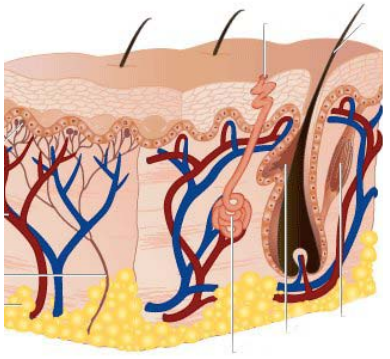
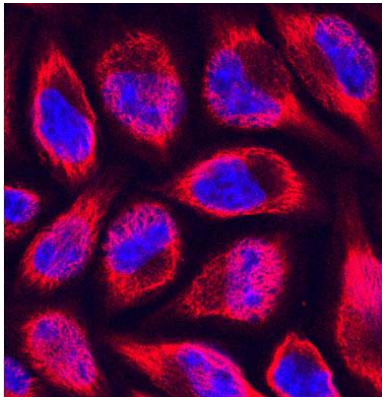
HUG
Hôpitaux Universitaires de Genève
Service de Dermatologie



Severe Skin Diseases:

Integrating new concepts of basic research into a clinical perspective



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UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

Charles Institute Seminar Series, UCD 2009

Outline

- Introduction
- Dendritic cells, Autophagy and Pathogens
- Skin cancer: Melanoma and Cutaneous lymphoma
- Clinical perspectives

Several pathological processes lead to severe skin diseases

Skin

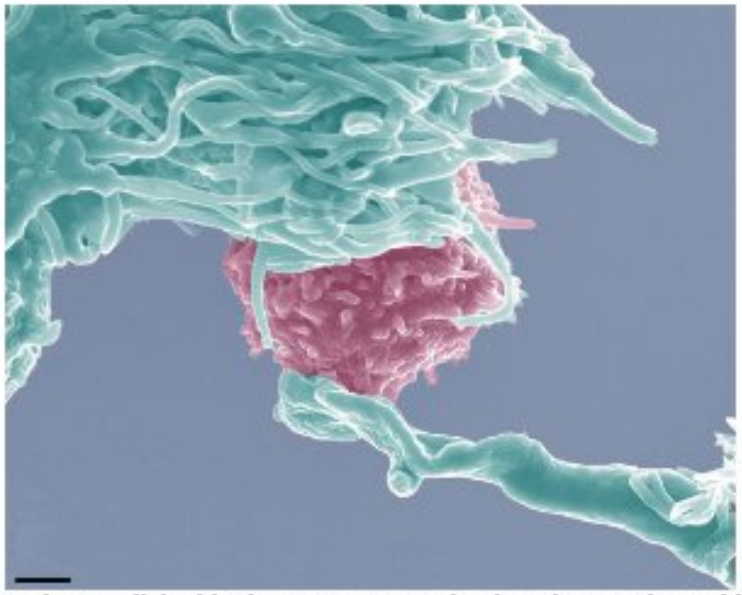
Skin cancers	Autoimmune/inflammatory diseases	Adverse drug reactions	STI skin infections	Other: Wounds, Genetic diseases…





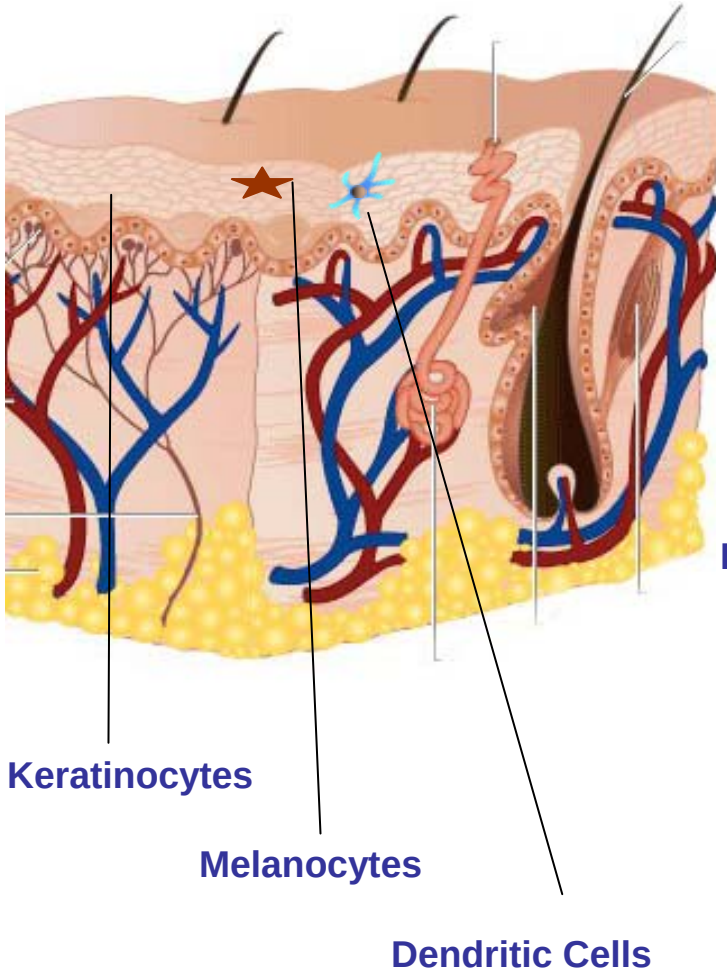
Severe skin diseases and severe diseases with an important involvement of skin and mucosal tissues are frequent when combined

Cell types in the skin are at the core of the pathological processes



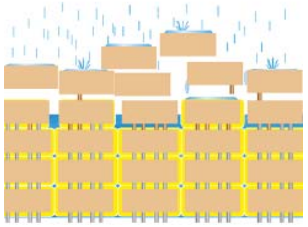
Dendritic cell (in blue) interacting with a lymphocyte (in pink)

O.Schwartz, Institut Pasteur, Paris



A major role for the skin is protection

1. Mechanical
2. Thermal, chemical
3. Liquid loss
4. Pathogens (bacteria, viruses…)
5. UV radiation



= **Healthy skin**

= **skin with barrier alterations (atopic eczema)**

Inflammation, infections

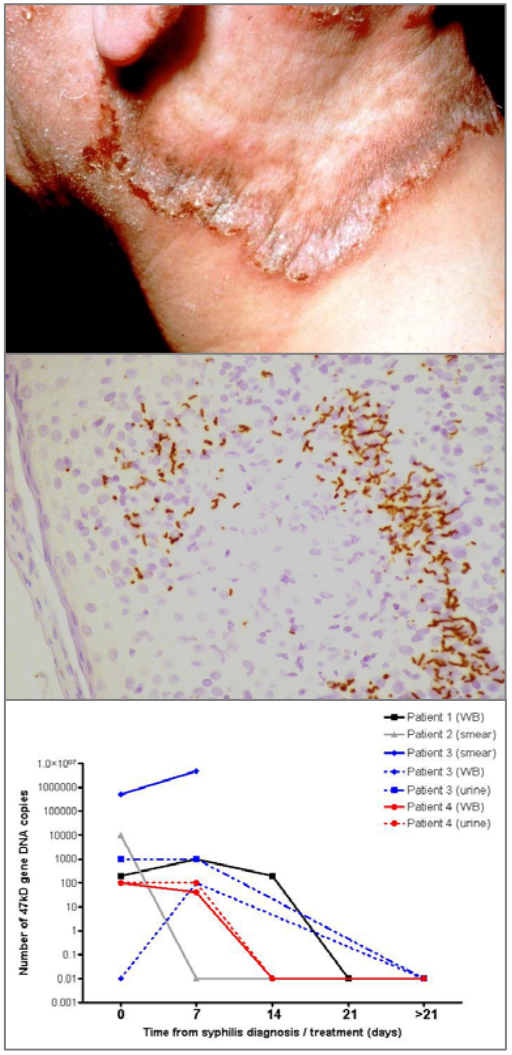
= **Skin with defects in DNA repair (XP)**

XP: Tumors

Dermatology has become a cross-disciplinary field

Historically based mainly on morphology

Towards a medical and surgical field integrating patient-oriented clinical and basic research

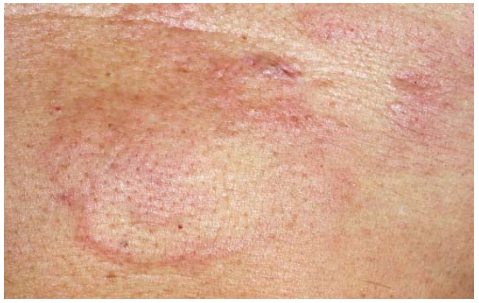


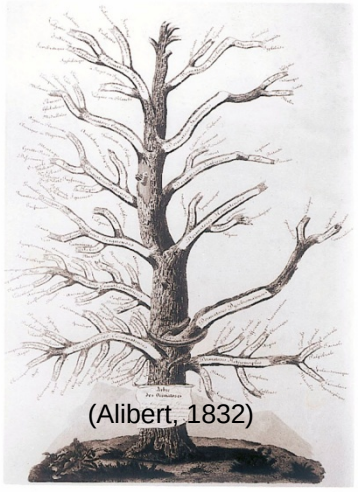
Dermatological science is rapidly evolving

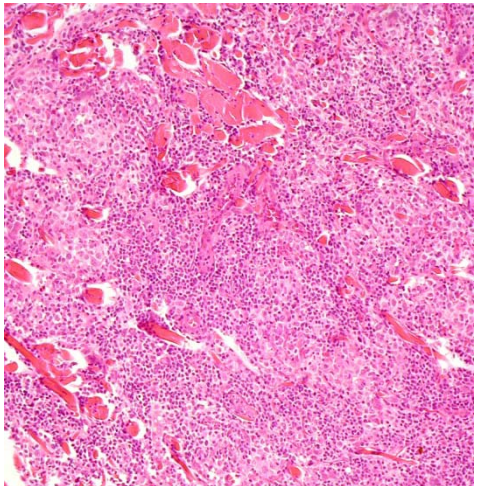
19th → 20th → 21st

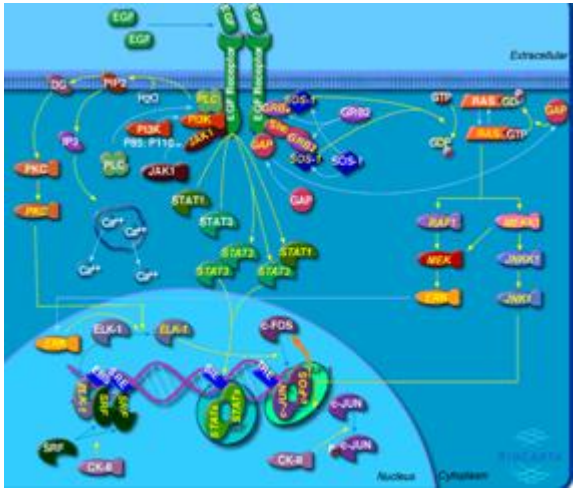
Visual diagnosis + Cinical/dermatopathology correlations + Mechanisms of diseases and Targeted therapies


Hebra, 1856




(Alibert, 1832)



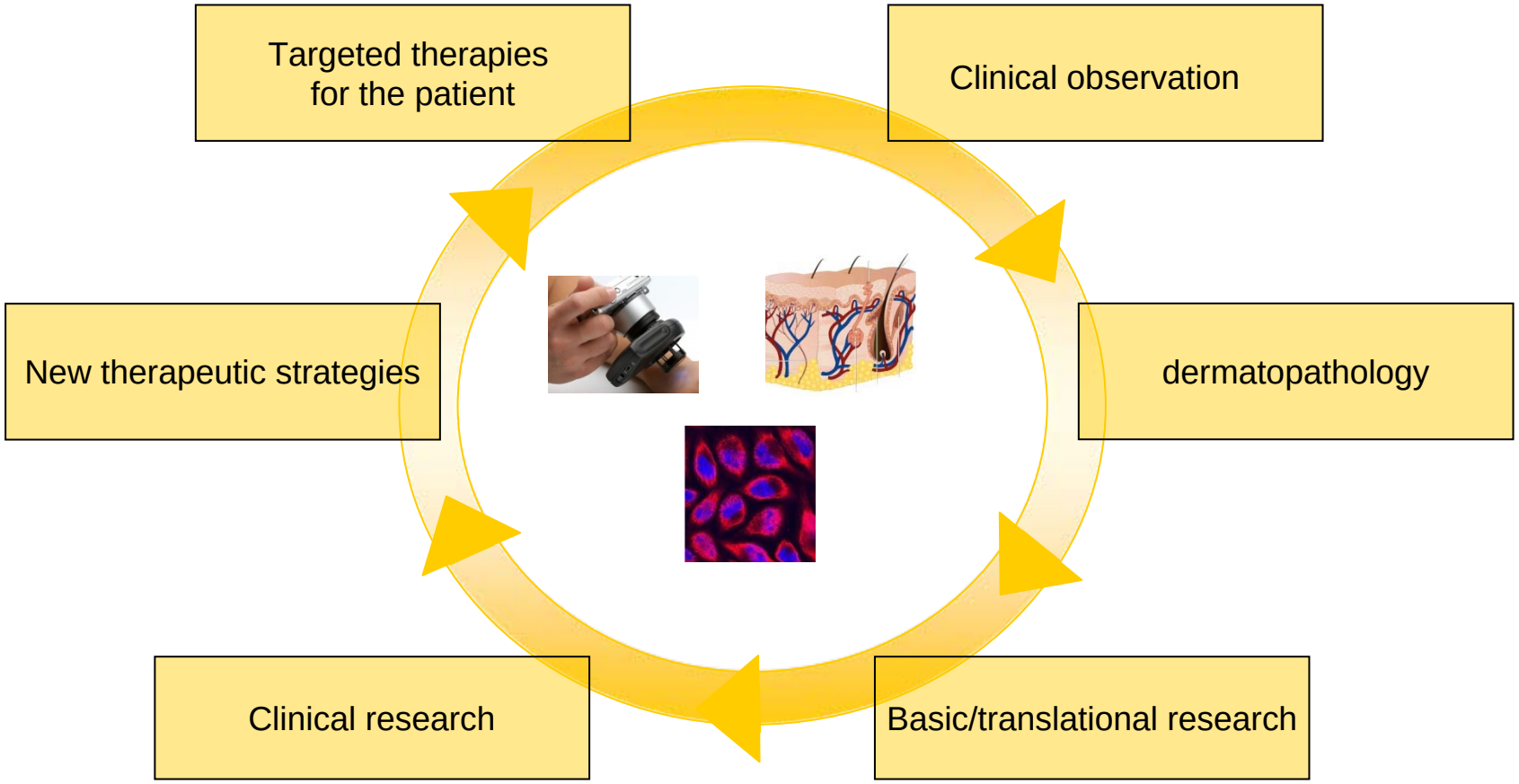


Historical “treatments” mercury

Non-targeted therapies corticosteroids

Targeted therapies, biologics

An integrated approach is required for complex skin diseases



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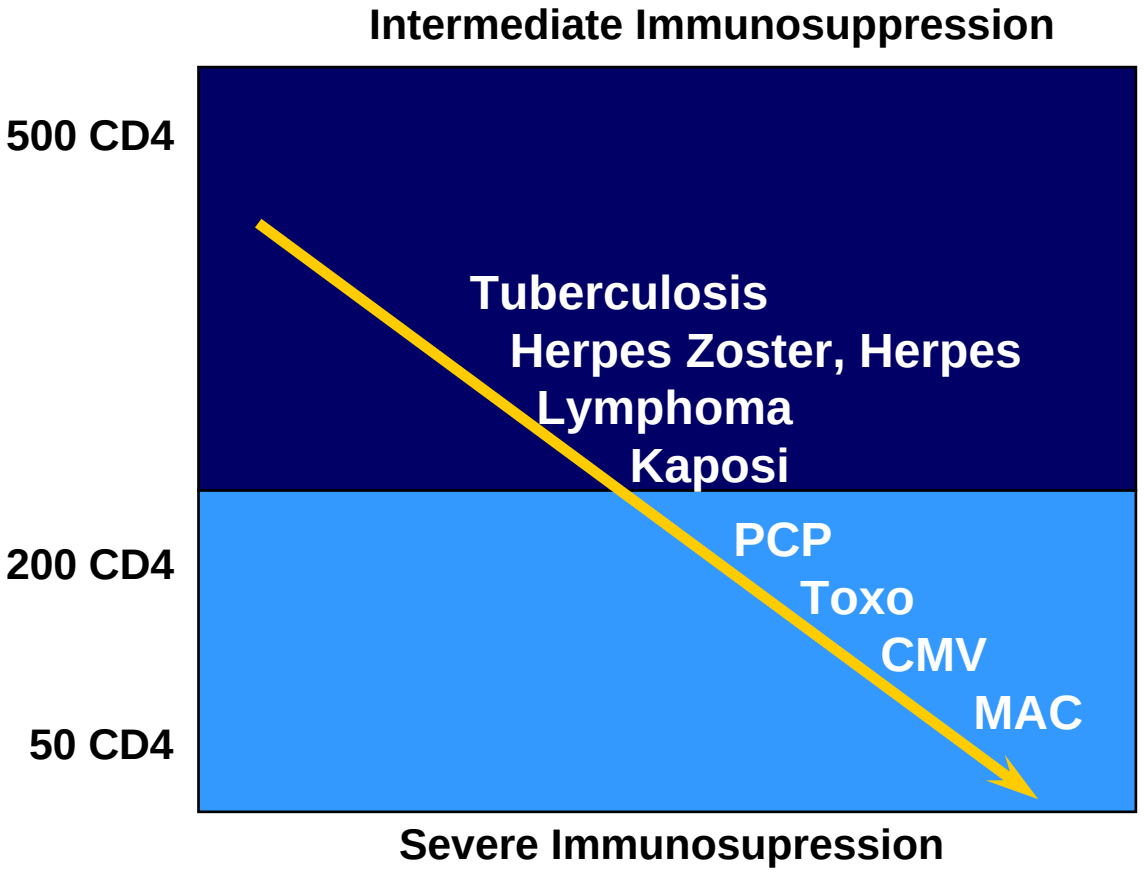
- Dendritic cells, Autophagy and Pathogens
- Skin cancer: Melanoma and Cutaneous lymphoma
- Conclusions and perspectives

