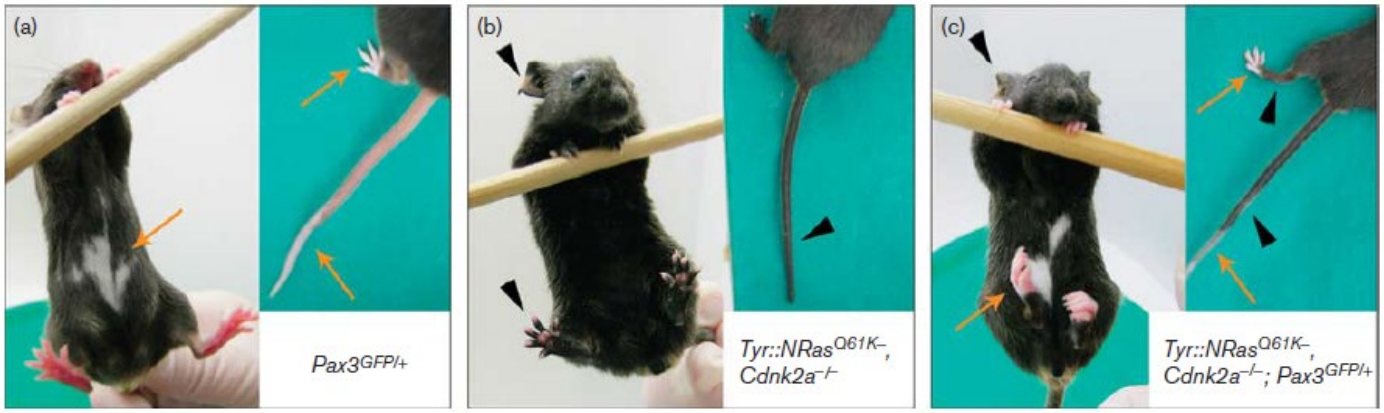
INRA SCIENCE & IMPACT

EnvA École nationale vétérinaire d'Alfort

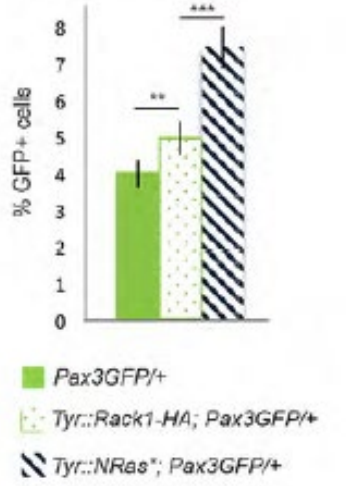
Model of RACK1 overexpression targeted to melanocytes

- *Tyr::*Rack1-HA*; Pax3*$^{GFP/+}$ mouse





Campagne et al. Melanoma Research 2016

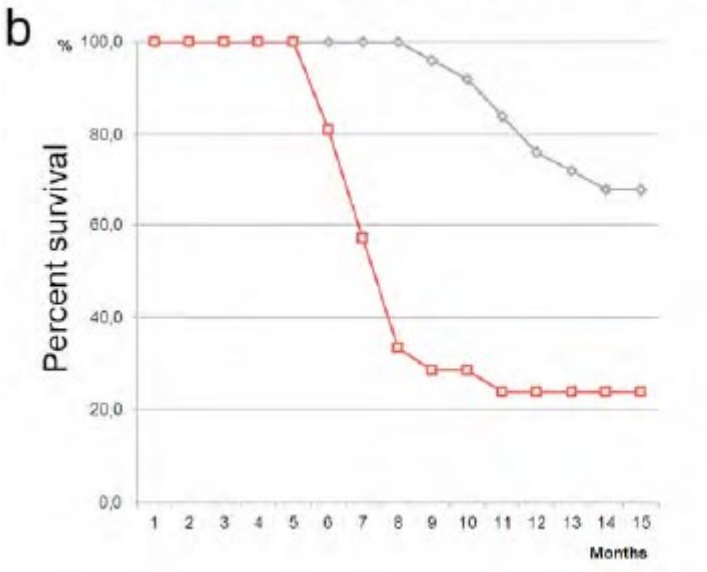


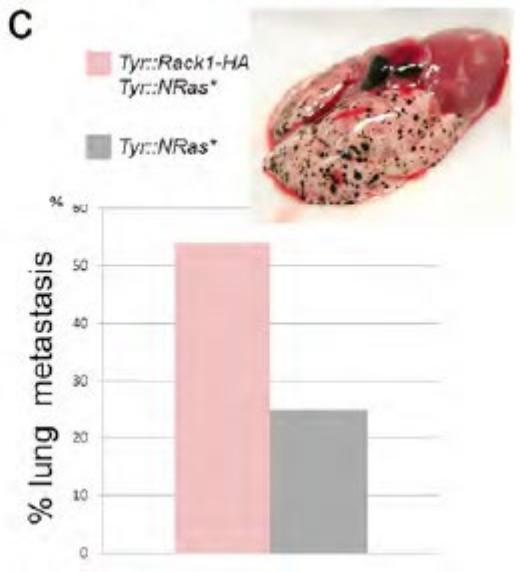
RACK1 overexpression increases neonatal skin melanocyte number