HIV and skin complications

Dealing with skin complications of HIV infection

Great frequency in VIH+ patients and complex cases



Dendritic cells are targets of pathogens during mucosal transmission

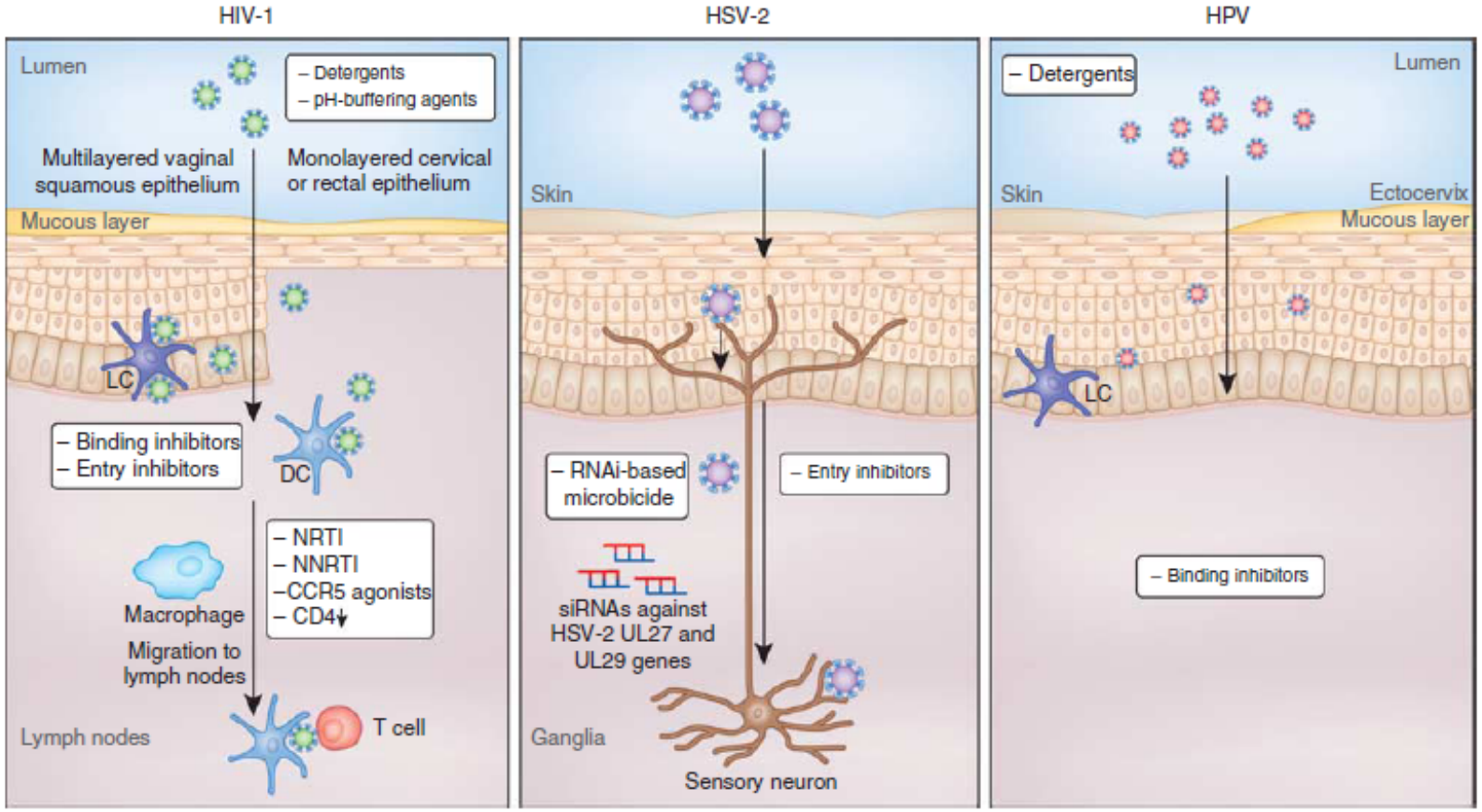
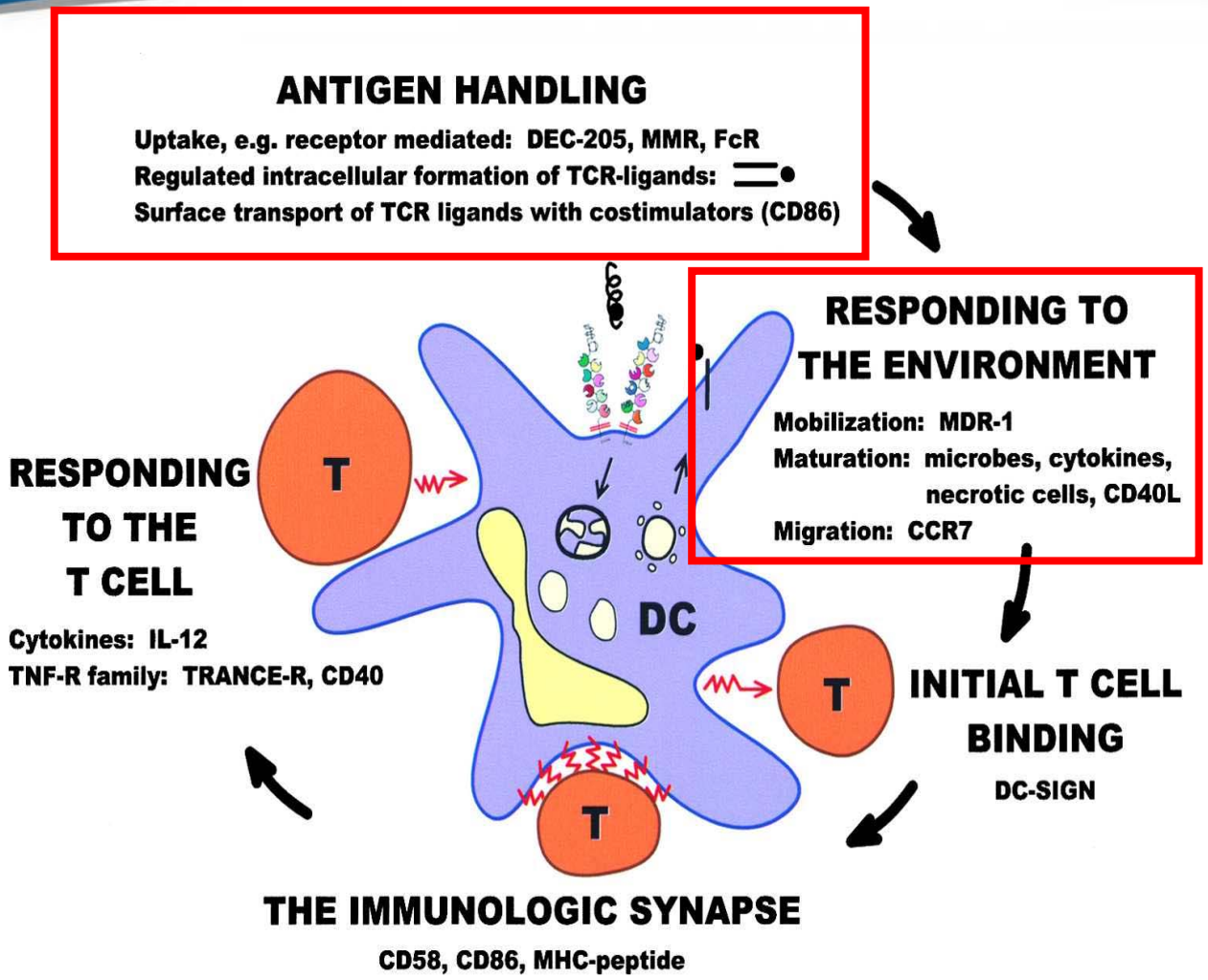


Figure 2. Microbicides against HIV-1/herpes simplex virus type 2 (HSV-2)/human papillomavirus (HPV). Microbicides for HIV-1 (left), HSV-2 (center), and HPV (right). Microbicides inhibiting STI transmission are active either on or directly beneath the mucosal surface. Nonspecific microbicides, such as detergents, usually act in the first steps of viral contact with the mucosal barrier. Specific compounds generally impair viral binding or entry into host cells. Finally, highly specific microbicides act at later stages, such as non-nucleoside reverse transcriptase inhibitor-based microbicides against HIV-1. Alternative methods, such as RNA-interference-based microbicides against HSV-2, are also depicted.

Reviewed in Nikolic and Piguet, J Invest Dermatol, 2009

Dendritic Cells: sentinels of the immune system



Defense against pathogens

- Viral infections (HIV, HPV, HSV)
- Bacterial infections (S.Aureus)
- Yeast infections (candida)

Immune response against tumors

- Lymphomas
- Melanoma (DC- based vaccines)

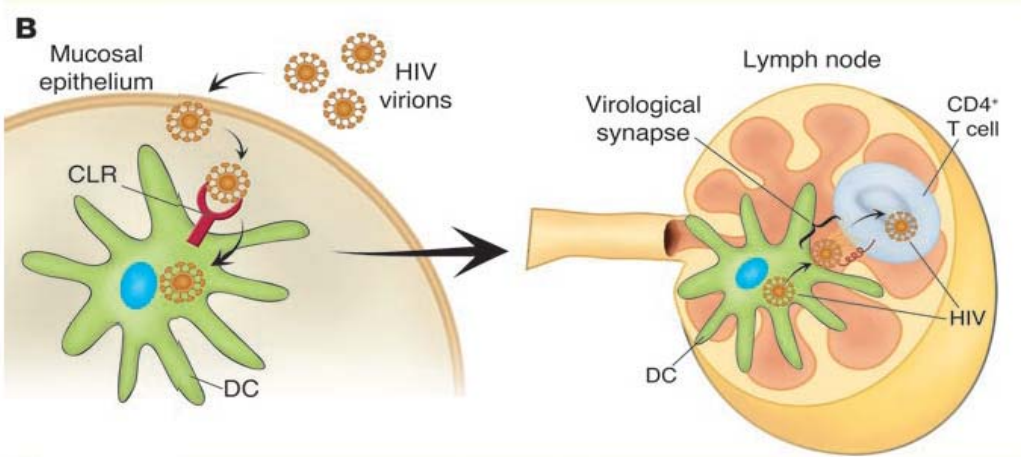
Skin Diseases

- Eczema
- Atopic Dermatitis
- Psoriasis
- Lupus Erythematosus

(Steinman, Cell 2000) (Reviewed in Piguet and Steinman, Trends Immunol, 2007)

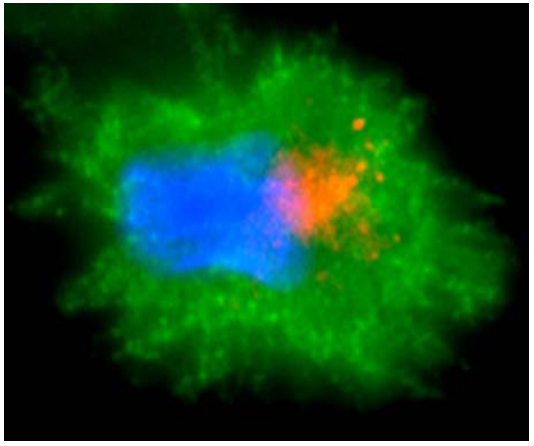
Mechanisms of pathogens mucosal transmission

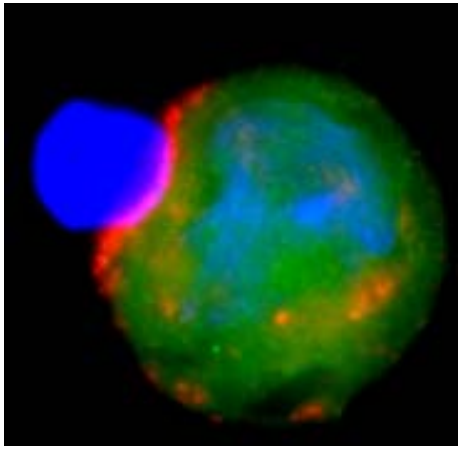
HIV: a paradigm for mucosal transmission



- Pathogens interact with mucosal tissues and other cellular targets in order to invade the host

- Study of transmission of HIV and other STI

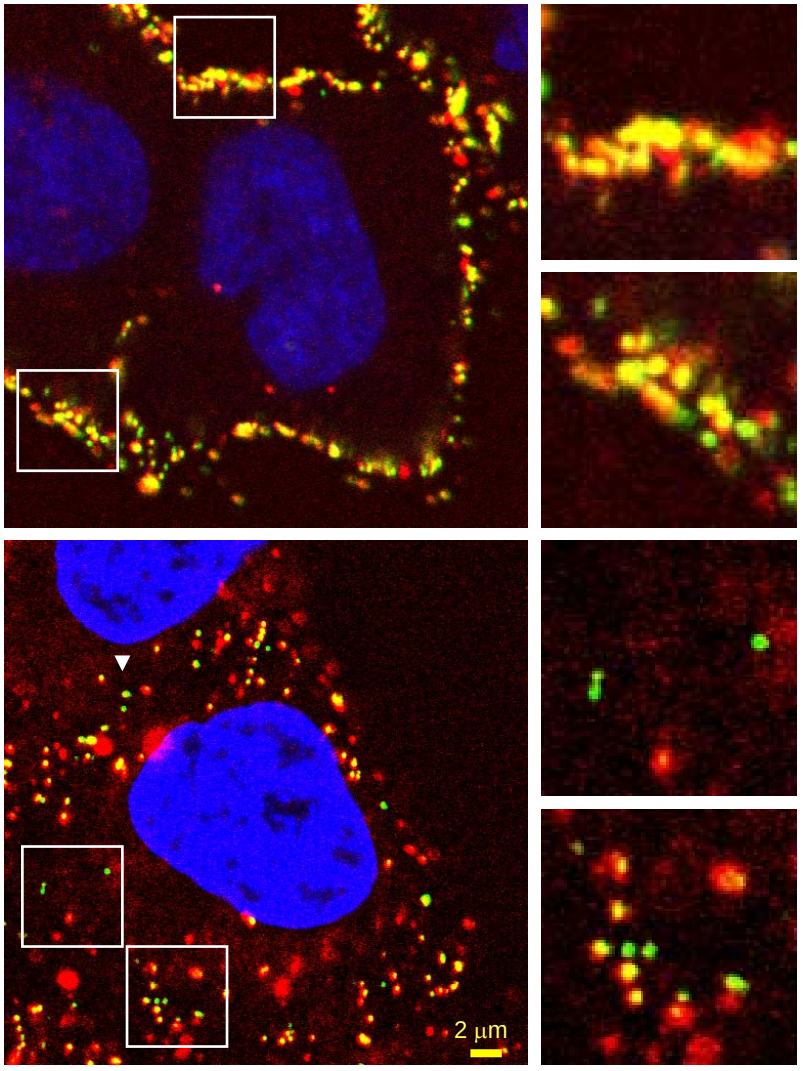




Piguet et al., Cell, 1999
Piguet et al., Nature Cell Biol, 2000
Piguet and Sattentau, J Clin Invest, 2004
Piguet and Steinman, Trends in Immunology, 2007

Virological studies: tracking HIV in Dendritic Cells

0h



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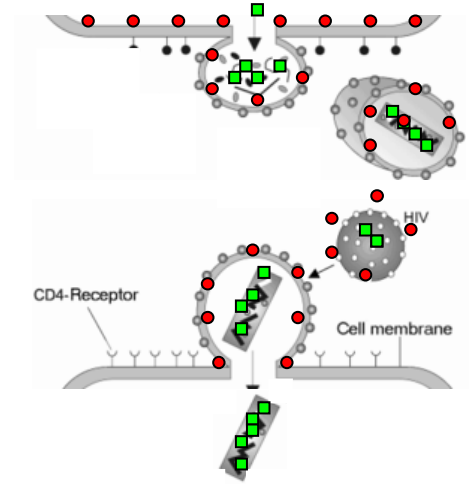
Dapi eGFP vpr S15 mCherry