Campagne et al. Cell Signaling 2017

Model of RACK1 overexpression targeted to melanocytes

- *Tyr::*Rack1-HA*; Pax3*$^{GFP/+}$ mouse



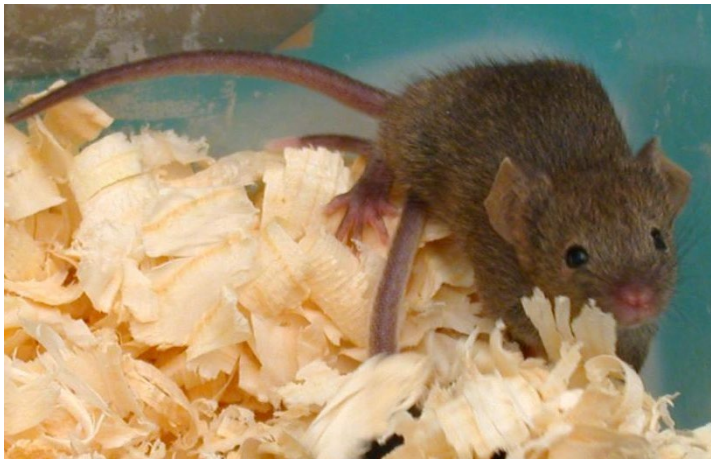


RACK1 accelerates melanoma development

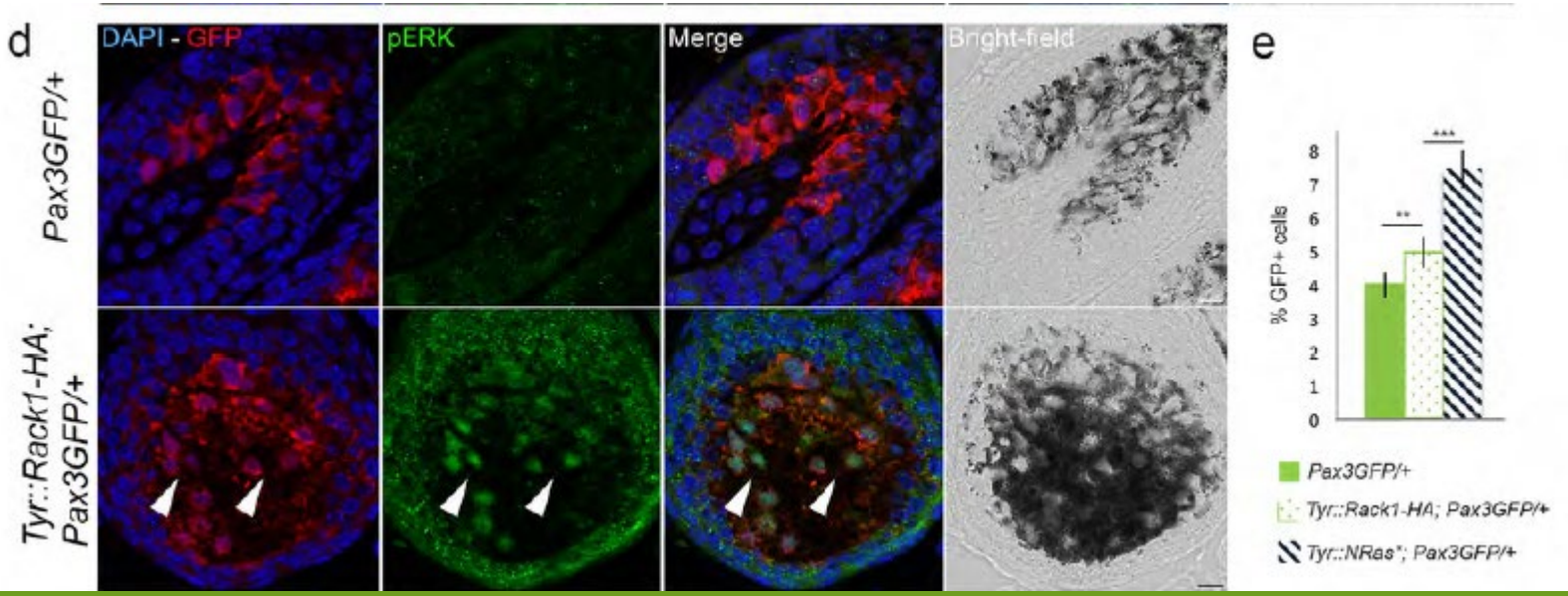
Campagne et al. Cell Signaling 2017

Model of RACK1 overexpression targeted to melanocytes

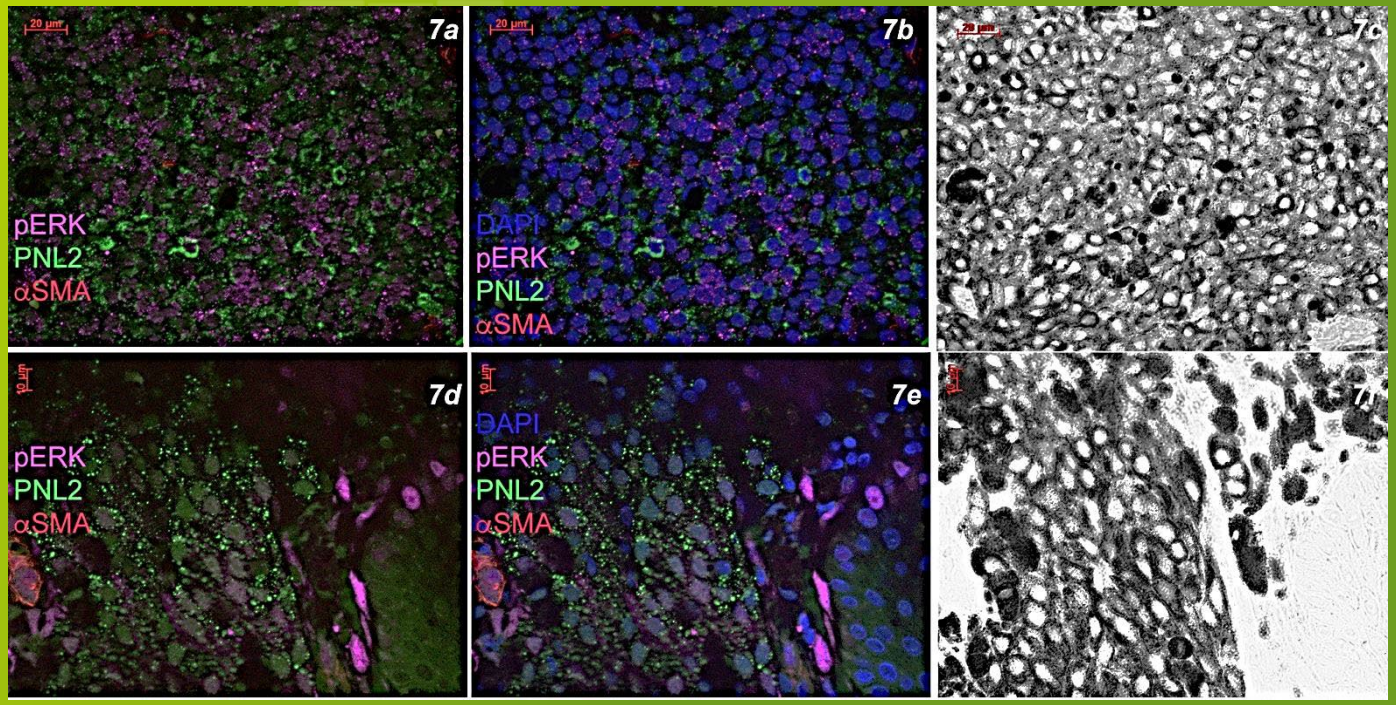
- *Tyr::*Rack1-HA*; Pax3*$^{GFP/+}$ mouse



Campagne et al. Cell Signaling 2017



Proliferative signaling pathways in MeLiM melanomas



Young tumors
(pigs <21 days)

«Older» tumors
(>21 days)

pERK : activation MAPK-ERK pathway
PNL2 : marker of melanocytes/melanoma cells
aSMA : marker of vascular cells
DAPI: nucleus

Similar results with pAKT, pJNK (+ other pathways)

→ Proliferation signals activated in young tumors, less as tumors progress

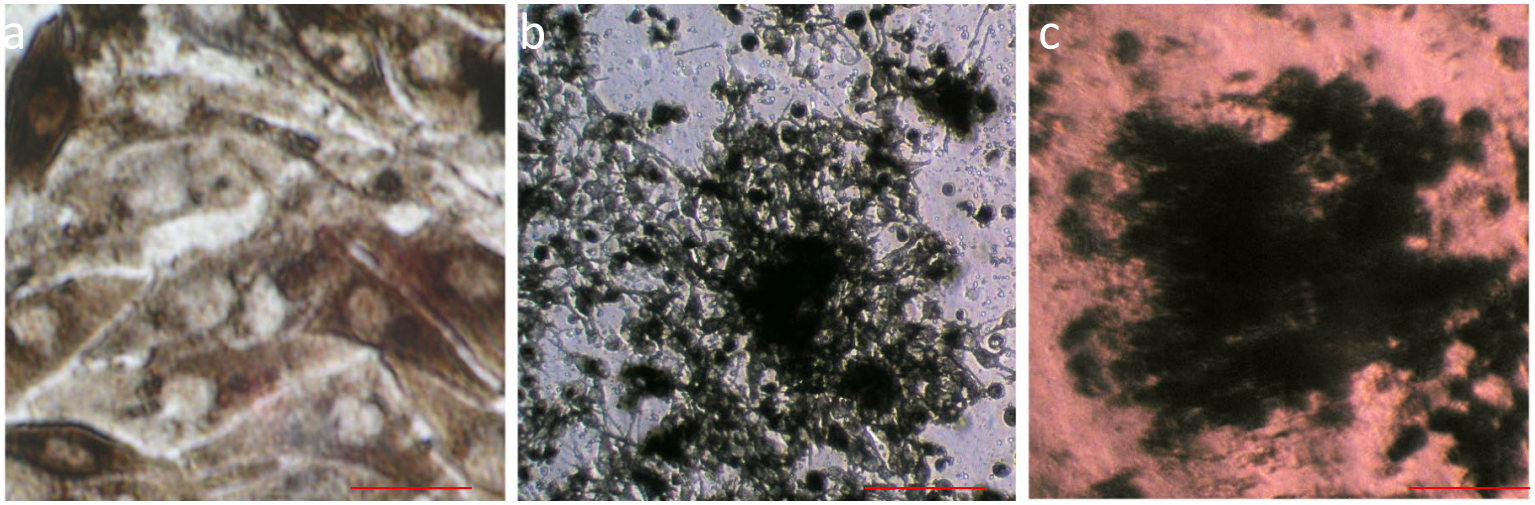
Egidy *et al*, in preparation

Malignancy traits in MeLiM melanoma in vitro and xenotransplantation assays

MeLiM melanoma cells in culture Bar:10µm.

Foci at confluent growth 100µm.

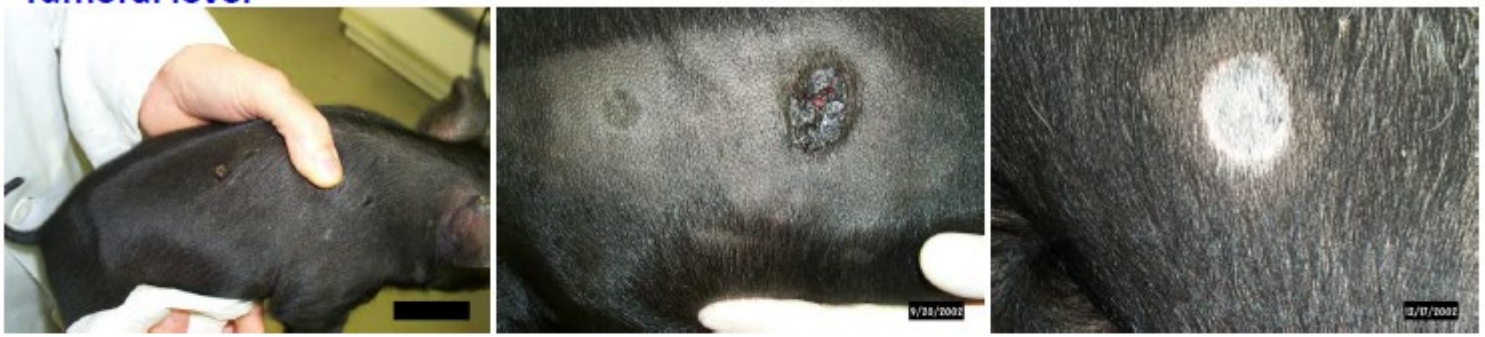
Soft agar colony formation 50µm.



➢ **MeLiM melanoma cells are contact insensitive, anchorage independent and serially transplantable in as transformed cells**

Melanoma development in MeLiM

Tumoral level

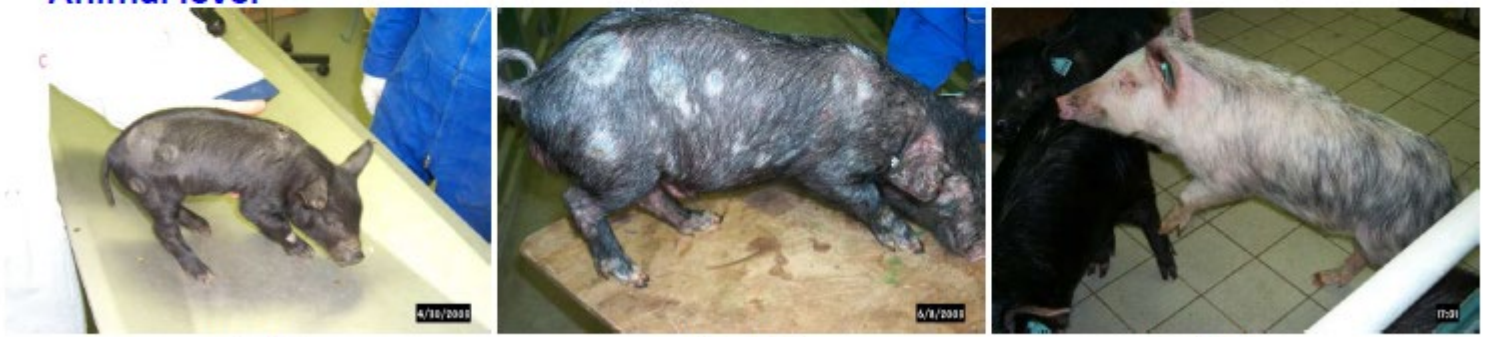


D+7 Growing ulcerated tumor (1 cm)

D+36 Ulcerated exophytic tumor (4 cm)

D+96 Regressed lesion with **depigmentation of hair and skin**

Animal level



D+15

D+113

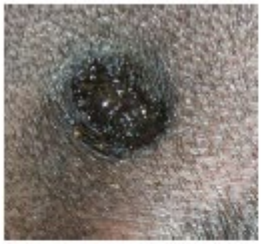
D+214

Depigmentation

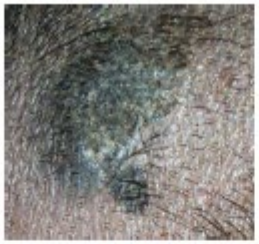
In MeLiM minipigs, melanomas spontaneously regress

Vincent-Naulleau *et al,* PCR 2004

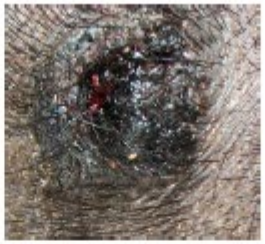
The MeLiM model : time course of the disease



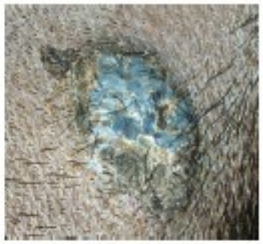
Growth, ulceration



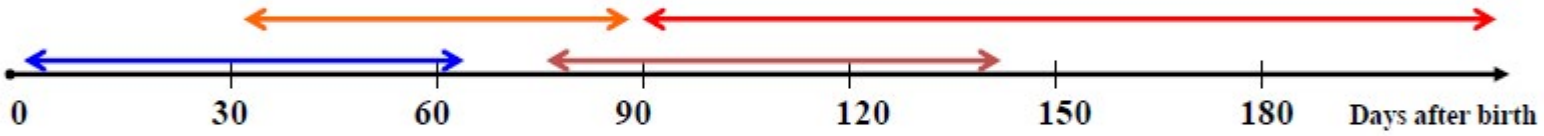
Surface flattening, greying*

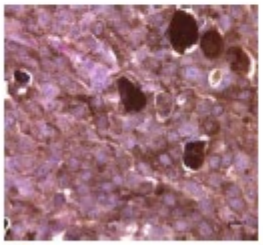


Relapse, increasing size, ulceration

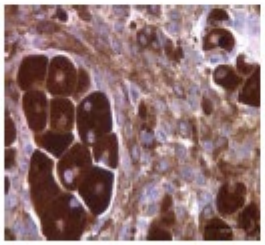


Surface drying and smoothing, bleaching, hair/skin depigmentation

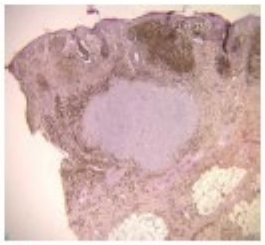




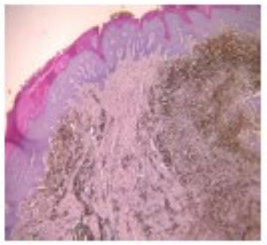
Tumour area composed of melanoma cells with scattered histiocytes