Human Frontier Science Program

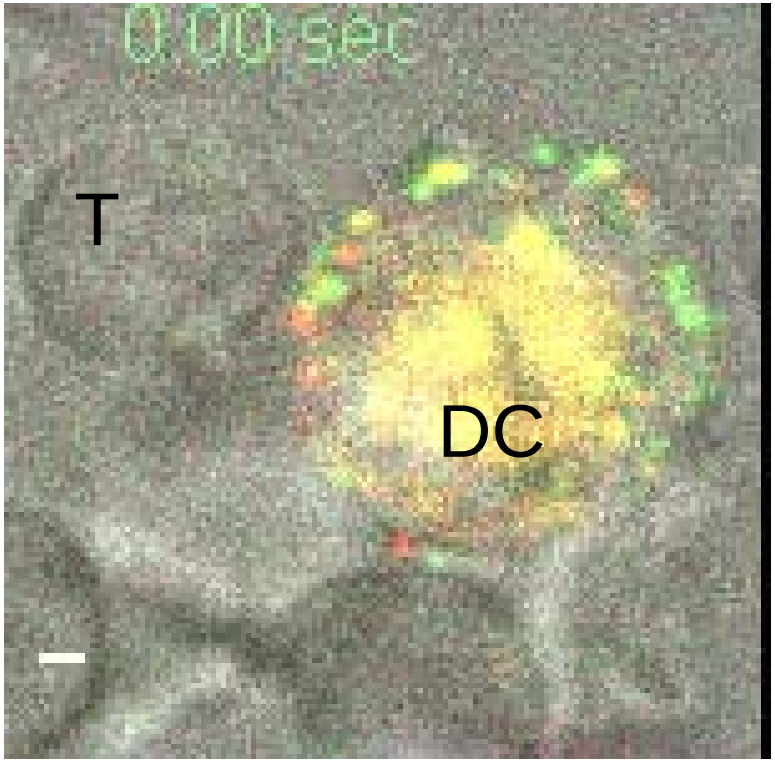
BILL & MELINDA GATES foundation

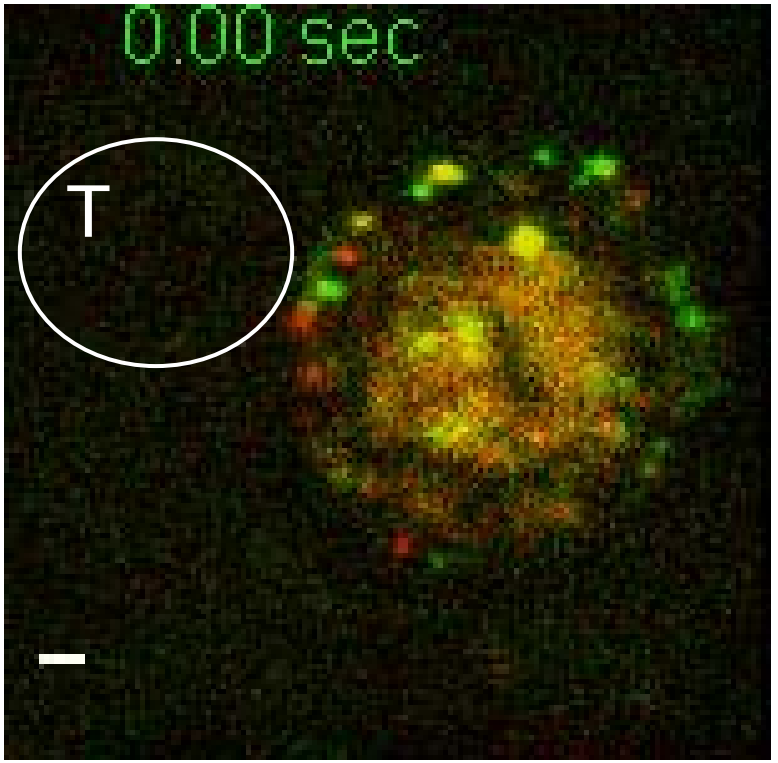
S15 mCherry
eGFP vpr

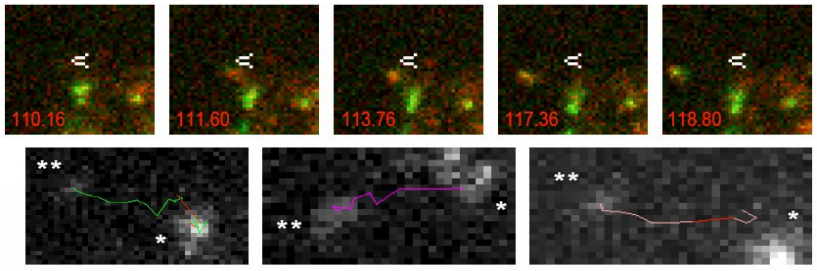


Pion et al, J Exp Med, 2006
Pion et al, J.Virol, 2007
De Witte et al, Nature Medecine,2007
Pion et al, J Invest Dermatol, 2007
Garcia, Traffic, 2008
Mangeat, PLoS Pathog, 2009

Live confocal studies: following viral particles across infectious synapses







Speed of HIV-1 Transfer :
0.40 +/- 0.23 μm/s

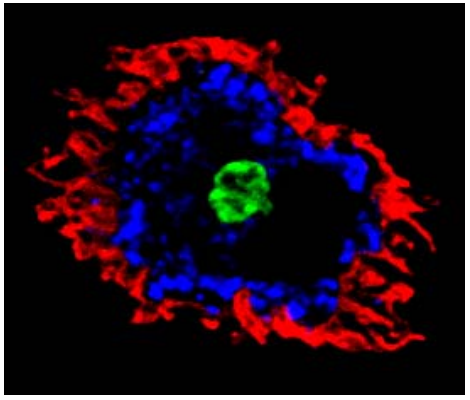
“Hot” areas for Dendritic cells biology investigation

How does HIV escape full degradation
in Dendritic Cells ?

Are Lysosomes, autophagosomes, amphisomes involved ?

Does HIV modulate autophagy in Dendritic cells?

What are the consequences for HIV antigen presentation ?



Autophagy or cellular self-digestion is a cellular pathway involved in protein and organelle degradation

Neurodegeneration

Pro: Basal autophagy is a homeostatic process that prevents intracellular proteins from accumulating to toxic levels.

Con: Inefficient lysosomal clearance results in intracellular accumulation of autophagosomes, which may process the amyloid precursor protein into toxic forms.

Myopathies

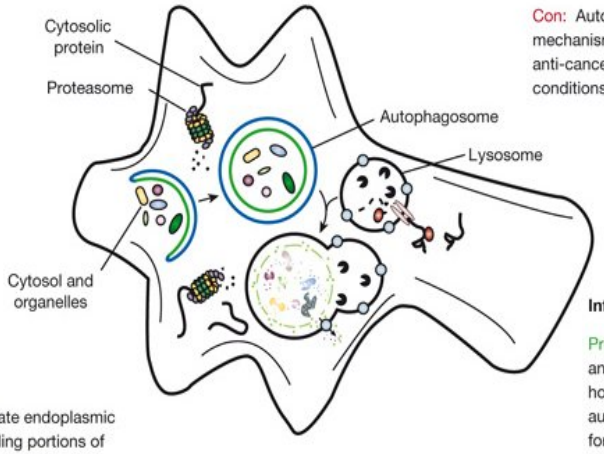
Pro: Autophagy prevents aggregate-prone protein accumulation that leads to physiological dysfunction.

Con: Autophagy may contribute to muscle wasting and defective autophagosome clearance may interfere with cellular function.

Cancer

Pro: Autophagy acts in tumour suppression by removing damaged organelles and possibly growth factors, and reduces chromosome instability.

Con: Autophagy acts as a cytoprotective mechanism that helps cancer cells resist anti-cancer treatments and survive in conditions of low nutrient supply.



Ageing

Pro: Autophagy removes damaged organelles and can limit production of reactive oxygen species.

Infection and immunity

Pro: Intracellular bacteria, viruses and protozoans are removed from host removed from host cells by autophagy, and antigens are processe for MHC class II presentation. Autophagy may prevent auto-immune and inflammatory diseases.

Con: Some microbes have evolved to subvert autophagy to establish a replicative niche.

Liver disease

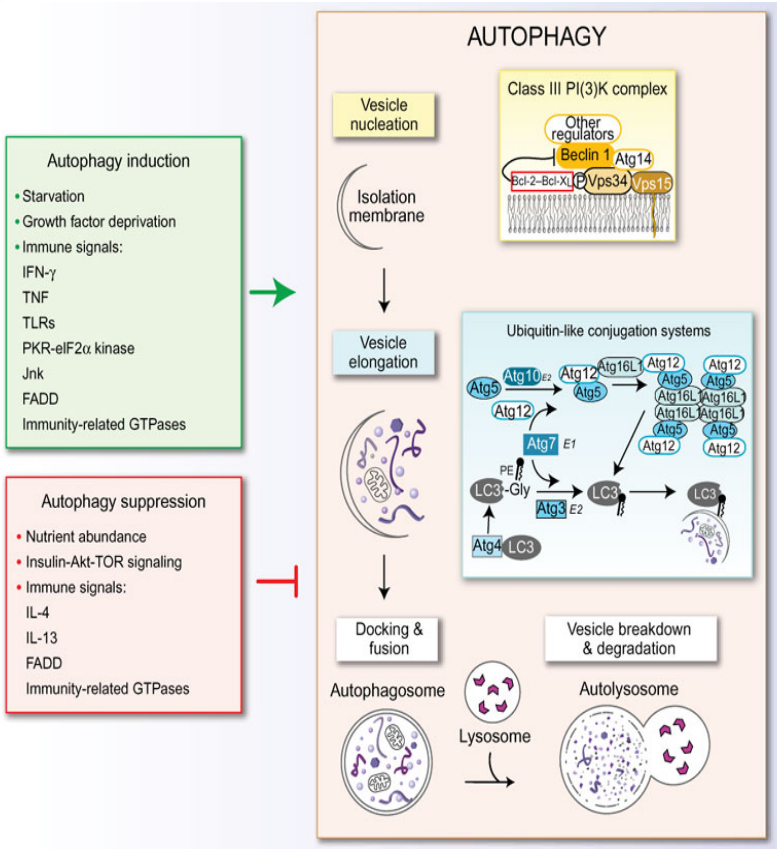
Pro: Autophagy can alleviate endoplasmic reticulum stress by degrading portions of the organelle containing misfolded proteins.

Con: Excessive autophagy may cause liver damage.

Heart disease

Pro: Autophagy may be protective during ischaemia and pressure overload.

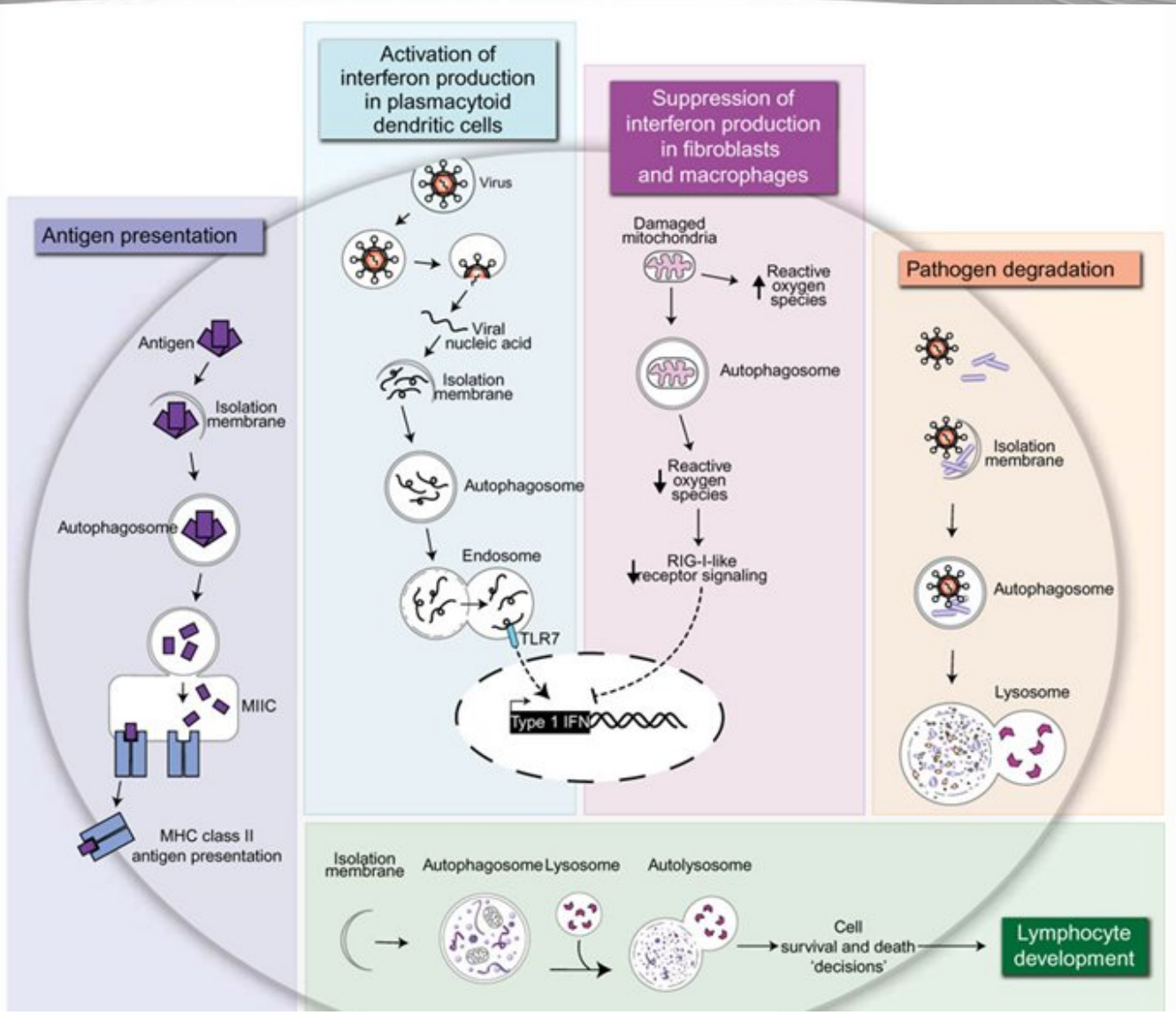
Con: Autophagy is harmful during reperfusion.