Dense pigment-laden histiocytes infiltrate



Tumour recurrent area



Melanoma cells disparition, lymphoid infiltrate, foci of fibrosis, increased vascularity

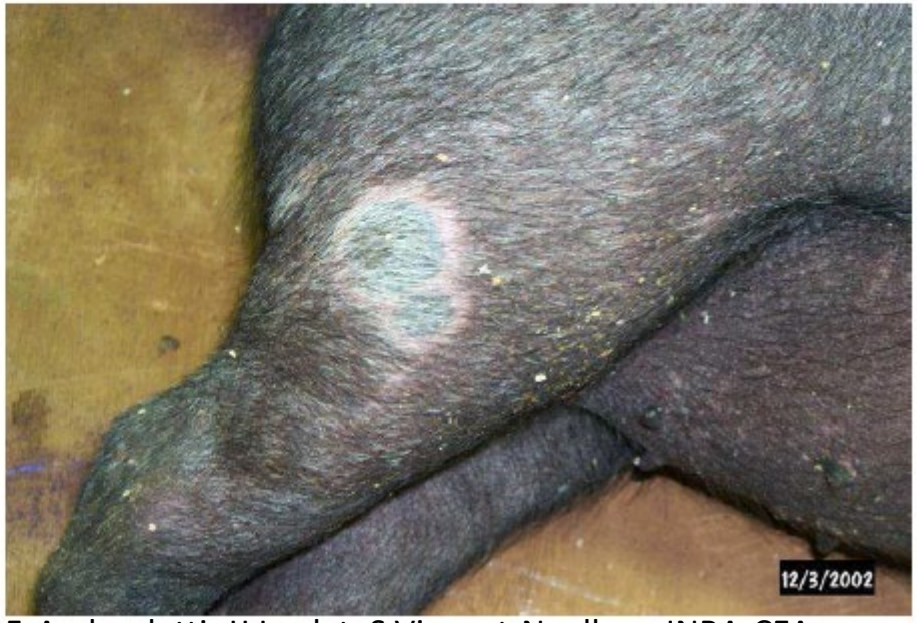
S. Vincent-Naulleau et al. Pigment Cell Res. 2004, 16: 1-12

Melanoma regression in MeLiM

Spontaneous regression (SR) is defined as the disappearance of the malignant tumor mass without treatment or as a consequence of an indirect action (i.e. treatment against another disease or symptoms) (William Coley, 1891).

Melanomas begin to dry and reduce in size, then become greyish and their surface flattens and smooths

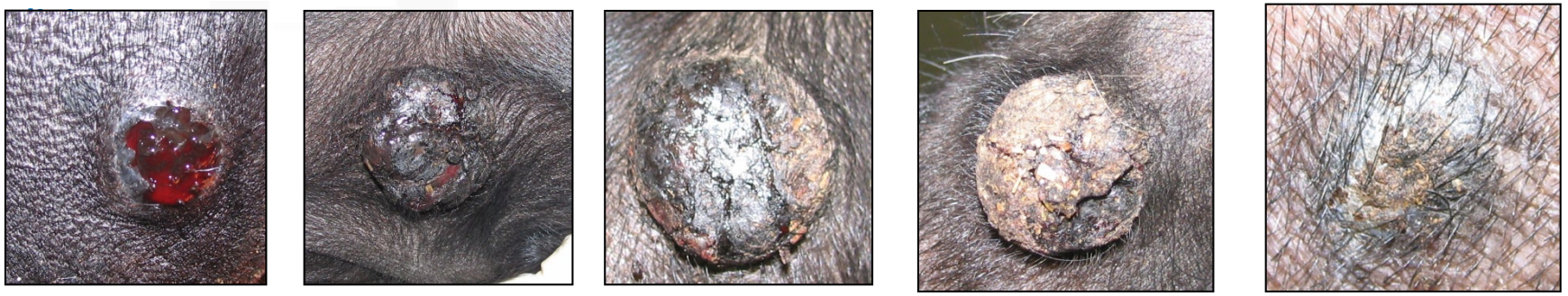
Depigmentation of skin and hair



F. Andreoletti, JJ Leplat, S Vincent-Naulleau INRA-CEA

Molecular mechanisms of regression

Clinical phenotypes



Cell cycle, Inhibition apoptosis, proliferation ↓

CDC2,CCNB1,**CDC6**, KIFs, **BIRC5**, TOP2A, **BUB1**, MCMs

Immune response ↑

TCRs,KLRC,CD83,CD86, **SLA cl I et II**, Igs, CSF1, **CCL5**,**CCR1**, IL15

Pigmentation ↓

PAX3, MITF, SLC24A5, TYR, SLC45A2, SILV,OCA2

Array results

Cell movement, Invasion ↑

FN1, ITGB2, ITGB3, CDH1, MMPs, ICAM2

Rambow *et al,* Neoplasia 2008

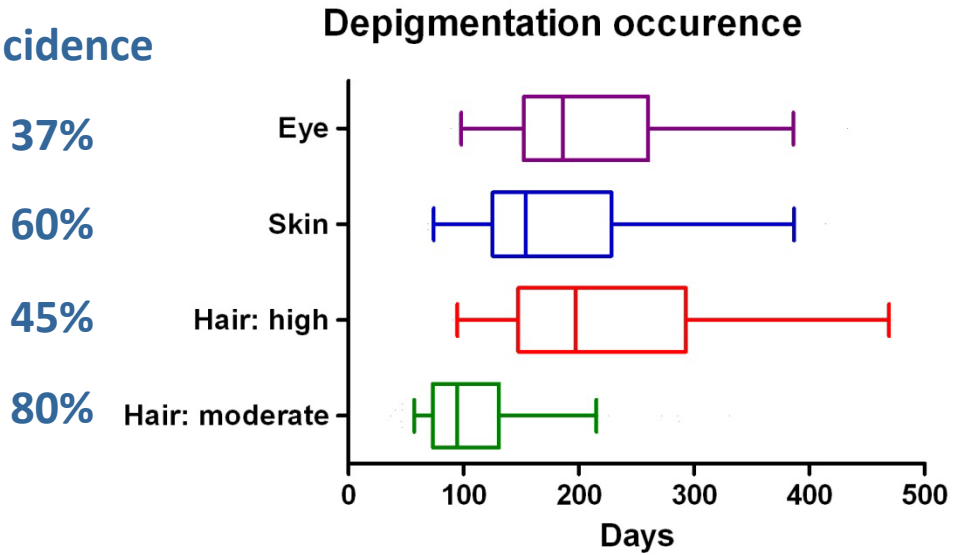
Depigmentation on MeLiM with melanoma regression

- Follow up of clinical observations on hair, skin, eyes depigmentation in minipigs resembling vitiligo in humans