Reviewed in Mitzushima et al, Nature, 2008

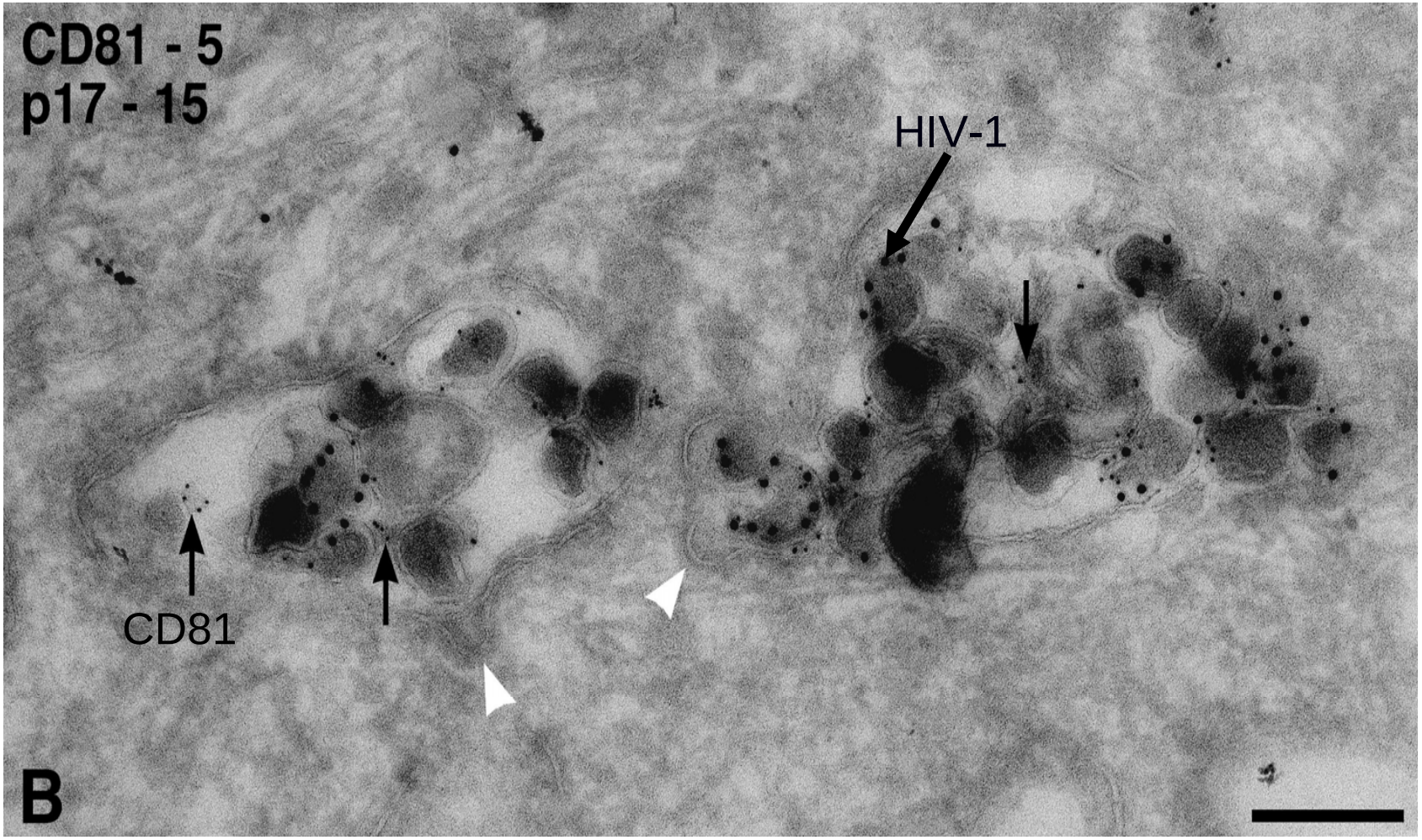
Hotchkiss, NEJM, 2009 Oct , Virgin and Levine Nat Immunol. 2009

Autophagy is involved in several immunological processes



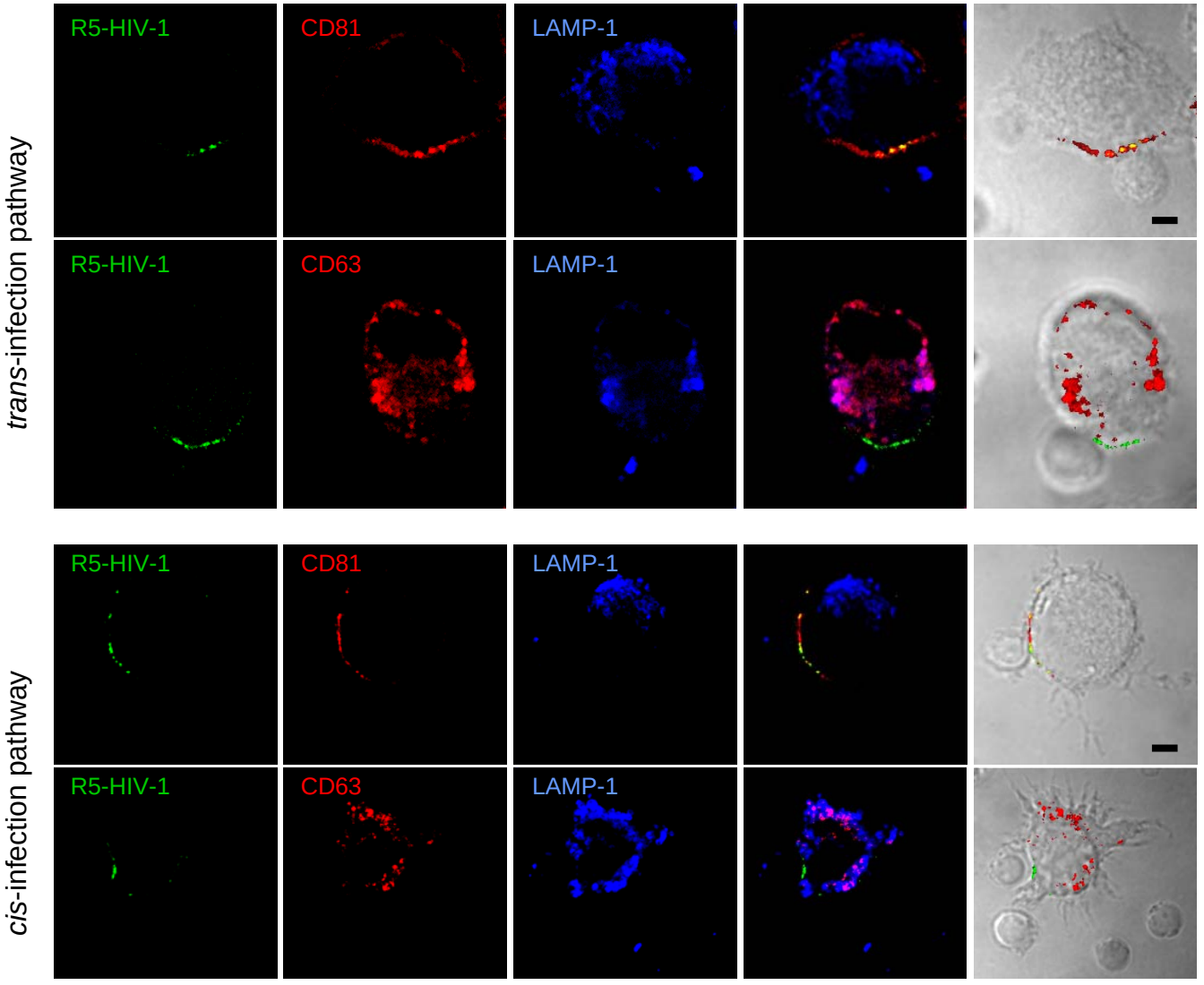
Reviewed in Virgin and Levine Nat Immunol. 2009

HIV-1 accumulates in a CD81- rich viral compartment (in part accessible from the cell surface)



Garcia, 2005, Traffic
Wilflingseder, 2007, J Immunol
Garcia, 2008, Traffic

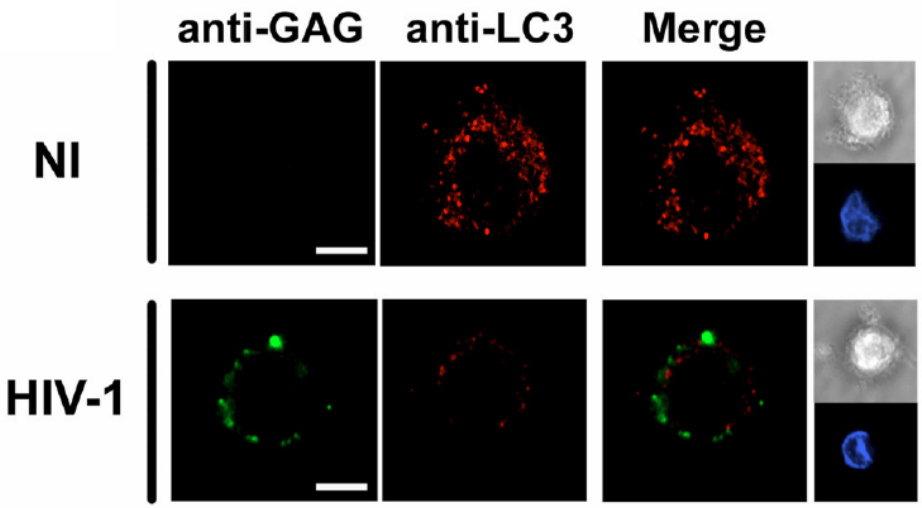
Dendritic cells transfer HIV infection to CD4+ T cells across Infectious synapses

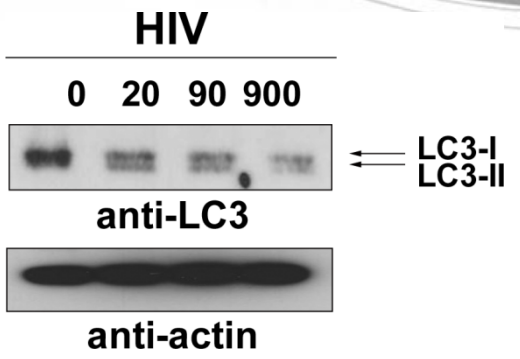


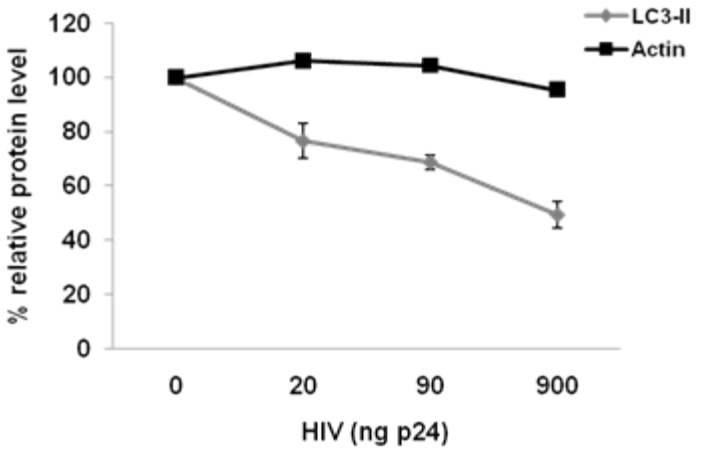
Garcia, Traffic, 2008

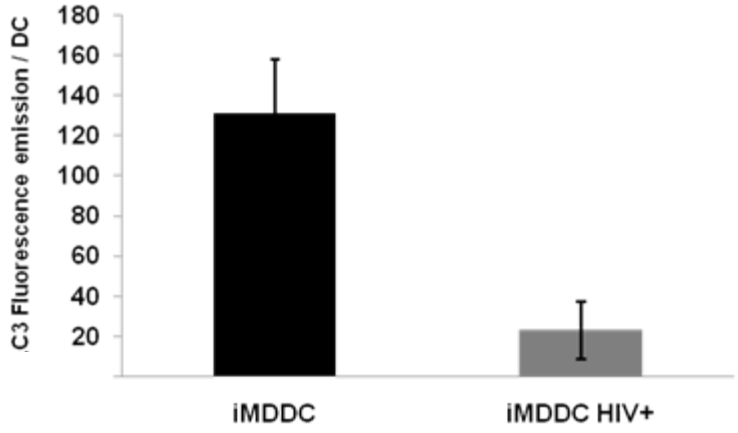
Arhel, J Clin Invest, 2009

Profound loss of autophagosomal LC3 -II in HIV-infected DC









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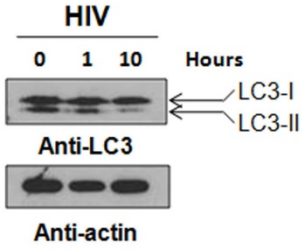
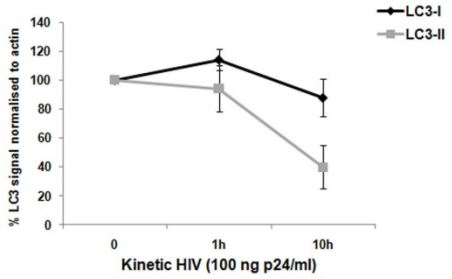
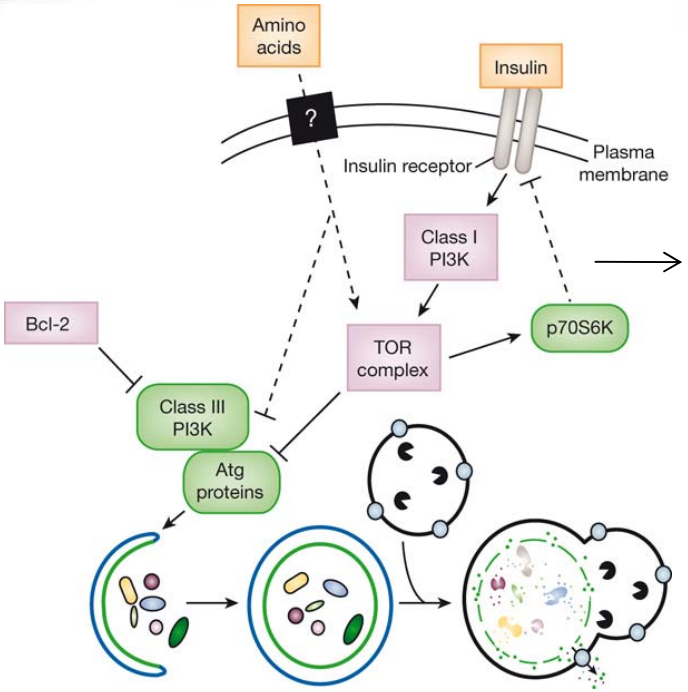




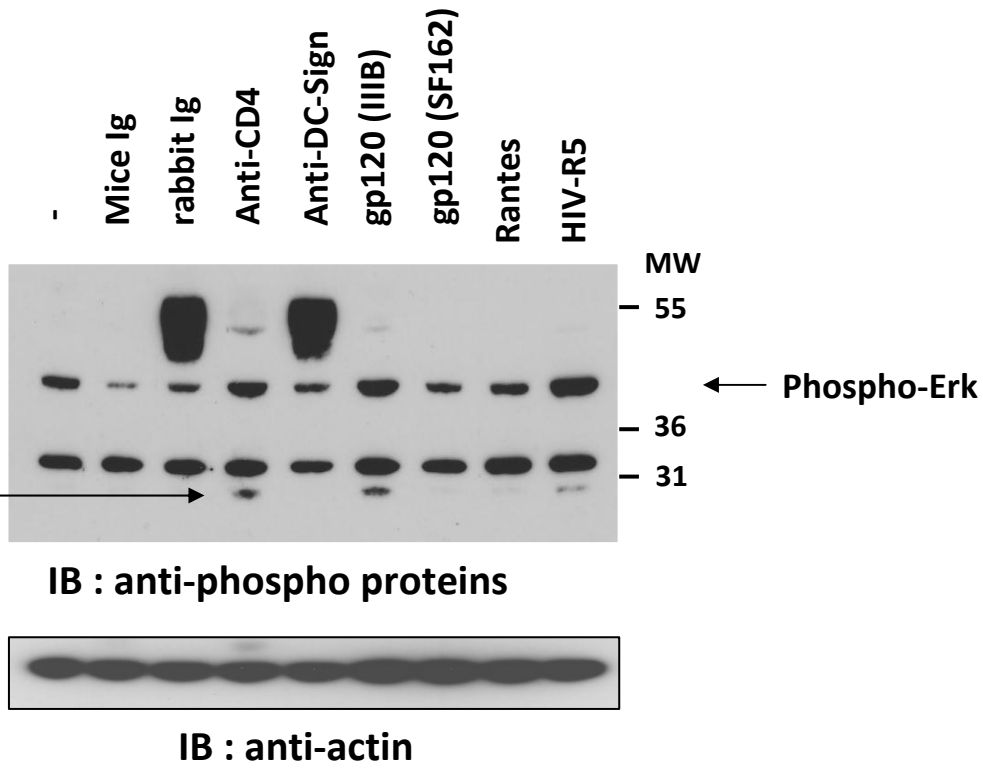
Env mediated signaling in DC leads to activation of mTor and Autophagy Block