No depigmentation

Moderate

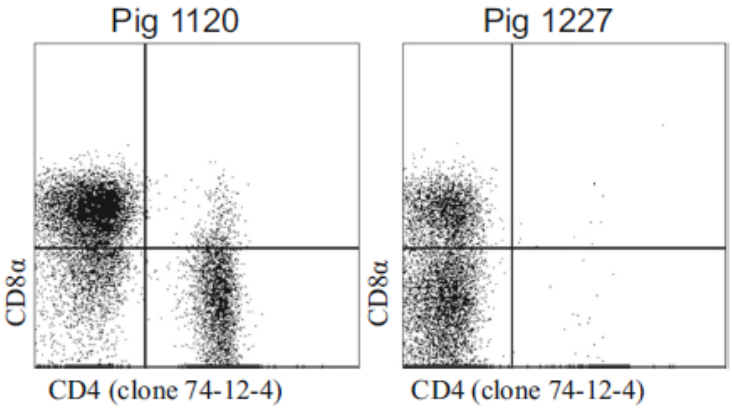
High



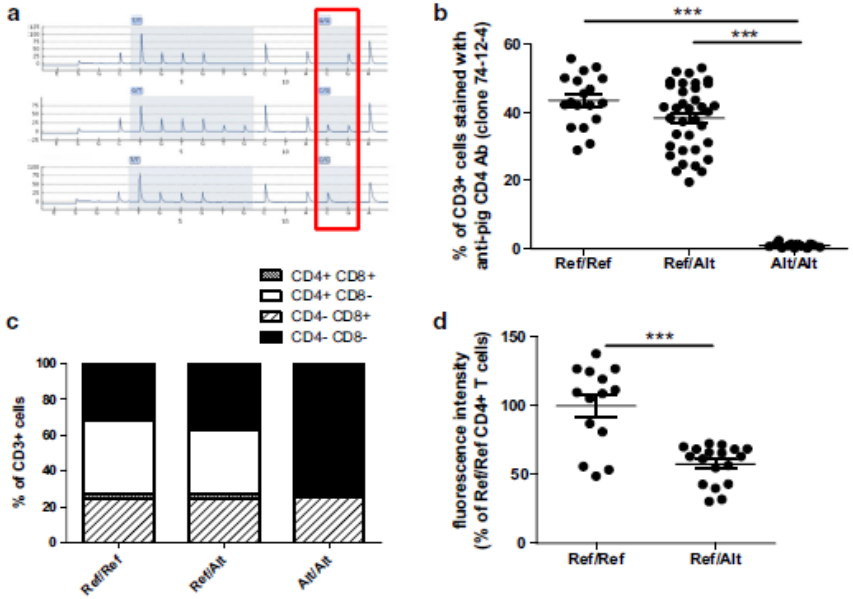
Blanc *et al*, Immunogenetics 2017

CD4 alleles may loose antibody binding

Some MeLiM did not show CD4 staining




```
Ref Haplo.1    24 GTQEKYLVLGKAGDLAELPCHSSQKKNLPFNWKNSNQTKILGGHGSFWHT 72
Ref Haplo.2    24 ---------------------------------S---------------K 72
Ref Haplo.3    24 ---------------------------------------------------K 72
Alt Haplo      24 -------------------------------S----D-I---RS-RNL--K 72
CD4.2          24 -------------------------------S----D-I---RS-RNL--K 72
CD4.B          24 -------------------------------S----D-I---RS-RNL--K 72
                  ********************************.**.*:* *** .* .:**.
```



A CD4 epitope deficiency is produced by a specific haplotype in MeLiM

CD4 alleles may loose antibody binding

SCANDINAVIAN JOURNAL OF Immunology
AN INTERNATIONAL MONTHLY JOURNAL
Explore this journal >

Identification of a Type 1 Diabetes-Associated *CD4* Promoter Haplotype with High Constitutive Activity

O. P. Kristiansen, A. E. Karlsen, Z. M. Larsen, J. Johannesen, F. Pociot, T. Mandrup-Poulsen The Danish IDDM Epidemiolog

First published: 2 June 2004 Full p

0022-1767/92/14810-3195$02.00/0
THE JOURNAL OF IMMUNOLOGY
Copyright © 1992 by The American Association of Immunologists
Vol. 148, 3195–3201, No. 10, May 15, 1992
Printed in U.S.A.

CHARACTERIZATION OF A POLYMORPHISM OF CD4 IN MINIATURE SWINE

THORALF M. SUNDT III,* CHRISTIAN LEGUERN,† SHARON GERMANA,* CRAIG V. SMITH,† KAZUAKI NAKAJIMA,* JOAN K. LUNNEY,* AND DAVID H. SACHS†

The Journal of Immunology
Copyright © 1993 by The American Association of Immunologists
Vol 151, 1365–1370, No. 3, August 1, 1993
Printed in U.S.A.

CED Clinical and Experimental Dermatology
Explore this journal >

Extensive Allelic Polymorphism in the CDR2-Like Region of the Miniature Swine CD4 Molecule

Sundt III,* David H. Sachs,† and

Possible association of the *CD4* gene polymorphism with vitil population

M. Zamani, M. A. Tabatabaiefar, S. M P. Mansouri

First published: 19 October 2009 Full publi

ELSEVIER
Veterinary Immunology and Immunopathology
Volume 168, Issues 3–4, 15 December 2015, Pages 176-183

Infant CD4 C868T polymorphism is associated with increased human immunodeficiency virus (HIV-1) acquisition

R. Y. Choi, C. Farquhar, J. Juno, D. Mbori-Ngacha, B. Lohman-Payne, F. Vouriot, G. John-Stewart, K. Fowke

First published: 2 February 2010

Identification of a CD4 variant in Microminipigs not detectable with available anti-CD4 monoclonal antibodies

Tatsuya Matsubara [a], Naohito Nishii [a, b], Satoshi Takashima [b], Masaki Takasu [a, b], Noriaki Imaeda [b], Kayo Aiki-Oshimo [b], Kazuaki Yamazoe [a, b], Yoshie Kametani [c], Asako Ando [c], Hitoshi Kitagawa [a, b]

BMC Veterinary Research
Open Access

A Common *CD4* Gene Variant Is Associated with an Increased Risk of HIV-1 Infection in Kenyan Female Commercial Sex Workers FREE

Julius O. Oyugi, Françoise C. M. Vouriot, Judie Alimonti, Stephen Wayne, Ma Luo, Allison M. Land, Zhujun AO, Xiaojian Yao, Rafick P. Sekaly, Lawrence J. Elliott ... Show more

The Journal of Infectious Diseases, Volume 199, Issue 9, 1 May 2009,

Identification and characterization of two CD4 alleles in Microminipigs

Tatsuya Matsubara[1,2], Naohito Nishii[1,2*], Satoshi Takashima[2], Masaki Takasu[1,2], Noriaki Imaeda[2], Kayo Aiki-Oshimo[2], Kazuaki Yamazoe[1,2], Michinori Kakisaka[3], Shin-nosuke Takeshima[3], Yoko Aida[3], Yoshie Kametani[4], Jerzy K. Kulski[4,5], Asako Ando[4] and Hitoshi Kitagawa[1,2]