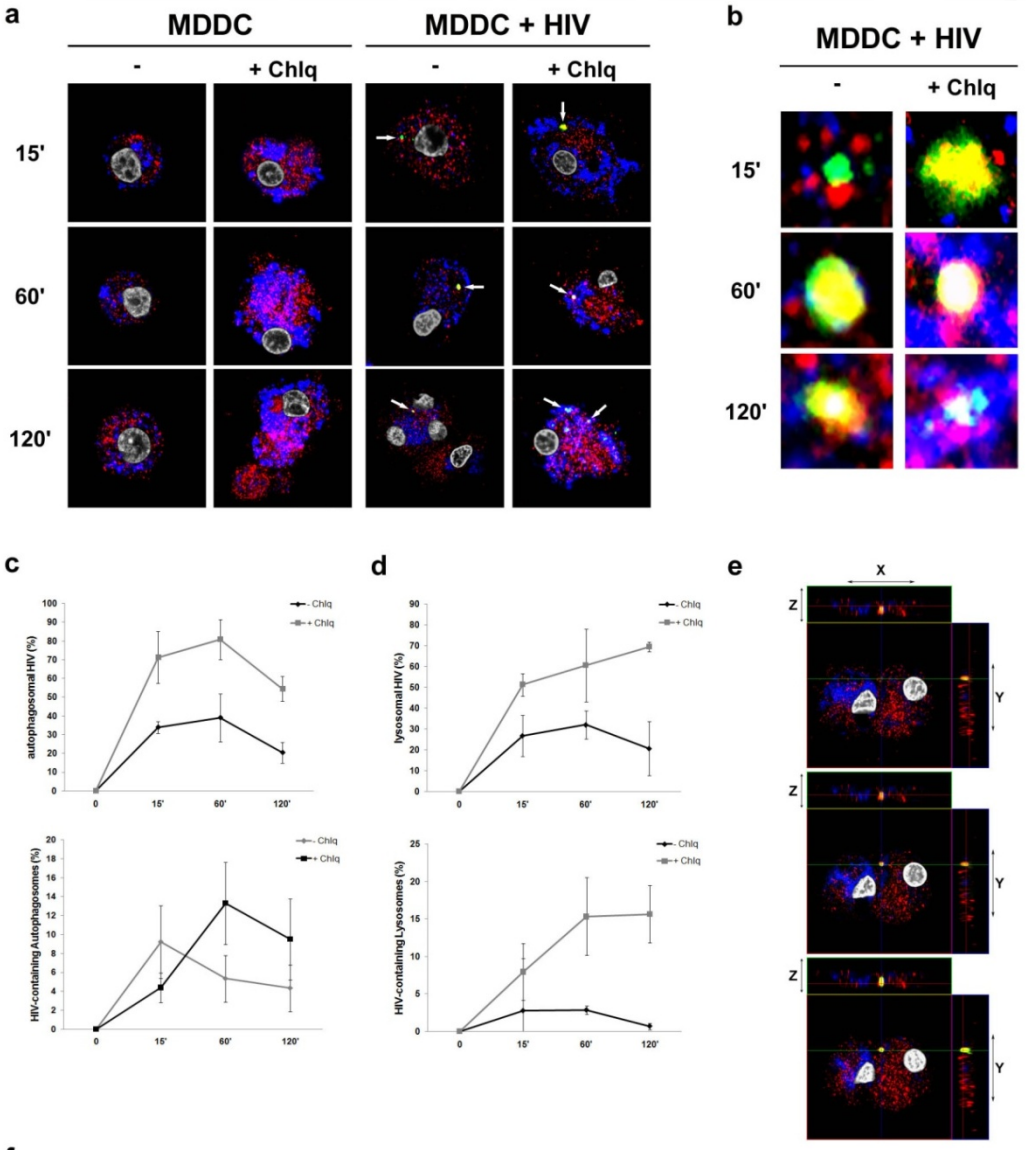
Adapted From Mizushima, Nature 2008

phospho-S6 correlates with 1) mTOR activation and 2) a block in autophagy initiation

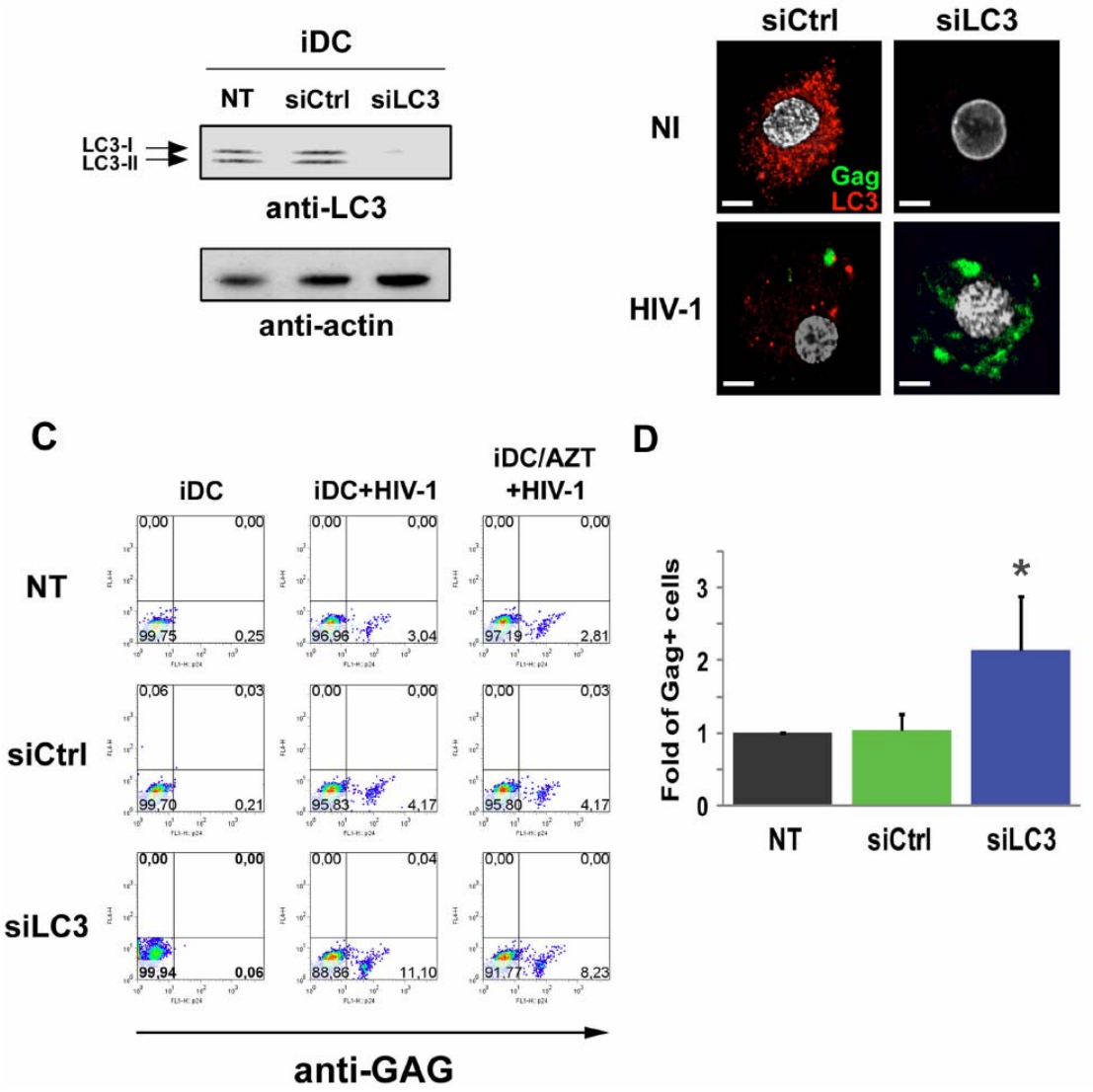
mTor target: Phospho-S6



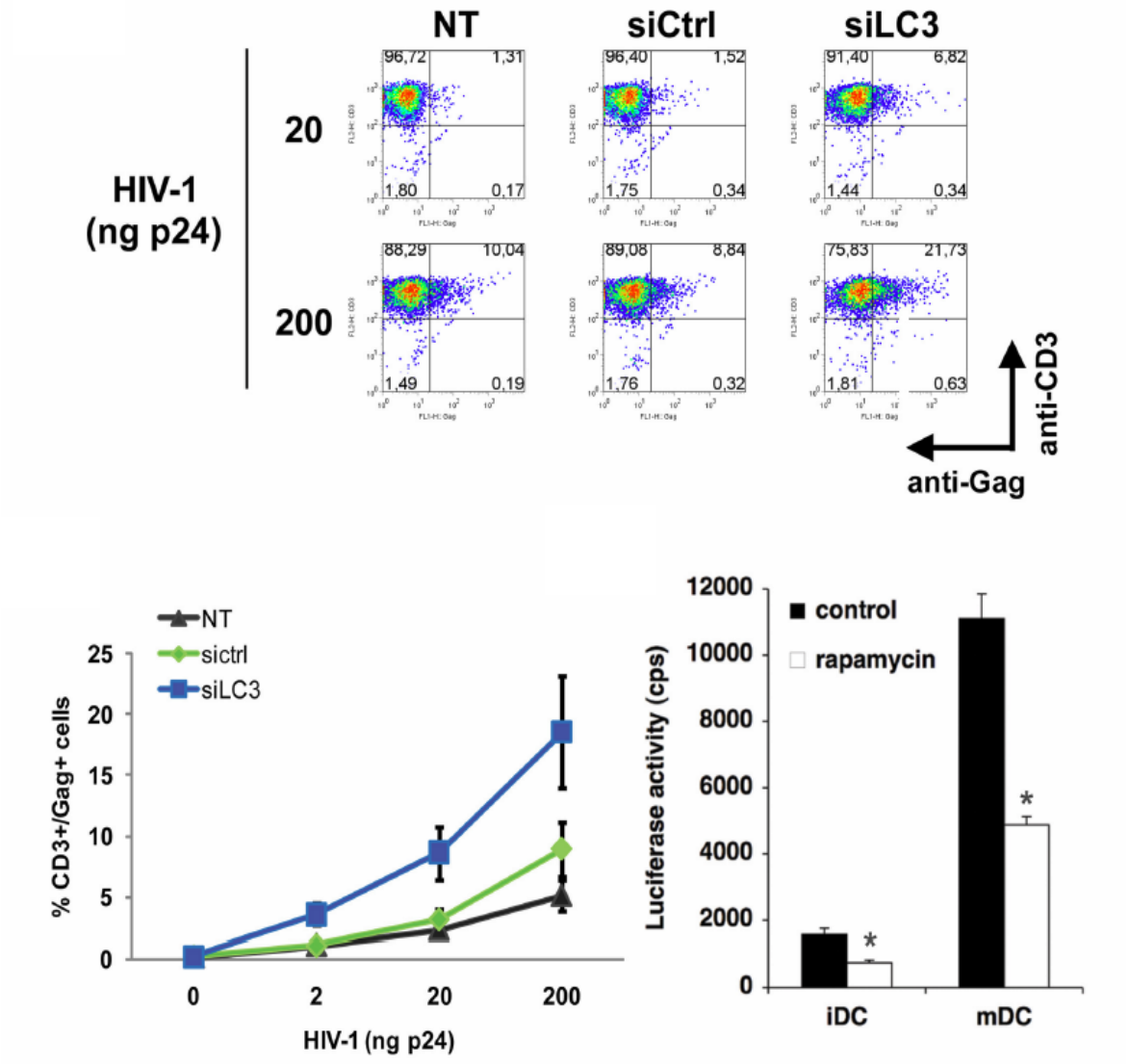
HIV is routed to lysosomes via autophagosomes in DC



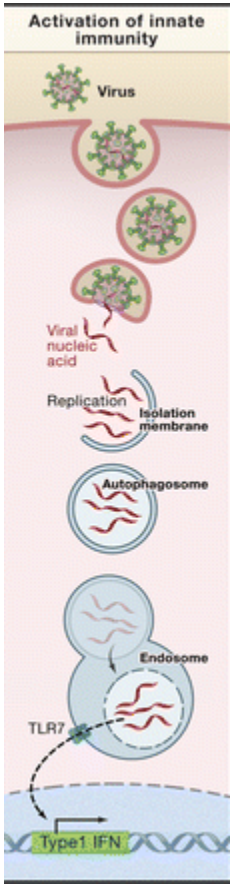
Autophagy inhibition Increases DC-associated Virus



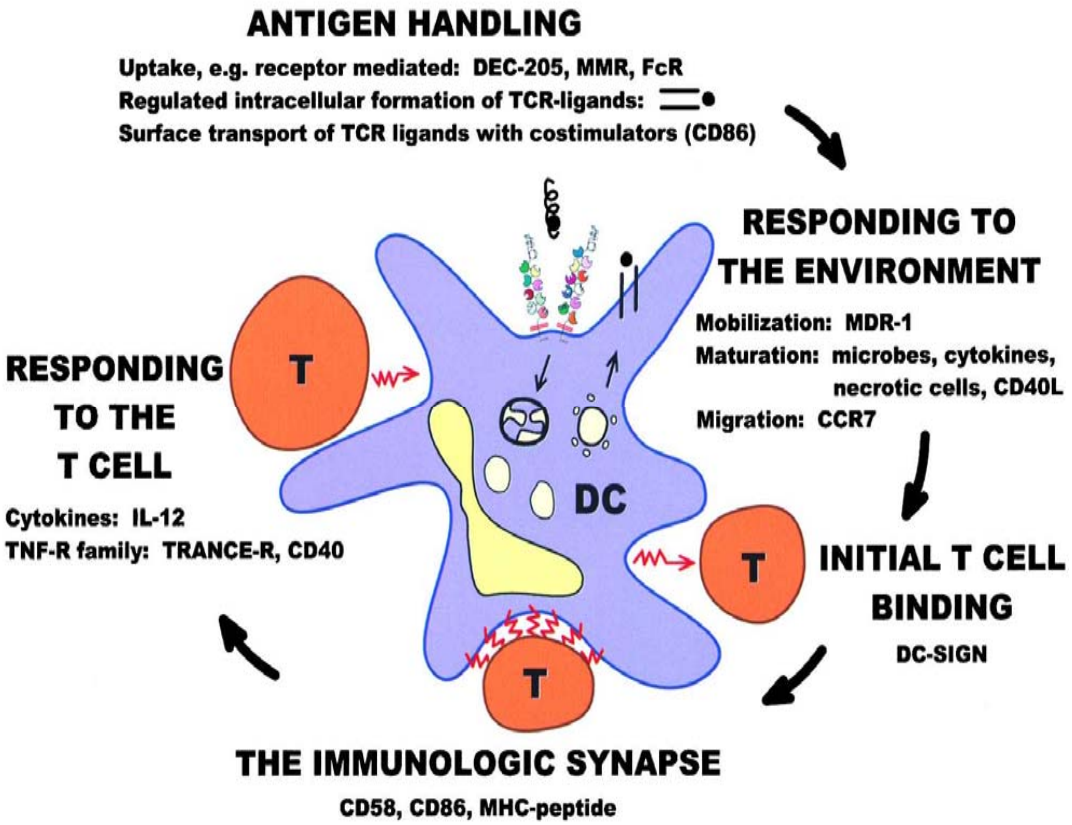
LC-3 depletion in DC enhances viral transfer to T cells



DC activation via TLR is required for efficient antigen presentation: any role for autophagy ?



From Levine and Kroemer Cell, 2008



Autophagy may be involved in the activation of innate immunity by delivering viral nucleic acids to endosomal compartments containing Toll-like receptor 7 (TLR7), which signals the induction of type 1 interferon (IFN) production.

Summary of findings of HIV downregulation of autophapgy in DC

- HIV is routed via a novel specialized endocytic structure in DC: "immunoamphisomes" (amphisomes = fusion between autophagosomes and endosomes)

- We propose that "Immunoamphisomes" in DC : 1) amplify virus degradation and 2) enhance innate and 3) adaptive immune responses

- Restoring autophagy (via mTOR inhibitors) in DC increases HIV degradation and HIV antigen presentation on MHC-II

- Autophagy has implications for early events of HIV infection and rational vaccine design

Results from our studies: New lead candidates for intervention

Langerin upregulation

DC-SIGN / Cdc42 inhibition (Secramin A, Rho gtpases inhibitors)

Autophagy (mTor inhibitors, sirolimus, everolimus…)

APOBEC3G/F upregulation

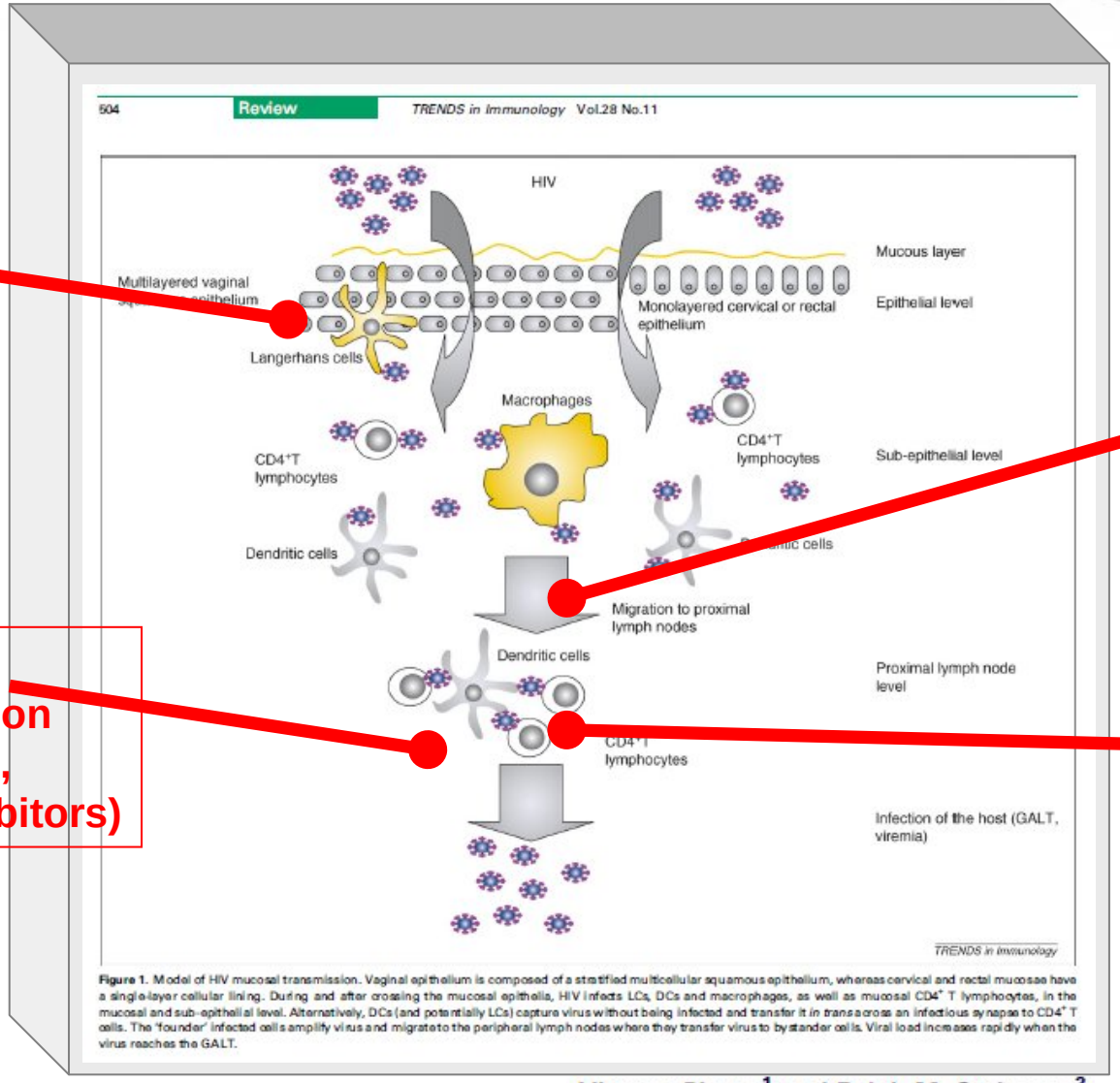


Figure 1. Model of HIV mucosal transmission. Vaginal epithelium is composed of a stratified multicellular squamous epithelium, whereas cervical and rectal mucosae have a single-layer cellular lining. During and after crossing the mucosal epithelia, HIV infects LCs, DCs and macrophages, as well as mucosal $CD4^+$ T lymphocytes, in the mucosal and sub-epithelial level. Alternatively, DCs (and potentially LCs) capture virus without being infected and transfer it *in trans* across an infectious synapse to $CD4^+$ T cells. The 'founder' infected cells amplify virus and migrate to the peripheral lymph nodes where they transfer virus to bystander cells. Viral load increases rapidly when the virus reaches the GALT.

Vincent Piguet[1] and Ralph M. Steinman[2]

Potential Applications

Enhancing vaccine potential by encapsulating antigens and autophagy inducers (mTor inhibitors) into nanoparticles for transdermal delivery

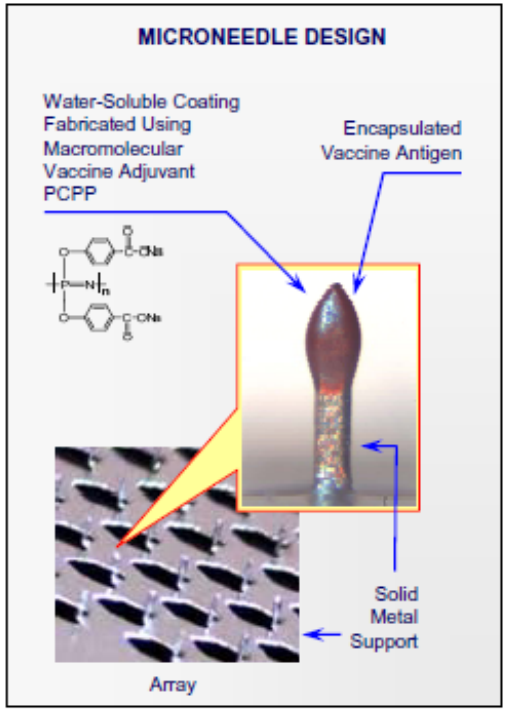


Figure 1. Structure of PCPP and schematic presentation of microneedle array design.

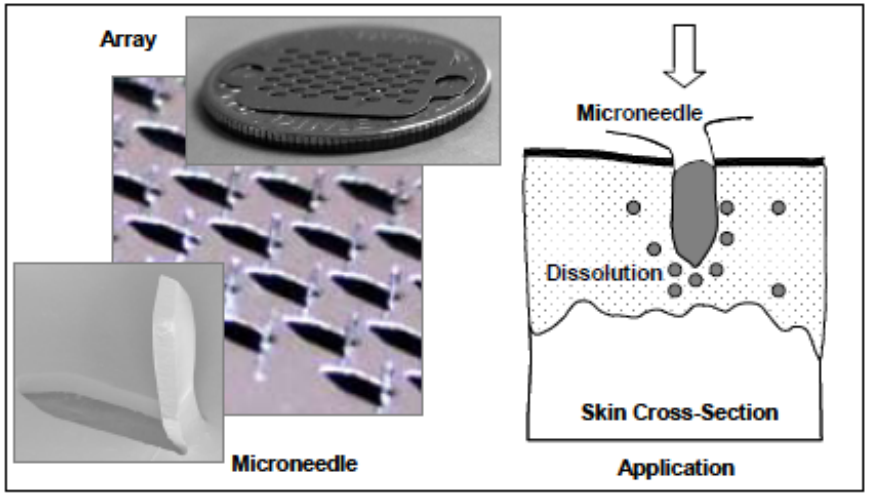


Fig. 1. Scanning electron microscopy image of a single microneedle, optical microscopy image of the microneedle array, and schematic presentation of the application process.

Adapted from Alexander K. Andrianov, Apogee, Boston

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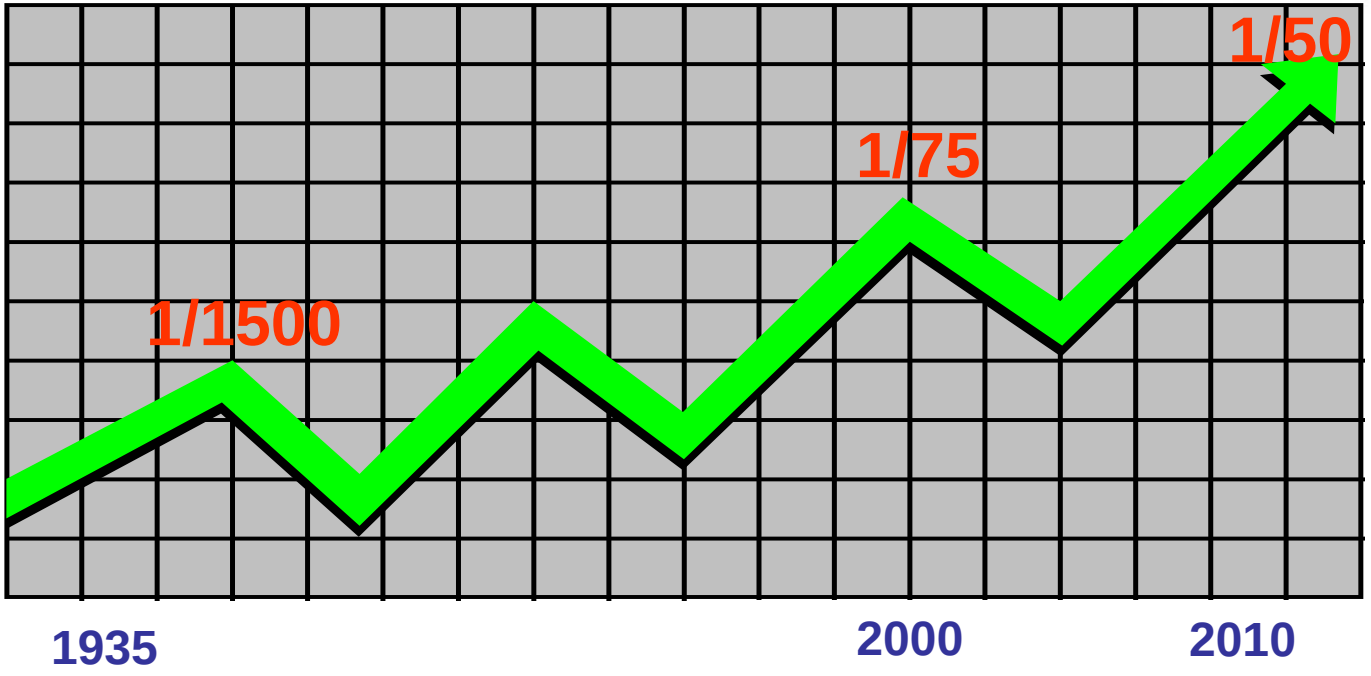
- Conclusions and perspectives

Melanoma: epidemiology et « epidemics »



Melanoma is - the most frequent tumor in women aged 25-29
- the third most frequent tumor among 20-39 (M+F)

Tumor type that leads to high numbers of years lost, only behind CNS tumors

Nevus

↓

Atypical Nevus

↓ ?

Malignant Melanoma

↓ ?

Metastatic disease

Translational research: Global genomic Analysis of melanoma

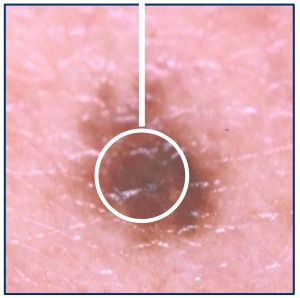
Genetic signatures

Mechanisms of tumor formation

Prognostic markers

Therapeutic targets

Atypical Nevus

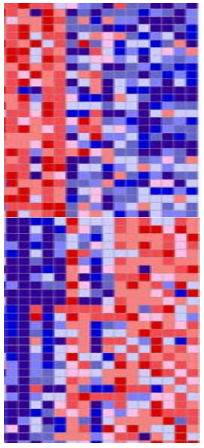


HUG Hôpitaux Universitaires de Genève Service de Dermatologie

Excision

Extraction RNA

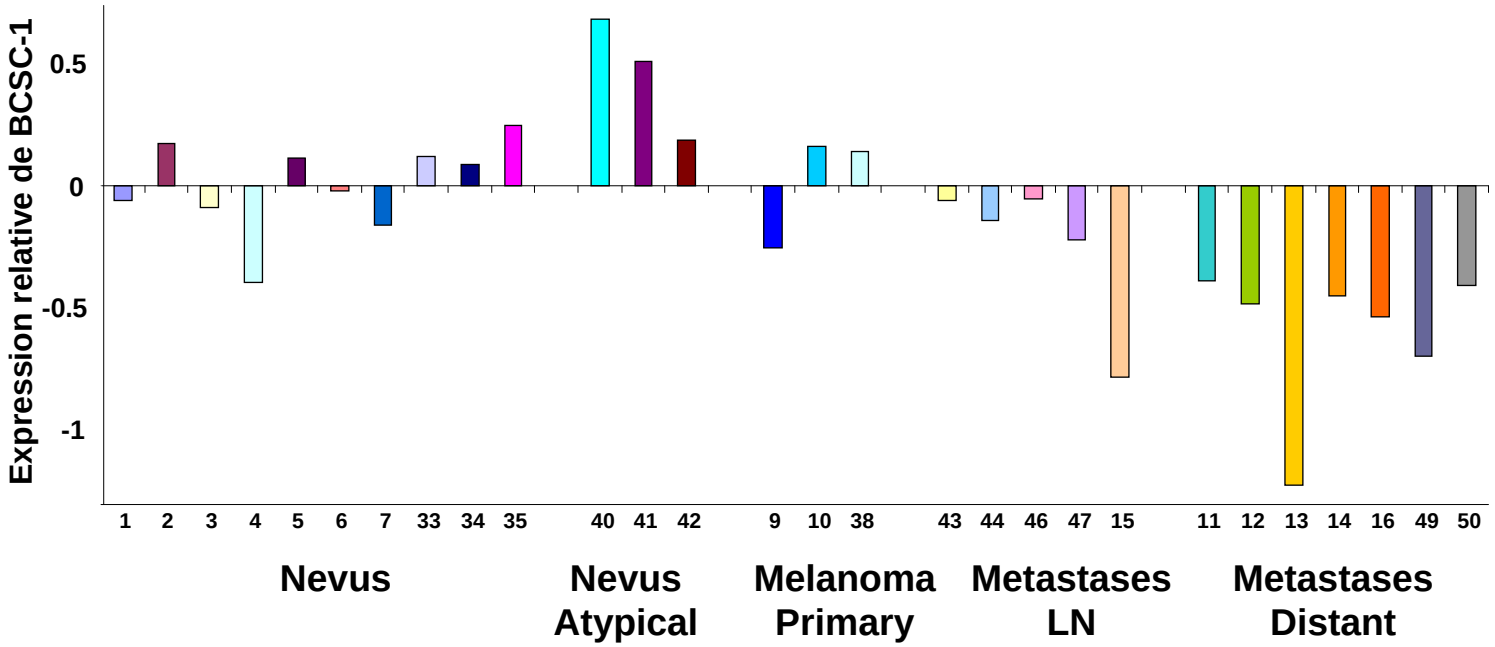
Alternative Splicing
DATAS
Gene Expression
microarrays



NATIONAL CENTER OF COMPETENCE IN RESEARCH nccr molecular oncology

Collaborations:
Stecca, PNAS, 2007
Preynat-Seauve, Cancer Research, 2007

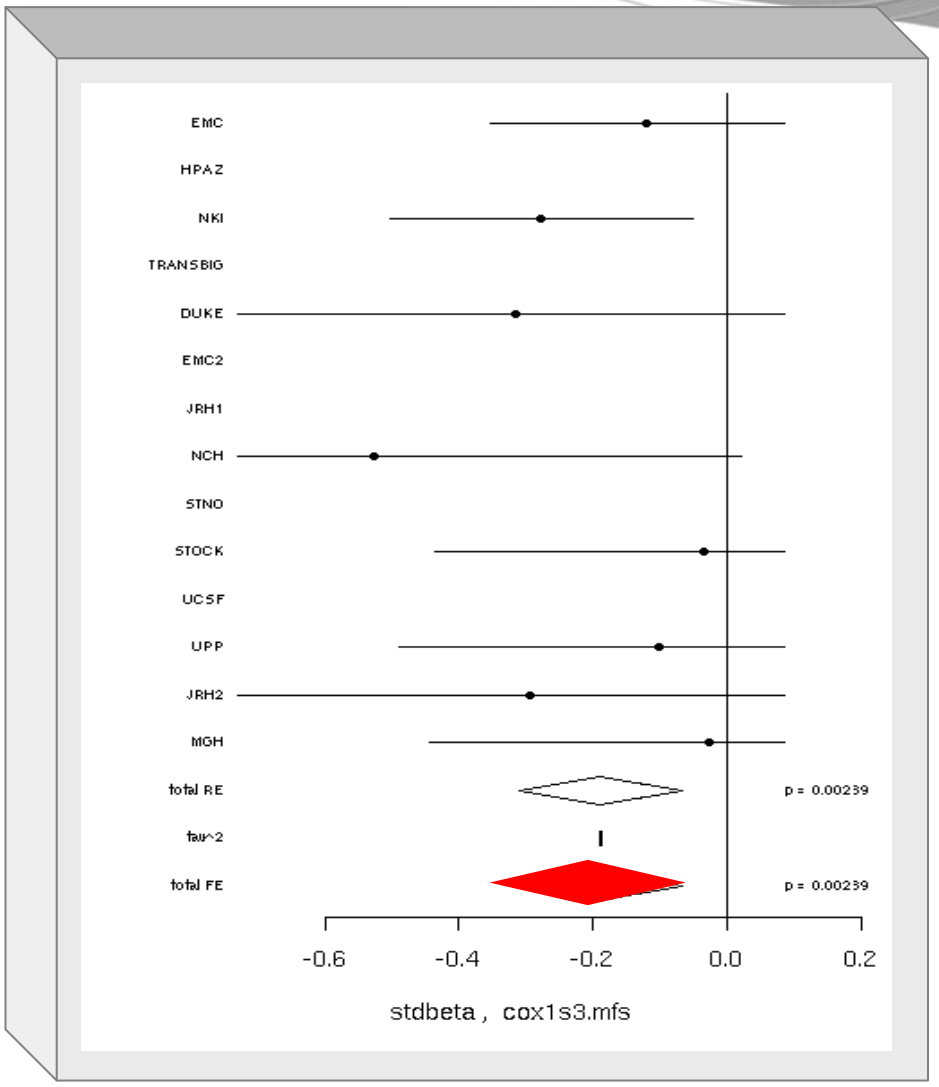
Validation of gene candidates potentially involved in tumor progression: BCSC-1