Animal Biotechnology ›
Latest Articles

INRA SCIENCE & IMPACT

Giorgia

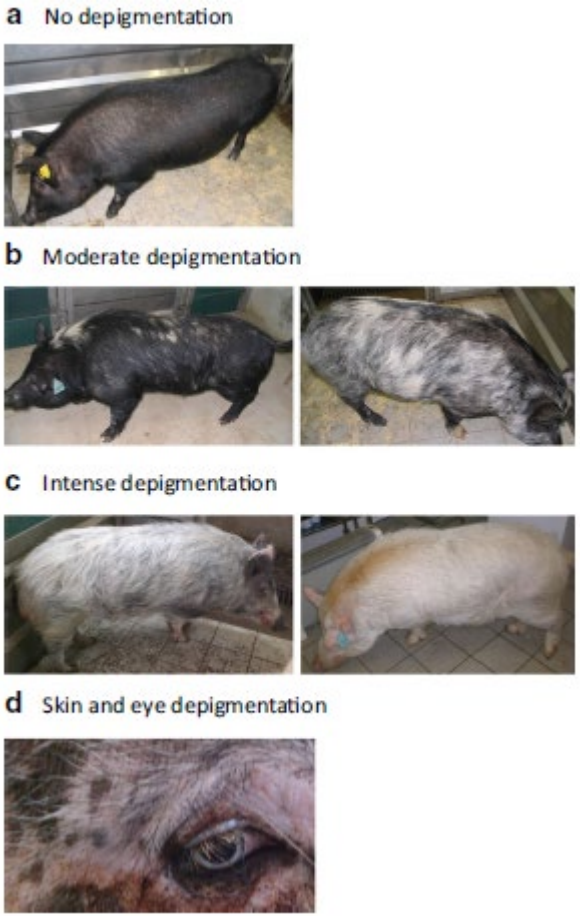
26 Views
0 CrossRef citations

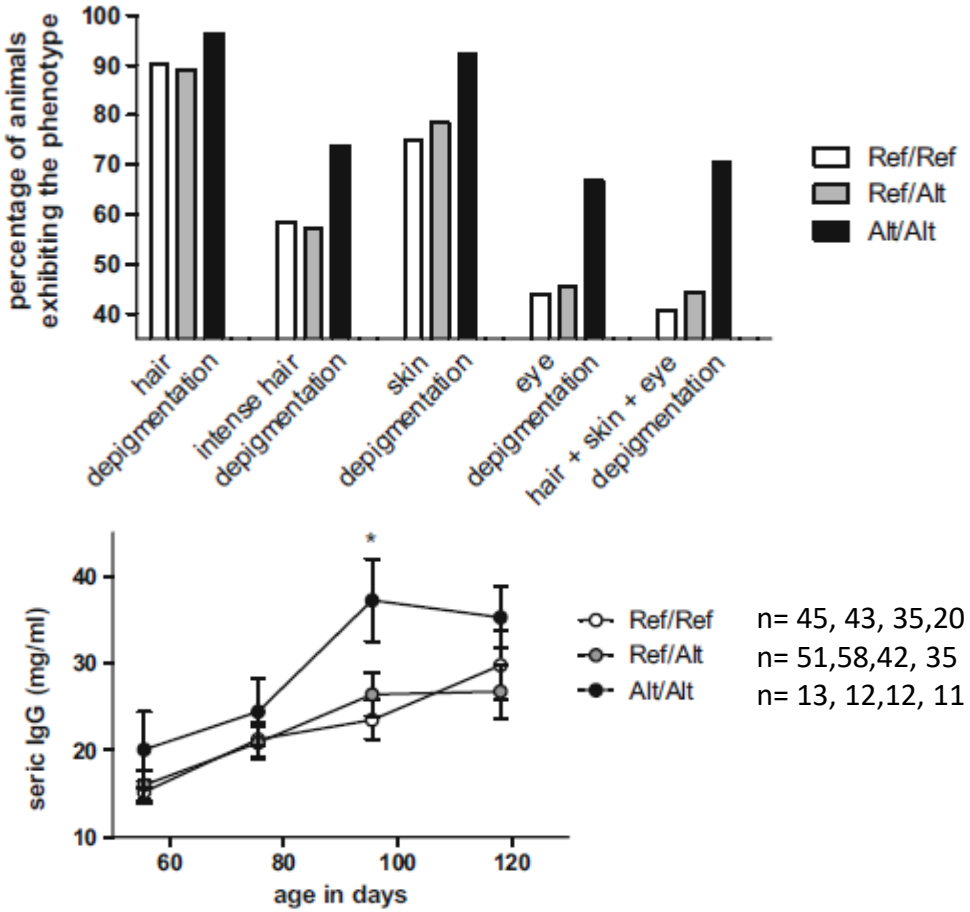
Original Articles

Distribution of the *CD4* Alleles in *Sus scrofa* Demonstrates the Genetic Profiles of Western Breeds and Miniature Pigs

Tomoko Eguchi-Ogawa, Tatsuya Matsubara, Daisuke Toki, Naohiko Okumura, Asako Ando,

Impact of CD4 haplotypes on depigmentation





CD4 is associated with immune response-related phenotypes in MeLiM minipigs

Blanc *et al*, Immunogenetics 2017

Melanoma regression in MeLiM

- Longitudinal transcriptomic studies: regression relates to melanoma cell's cell cycle arrest, & an immune reaction
- Senescence is detected by patches along tumor regression

  ➢ **Regression is partly the result of replicative potential loss in MeLiM**

- Regression goes along with depigmentation (*CD4* locus specific haplotype)
- Blood antibodies against porcine melanocytic and melanoma antigens appear during regression

  ➢ **Regression is partly the result of immunoreactivity in MeLiM**

Patients could not just survive with cancer, but live without cancer

Regression
WANTED

Characterisation of a minipig spontaneous regression model with no invalidating adverse effects to find novel pathways of immune activation

Is this capacity to regress transposable to human cancer?

INRA
SCIENCE & IMPACT

Characterisation of a minipig spontaneous regression model with no invalidating adverse effects to find novel pathways of immune activation

Functionally test:

1. whether regression is specific to MeLiM mutations
2. and/or dependent on the immune system
3. Identify novel markers of interaction immune / tumor cells
4. compare to human melanoma cells behaviour

Development and integration of new experimental models relevant for research in oncology: Optimisation of the 3R principle

INRA SCIENCE & IMPACT

PLAN CANCER 2014-2019

Inserm La science pour la santé From science to health

Oncogene (2016) **35,** 2166–2177

www.nature.com/onc

OPEN

ORIGINAL ARTICLE

Targeted inhibition of metastatic melanoma through interference with Pin1-FOXM1 signaling

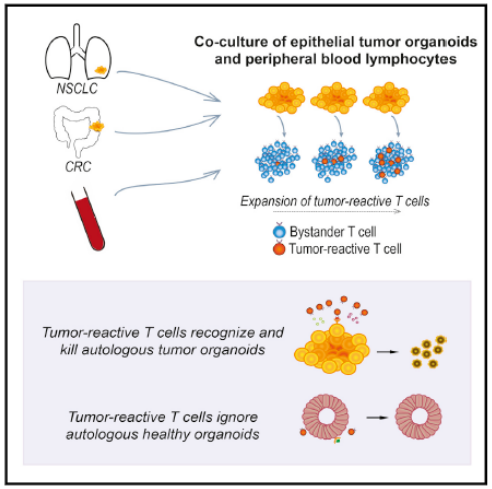
F Kruiswijk[1,8], SC Hasenfuss[1,8], R Sivapatham[2,8], MP Baar[3], D Putavet[3], KAT Naipal[3], NJF van den Broek[1], W Kruit[4], PJ van der Spek[5], DC van Gent[3], AB Brenkman[1,6], J Campisi[2,7], BMT Burgering[1], JHJ Hoeijmakers[3] and PLJ de Keizer[1,2,3]

Resource

Cell

Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids

Graphical Abstract



Authors

Krijn K. Dijkstra, Chiara M. Cattaneo, Fleur Weeber, ..., Hans Clevers, Ton N. Schumacher, Emile E. Voest

Correspondence

e.voest@nki.nl

In Brief

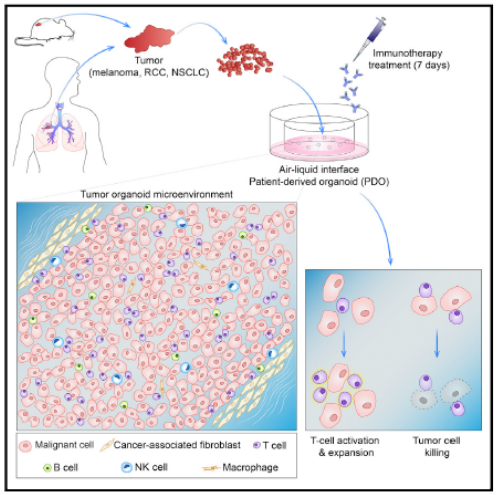
A modified patient-derived tumor organoids system allows the expansion of tumor-specific T cells from blood for personalized analysis of their anti-cancer properties.

Resource

Cell

Organoid Modeling of the Tumor Immune Microenvironment

Graphical Abstract



Authors

James T. Neal, Xingnan Li, Junjie Zhu, ..., Grace X.Y. Zheng, Mark M. Davis, Calvin J. Kuo

Correspondence

cjkuo@stanford.edu

In Brief

The tumor-immune microenvironment is modeled using a patient-derived organoid approach that preserves the original tumor T cell receptor spectrum and successfully models immune checkpoint blockade.

Hypoxia subverts the immune system and promotes tumor progression via HIF and STAT3

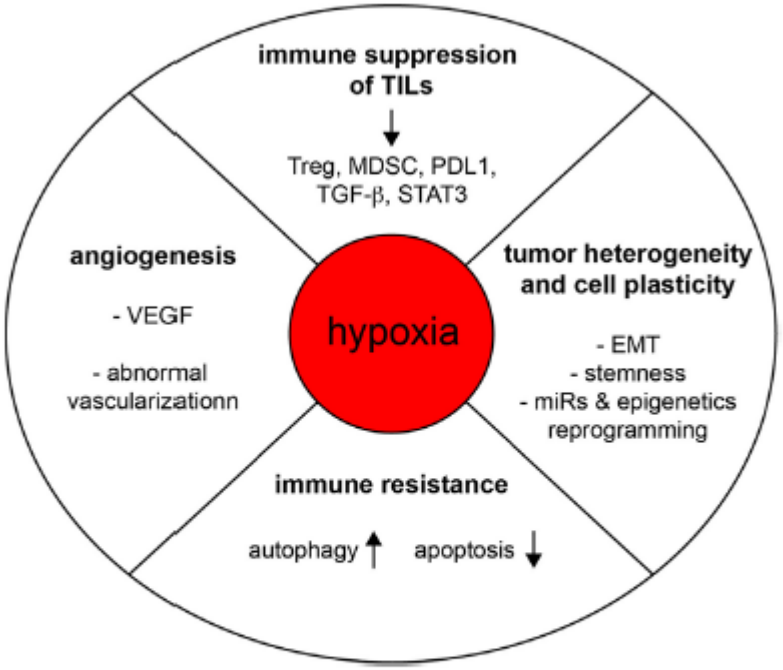


FIGURE 2 | Hypoxia subverts the function of the immune system and promotes tumorigenesis. Hypoxia regulates tumor progression through various mechanisms of action, including the promotion of angiogenesis, tumor heterogeneity, cell plasticity, immune resistance, and intratumoral immune suppression.

Terry et al Front Immunol 2017

Conclusions and perspectives

- Minipigs are medium size animal models with many features similar to humans

- Melanoma in MeLiM minipigs is genetically heterogeneous as in humans

- Mechanisms of tumour progression share genetic, histological and biochemical signatures with human triple wild type melanomas

- Efficient spontaneous tumour regression seems related to innate and acquired immunity pathways

- study of few non regressing MeLiM tumours on autonomous capacity to proliferate, senesce or die, and on immune activation are ongoing

Patients could not just survive with cancer, but live without cancer

Animal research is regulated

Since 1951, the Animal Welfare Institute has been dedicated to reducing animal suffering caused by people.
We seek better treatment of animals everywhere— in the laboratory, on the farm, in commerce, at home, and in the wild.