Loss of expression of BCSC-1 in metastatic melanoma

Analysis of survival in silico in human tumor gene banks

Better survival in patients correlates positively with BCSC-1 expression



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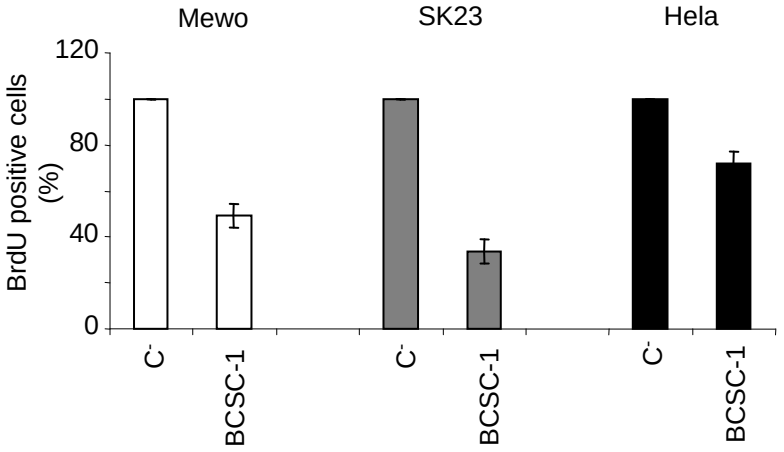
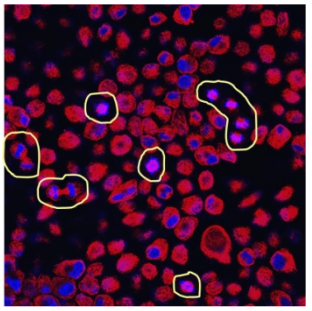
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

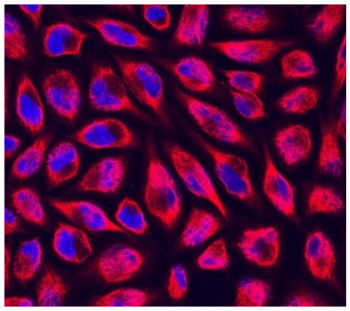
Manuscript in preparation

BCSC-1 reduces melanoma cells proliferation (block in G2/M) but increases their migration



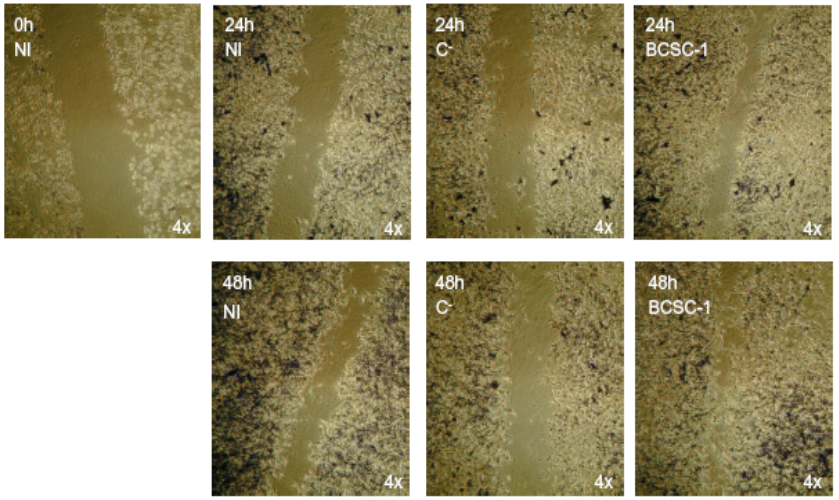


Ctrl (mitosis)

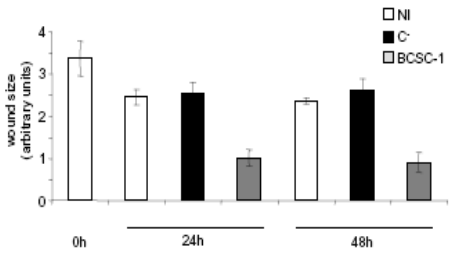


BCSC-1

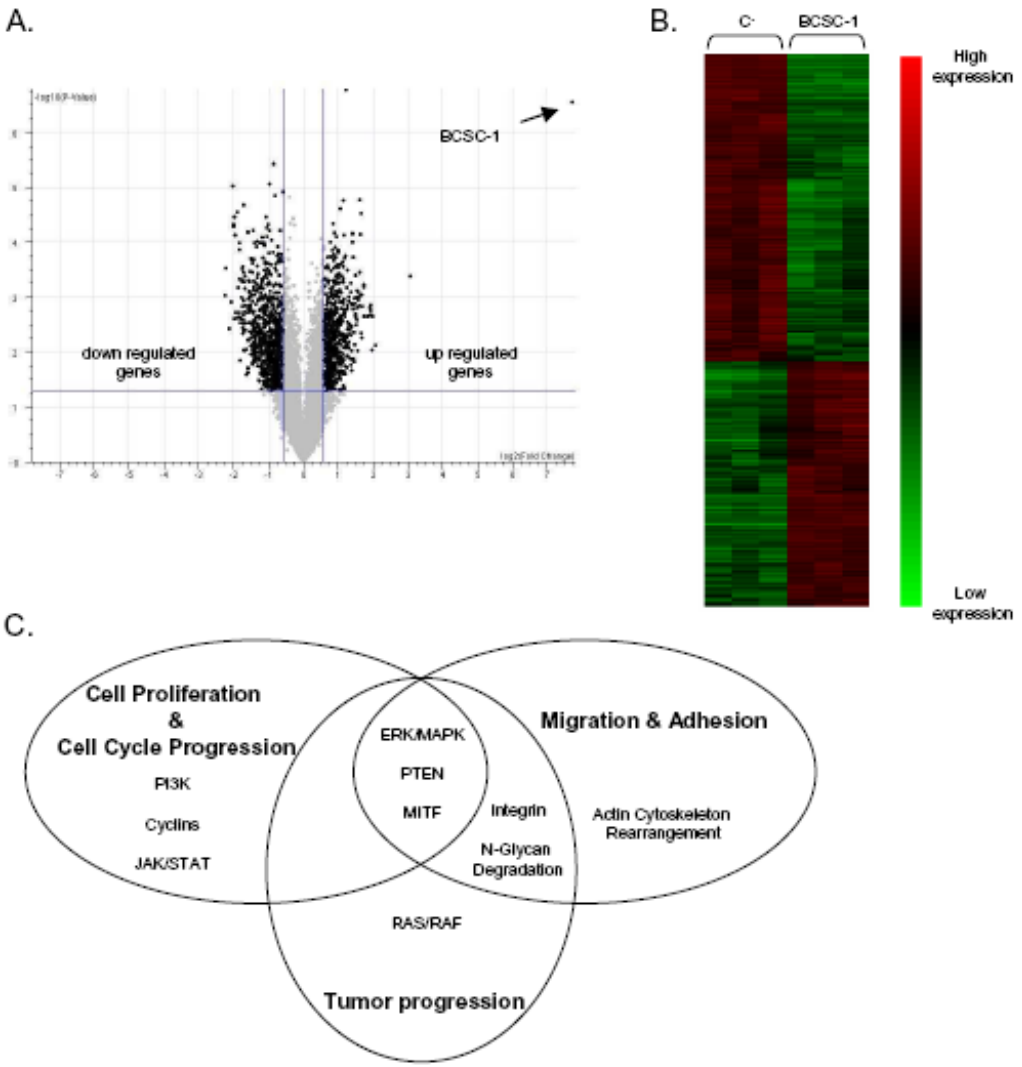
A.



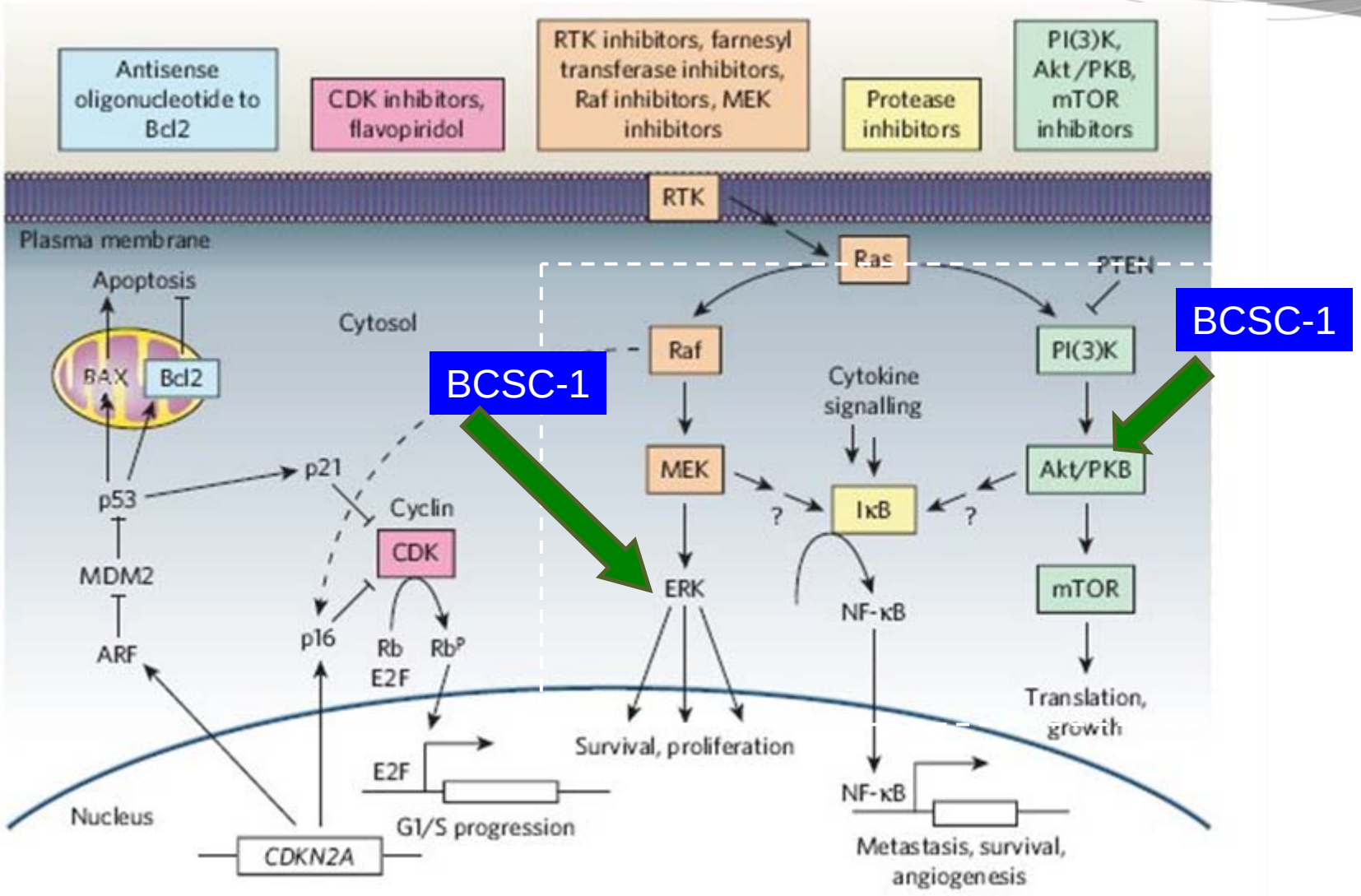
B.



BCSC-1 modulates ERK signaling and MITF



Therapeutic applications and melanoma genomics: Targeted therapies for melanoma



Modified from Gray-Schopfer et al, Nature, 2007

Outline

- Introduction
- Dendritic cells, Autophagy and Pathogens
- Skin cancer: Melanoma and Cutaneous lymphoma
- Conclusions and perspectives

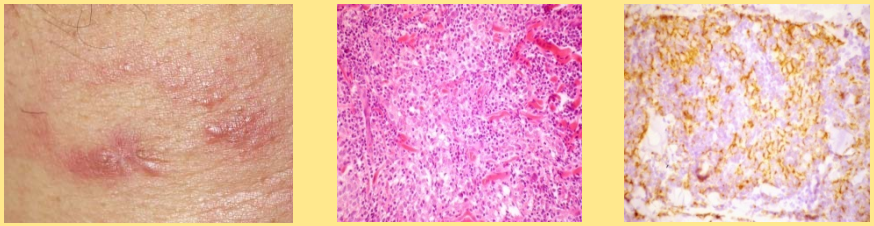
Cutaneous lymphoma

- Heterogeneous group of B and T neoplasia affecting primarily the skin
- Low grade lymphomas but aggressive forms (f.i. Sezary)
- Survival depends on early diagnosis
- Mechanisms still unknown: tumor-host interactions

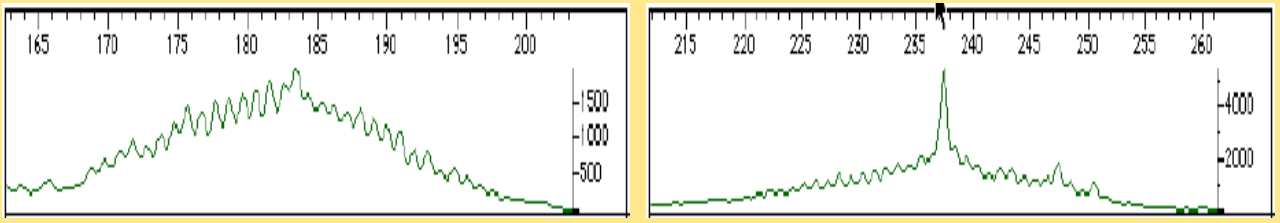
from JL. Alibert, 1833

Diagnostic: clinical, pathology, immunology et molecular biology

Clinical features , Histology and immunohistology: major criteria

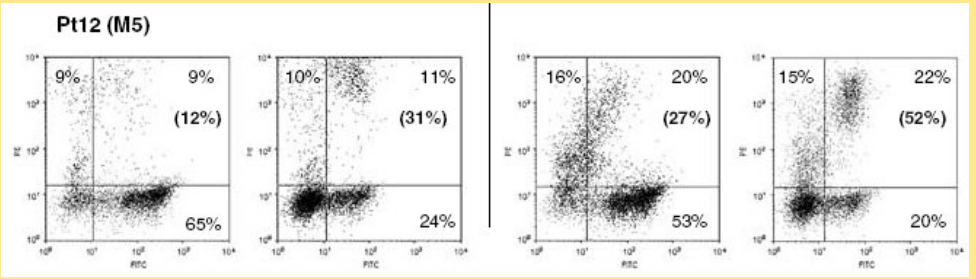


Molecular biology (T/B cell receptor rearrangements Skin/blood)



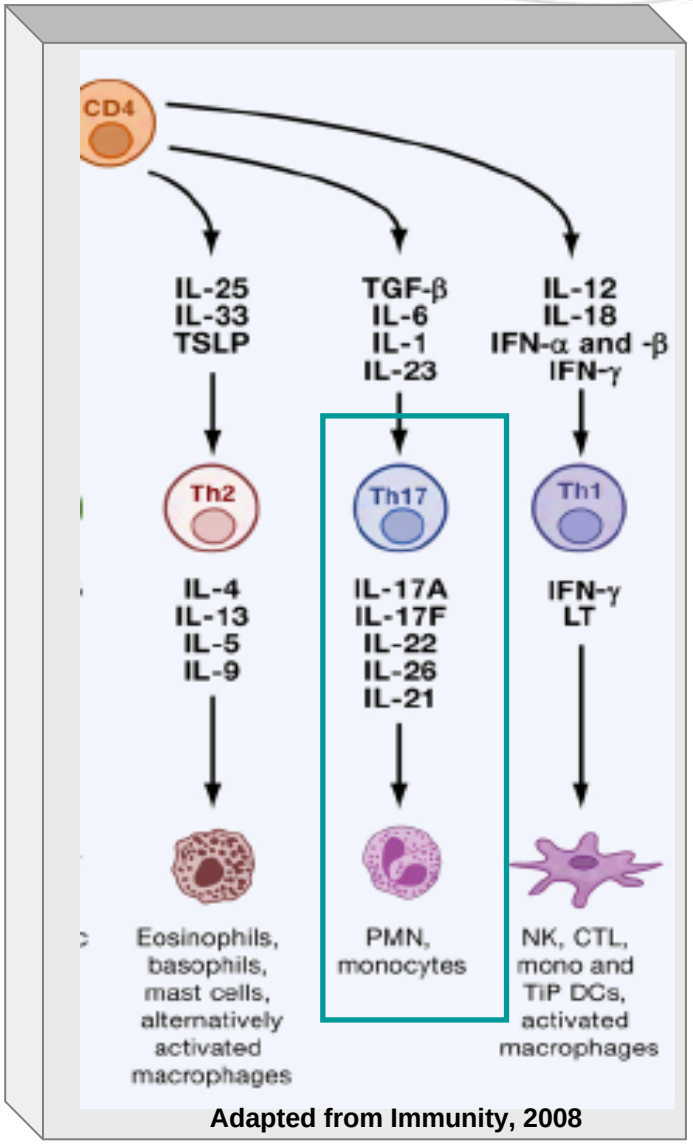
control **Cutaneous lymphoma**

Immunology : flow cytometry – Abnormal lymphocytic populations



Research: Immune polarization in cutaneous lymphoma

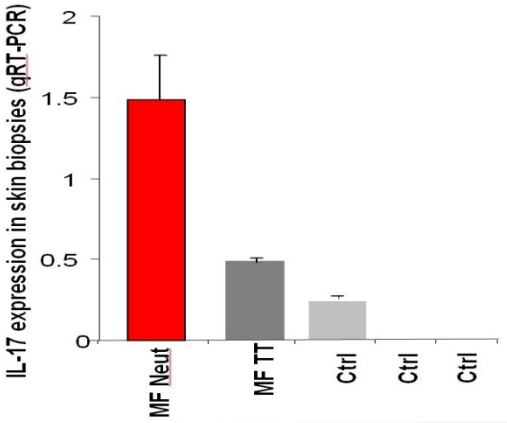
- Analysis of function of the immune system polarization during CTCL: Th2
- Sezary syndrome: deficit in CD40 ligand
  (collaborations: Huard and al, Blood, 2005)
- Study of a subgroup of mycosis fungoides associated with neutrophilic reactions