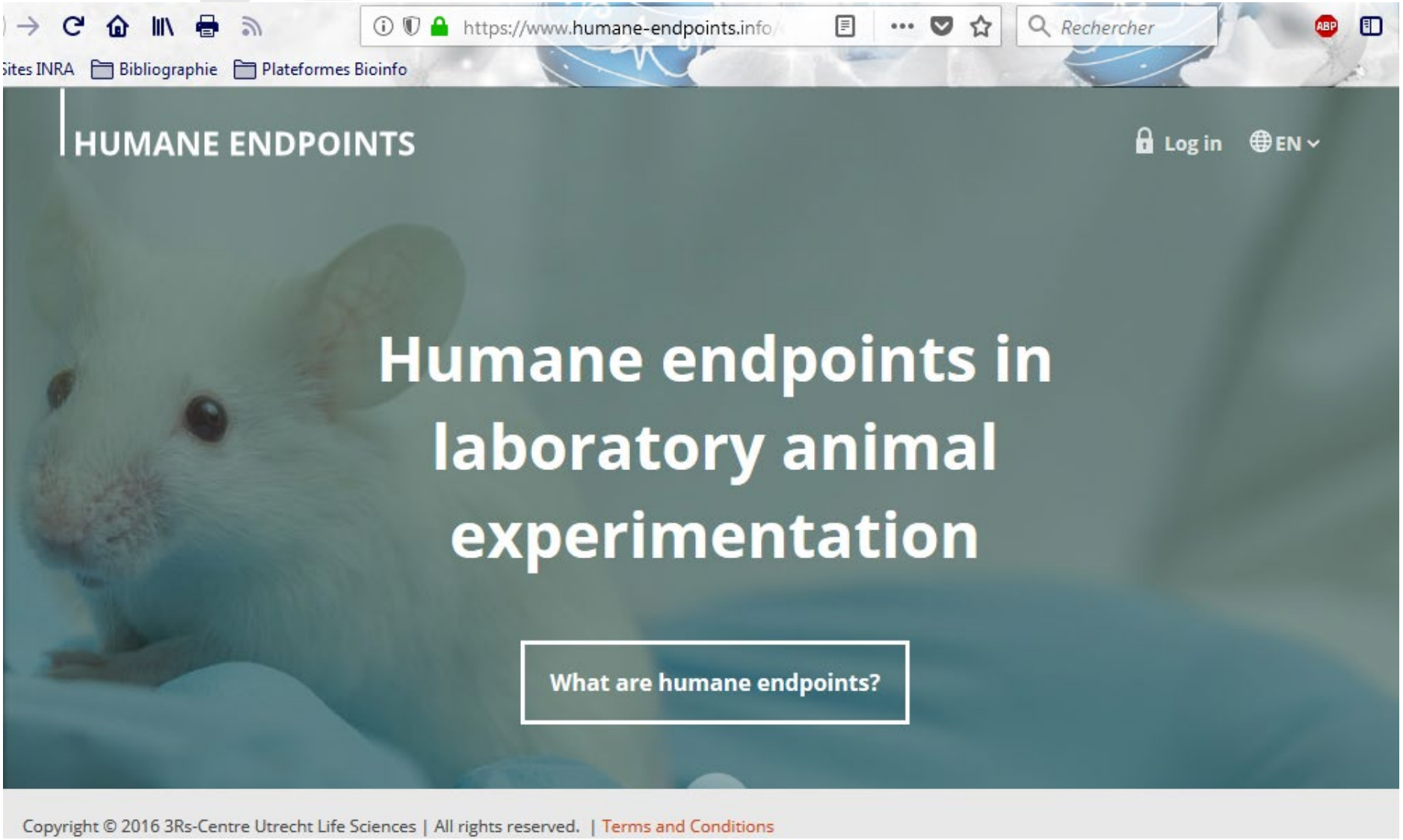
EXECUTIVE SUMMARY

Background

EU citizens are becoming increasingly concerned that all kinds of production systems and other activities should be sustainable. Animal welfare is an important aspect of sustainability, and also of product quality, and may result in consumers refusing to buy products. The welfare of an individual is its state as regards its attempts to cope with its environment. Welfare includes feelings and health and can be measured scientifically. It is a biological concept, quite different from rights, and refers only to living animals.

The terms welfare, stress, needs, humane and euthanasia are defined and some of the ways in which they are used imprecisely in EU documents and elsewhere are described. Animal health is principally of importance because it is a key part of animal welfare. It can also have economic and human disease consequences. The terms health and welfare have exactly the same meaning for humans and for other animal species, hence the current interest in 'one health' and 'one welfare'. When the welfare of individuals is poor, there is increased susceptibility to disease, hence improving welfare generally reduces disease. Preventing anti-microbial resistance is good for animal welfare and improved welfare can reduce the need for use of anti-microbial products. Those with a medical backround and those with a veterinary or other biological background benefit from exchanging information, in particular because of the similarities in disease and in other causes of poor welfare in humans and other species. Care for people and care for animals used by people is generally better if all are considered as individuals.

INRA SCIENCE & IMPACT


https://www.humane-endpoints.info
Rechercher
Sites INRA
Bibliographie
Plateformes Bioinfo
HUMANE ENDPOINTS
Log in
EN
Humane endpoints in laboratory animal experimentation
What are humane endpoints?
Copyright © 2016 3Rs-Centre Utrecht Life Sciences | All rights reserved. | Terms and Conditions

EXPERIMENTATION ANIMALE

RESSOURCES

Le dispositif d'expérimentation animale

REGLEMENTATION

La réglementation sur l'expérimentation animale

DAP

Comment faire une demande d'autorisation de projet?

COMETHEA

Comité d'éthique en expérimentation animale

CELLULE ANIMALIERS

Le réseau des animaliers

LIENS

S'informer et suivre l'actualité

What are humane endpoints?

A humane endpoint can be defined as:

'the earliest indicator in an animal experiment of (potential) pain and/or distress that, within the context of moral justification and scientific endpoints to be met, can be used to avoid or limit pain and/or distress by taking actions such as humane killing or terminating or alleviating the pain and distress' (Hendriksen

Some elements of this definition can

- '....**potential pain**....' (Hendrikse
  e.g. pre-clinical parameters such
  up/down regulations as an indic
  physiological parameters such a
- '...**within the context of the sc**
  endpoints should always be bal
- '....**taking actions such as**...' (C
  painful/stressful procedure are

The following conclusions can be drawn from this definition. A humane endpoint :

- Not necessarily means the humane killing of the animal, but could also result in interventions to alleviate the stressful/painful experimental procedure (e.g. performing surgery) or providing analgesics.
- Is not necessarily based on clinical signs but could also start from pre-clinical signs or from physiological or molecular biomarkers predictive of pain/distress later on in the disease process.
- Should be balanced against the scientific endpoints to be met. Thus, pain and distress might be intrinsic to a certain experimental model (e.g. arthritis). However, in this case the humane endpoint should never be beyond the scientific endpoint.
- Should never be beyond the level of moral justification.

A humane endpoint can be considered as a possible refinement alternative for those experiments that involve pain and discomfort to the animals. In the Netherlands annually about 2,7 percent of all animals used in research experience more than 'moderate/severe' (Zo Doende 2014, an annual report of the Netherlands Food and Consumer Product Safety Authority, only in Dutch).

Biomedical research areas with relatively high percentages of pain and distress are cancer research, toxicity studies, vaccine potency studies, infectious disease studies and autoimmune disease studies.

Applying humane endpoints should seriously be considered when animal experiments involve severe pain and suffering.

INRA SCIENCE & IMPACT

Acknowledgments :

UMR1313 GABI GIS & GeMS
Fabrice Andreoletti
Fany Blanc
Emmanuelle Bourneuf
Boubacar Coullibaly
Héloïse Debare
Jordi Estellé
Elisabetta Giuffra
Guillaume Piton
Jean-Jacques Leplat
Silvia Vincent-Naulleau
Claire Rogel-Gaillard

Master students :
Leyla Ahmadova
Samuel Badji
Kévin Désirabel,
Sihem Hamine
Sergiu Raileanu
Ameni Sdiri

CEA – Institut de Génomique
INRA -Get-PlaGE
INRA – MIMA2

Institut Gustave Roussy
Salem Chouaib
Stéphane Terry

-

UMR955 INRA-ENVA
Florence Bernex
Philippe Bossé
Cécile Campagne
Sophia Julé

CEPH *Florence Demenais*

Institut Cochin *Armelle Blondel*

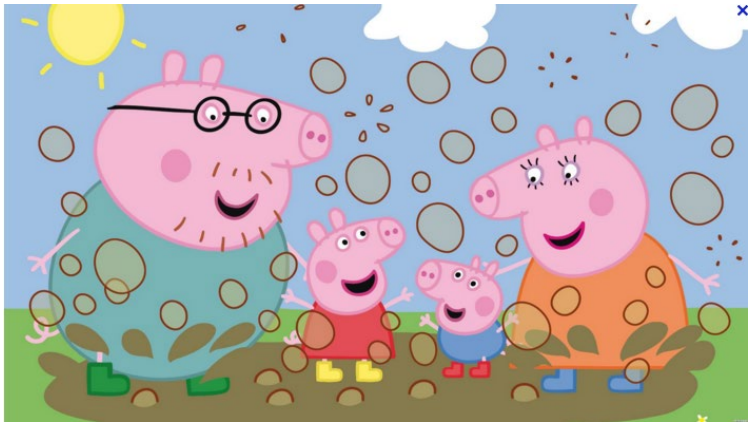
Institut Curie *Xavier Sastre-Garau*

Hôpital Saint Louis
Maxime Battistella
Anne Janin
Céleste Lebbé

IDEXX Alfortville *Mercedes Estrada*

Libechov Institute
Vlatislav Horak
Monika Cervinkova

CNRS URA 2578 –I. Pasteur
Jean-Jacques Panthier



Please, split in 2 groups

- Swine experimental Unit
- Histology facility

Pascal Lafaux

Julie Rivière
Marthe Vilotte

Interested? Mail Giorgia.egidy-maskos@inra.fr


Thank you for your attention