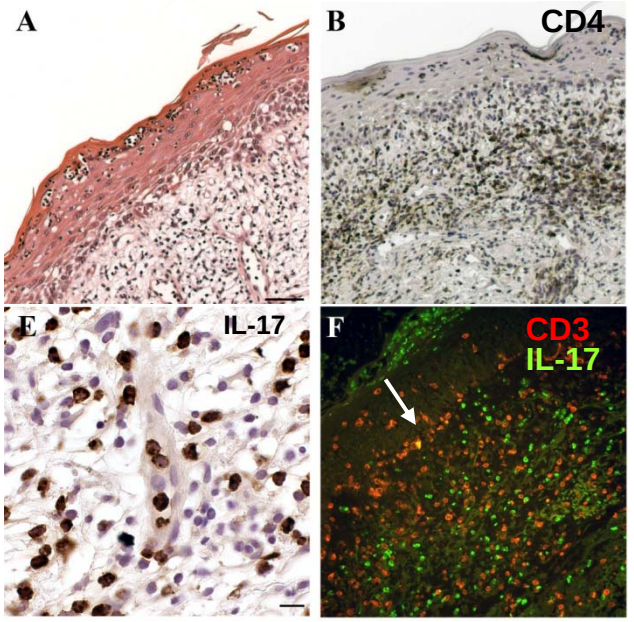


Adapted from Immunity, 2008

CTCL with neutrophilic reactions: IL-17

- patients Geneva-Paris (French CTCL group): subgroup of CTCL with poor prognosis
- Modulation of Th-17 cytokines





Fontao et al., in preparation

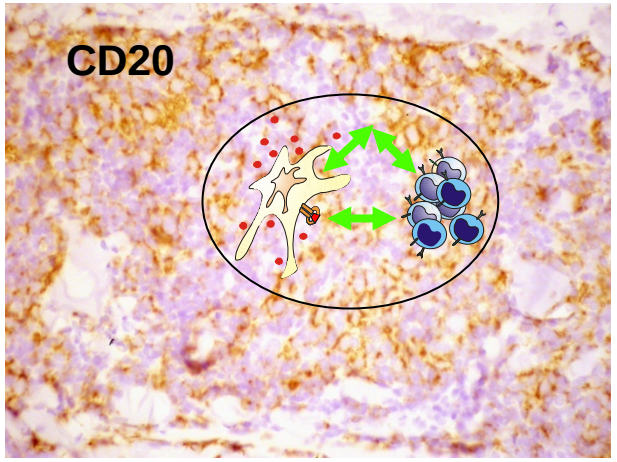
Therapeutic implications: Immunomodulation during B or T cell cutaneous lymphomas

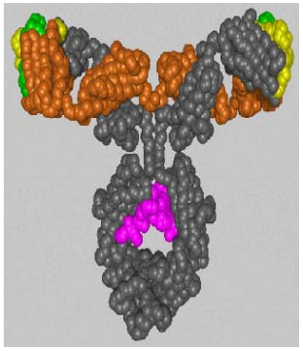
Already in the clinic:

- Alpha-Interferon
- Rexinoids (bexaroten)
- Photopheresis
- monoclonals (anti-CD20, CD52, CD4,CD25…)

In development:

- HDAC inhibitors (modulation cytokines Th2)
- mTor inhibitors (everolimus)
- New monoclonals





Monoclonal antibody

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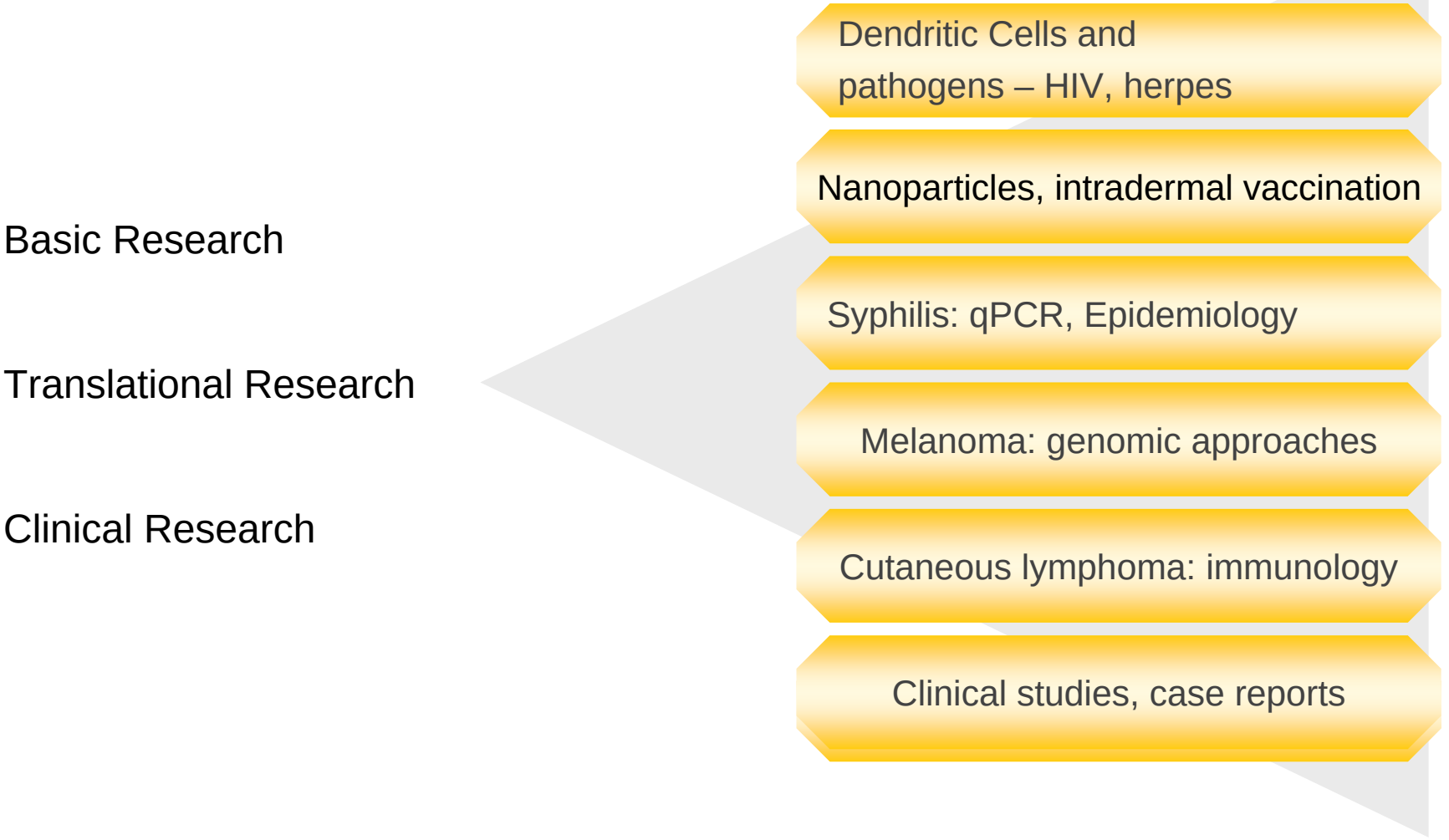
Conclusions

1. Investigative Dermatology is a broad scientific discipline including but also extending beyond “classical” skin diseases (eczema, psoriasis…)

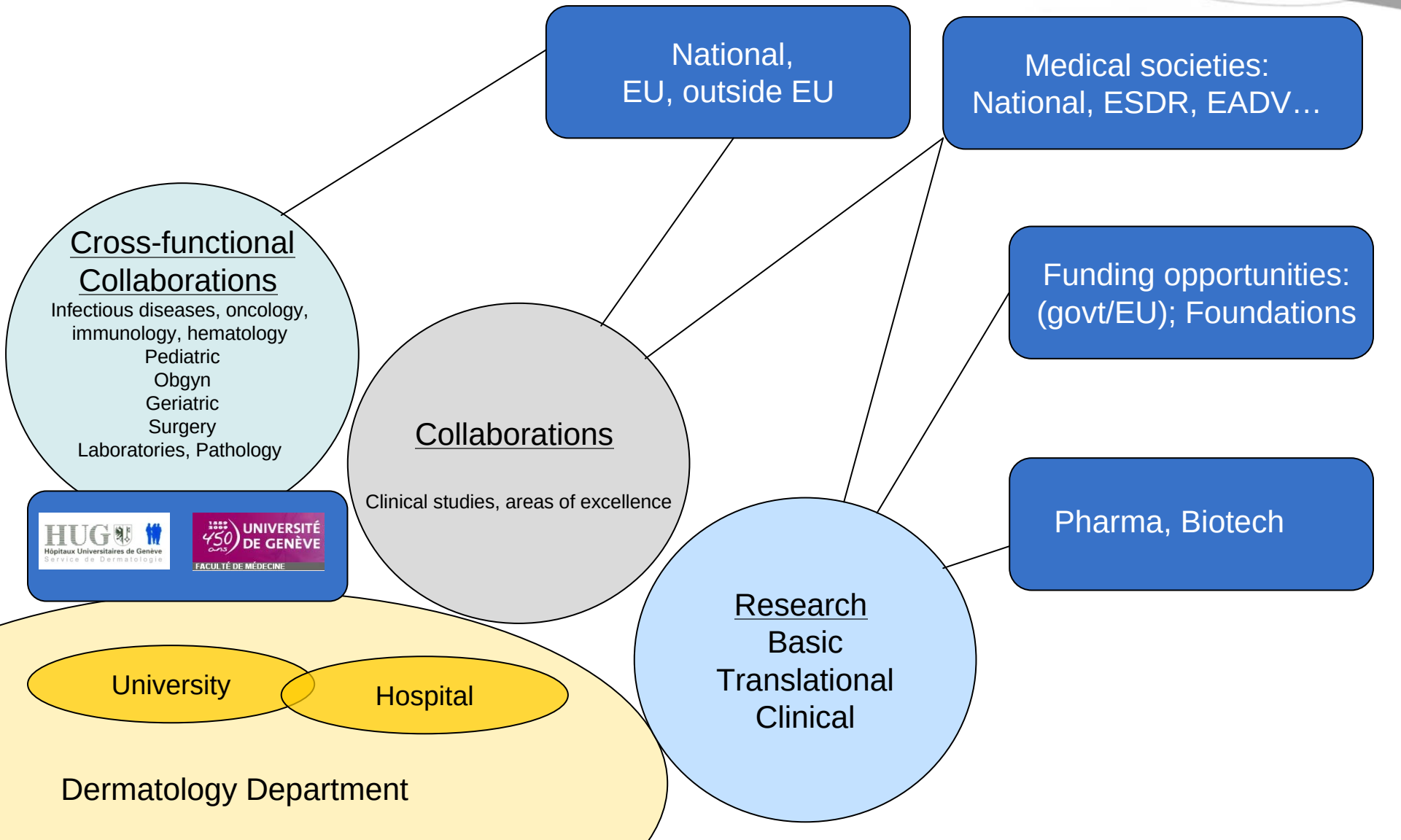
2. Several of the basic mechanisms we have studied have implications that apply to other skin conditions (f.i mTOR inhibitors) as well as other areas of medicine (infections/immunology)

3. Projects involving translational aspects (clinical-research) require a close collaboration between the laboratory and the clinicians

Future areas of investigation in Clinical and Investigative Dermatology



Local and international collaborations to support a dynamic program



Main events for investigative dermatology and sister societies

EUROPEAN SOCIETY FOR DERMATOLOGICAL RESEARCH

2010 **40th Annual ESDR Meeting**
Helsinki, Finland
8-11 September 2010

2011 **41st Annual ESDR Meeting**
Barcelona, Spain
7-10 September 2011

2012 **42nd Annual ESDR Meeting**
Venice, Italy
19-22 September 2012

2013 **International Investigative Dermatology**
Edinburgh, Scotland
8-11 May 2013

EADV

Society for Investigative Dermatology

The Japanese Society for Investigative Dermatology

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Dermatology

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R.Stalder(DIP)
G.Ghers (diplômant)

JF. Arrighi
M.Pion
G.Blot
JM.Escola

STI Clinical/epidemiology
L.Trellu, S.Abraham (HUG)

Melanoma and CTCL
R.Correa-Rocha (NCCR)
S.Anghel (MEDIC)
F.Jaunin
F.Goldenberg (Carigest)

Clinical research
C.Prins, S.Abraham, L.Fontao (HUG)

Collaborations

STI, Dendritic cells
R.Steinman, Rockefeller, NY, USA
V.Deretic, New Mexico, USA
G.Towers, UCL, London, UK
O.Schwartz, Institut Pasteur Paris
M. Marsh, UCL, London, UK
C.Aiken, Nashville, USA
Y. Van Kooyk, T. Geijtenbeek, Amsterdam
D.Trono, EPFL
J.Luban, MIMOL, Genève
C.Aiken, Nashville, USA
Q.Sattentau, Oxford, UK
B.Hirschel, Mal. Infectieuses, Genève
O.Levy, Harvard medical school, US

Melanoma and CTCL
T.Matthes, hématologie, Geneva
A.Ruiz i Altaba, Geneva
G.Kaya, dermatologie, Geneva
L.French, dermatologie, Zurich
J.Tschopp, IB, Epalinges
N.Dupin, Cochin, Paris
R.Braun, Zurich
L.Bracco, Exonhit Therapeutics, Paris
I.Stamenkovic, Pathologie, CHUV
C.Ruegg, Oncologie, CHUV
G.Ghanem, Brussels, Belgium
P. Decombes, NCCR-UNIGE, Geneva
M.Delorenzi, SIB, Epalinges

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Medic, Carigest

Pharma/biotech: Novartis, GSK, Sanofi-Pasteur, Exonhit …

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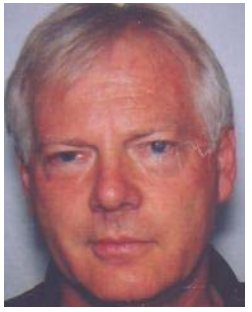
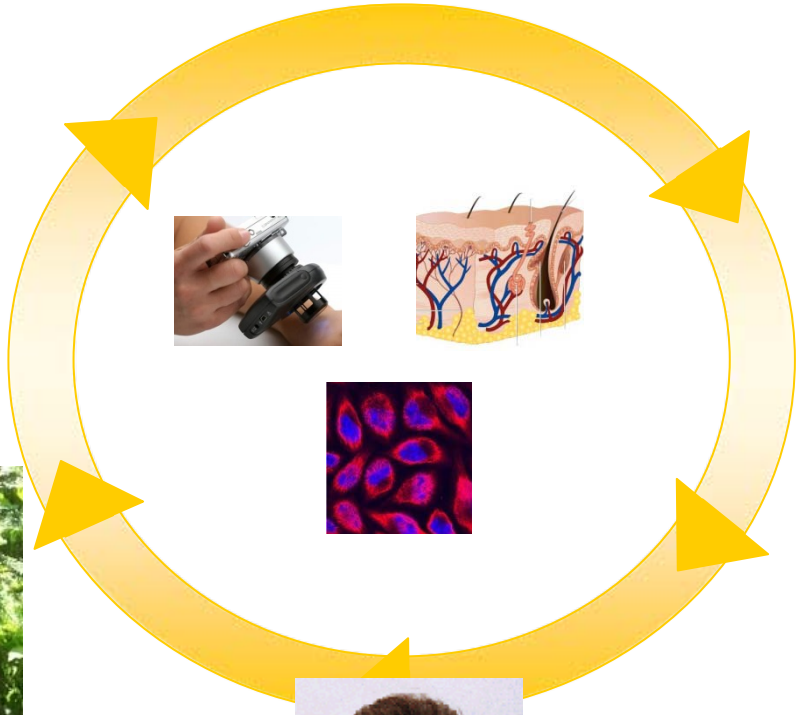
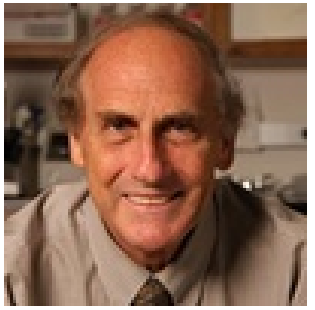
JH Saurat, Geneva



J.Tschopp, Epalinges, CH



D.Trono, Salk Institute, US
EPFL-Lausanne



R.Steinman, Rockefeller, US

M.Marsh, University College London