Universidade de São Paulo

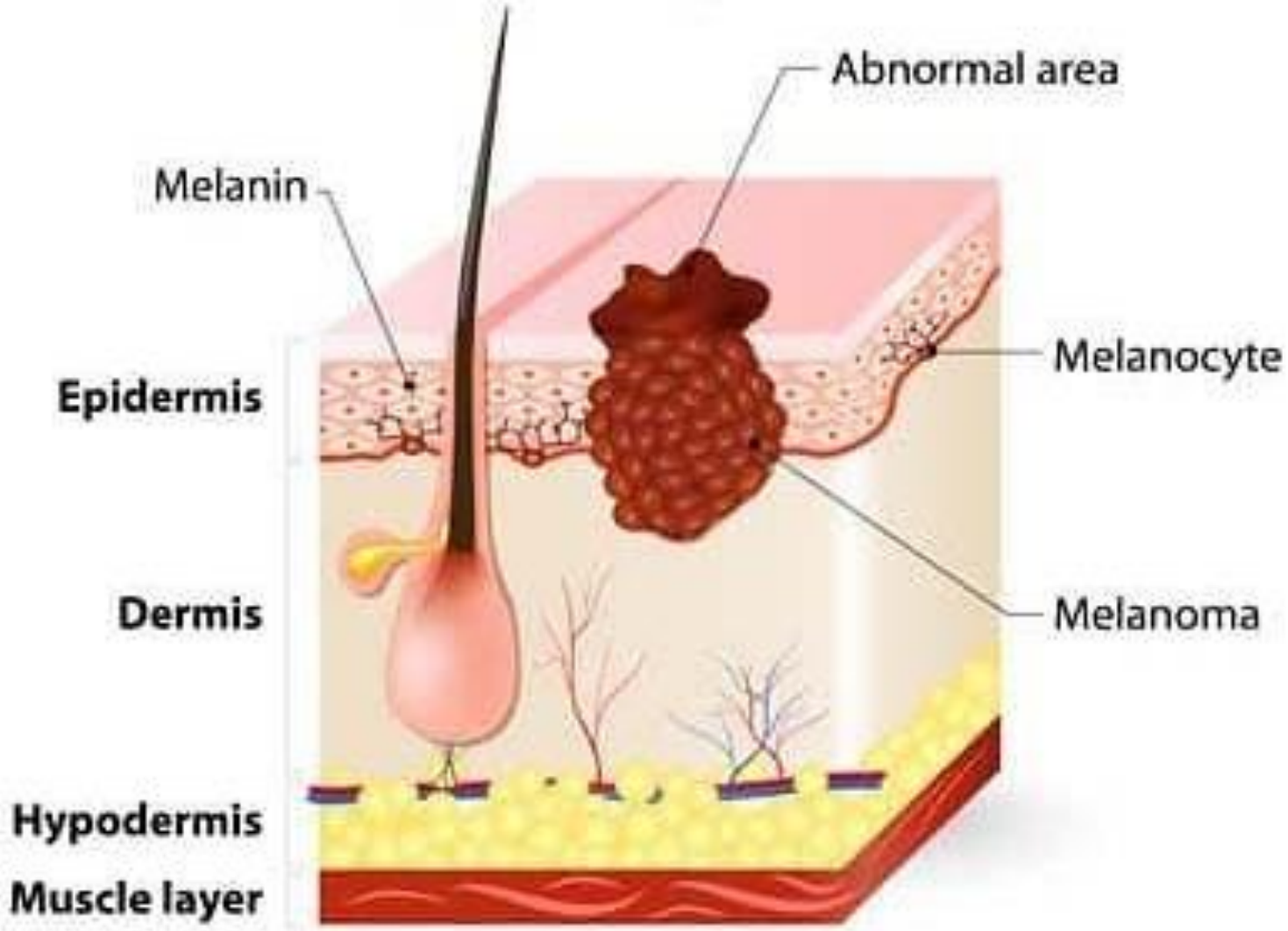
2nd
PAN-AMERICAN
Conference for Alternative Methods
August 23-24, 2018
Rio de Janeiro

A stochastic clonal selection for modeling intra tumor heterogeneity in human melanoma and clinical implications

Larissa A. C. Carvalho

lari.anastacio@gmail.com

Melanoma of the skin

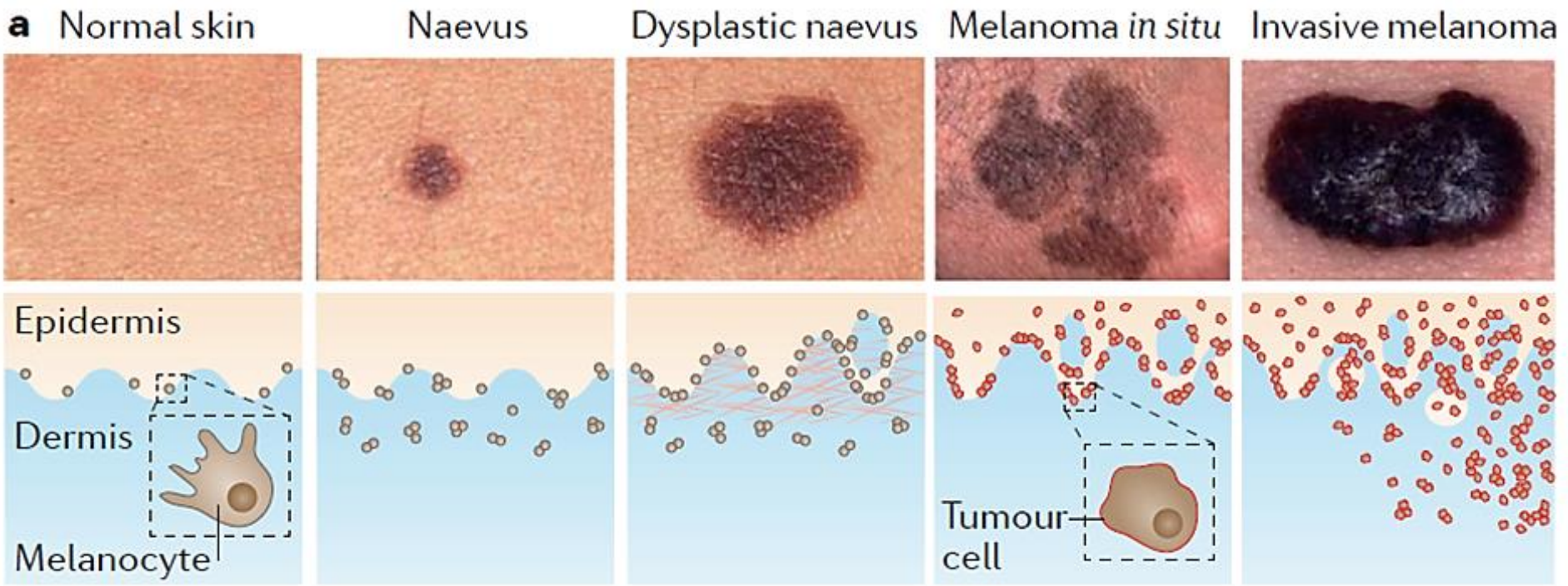


Melanoma progression: mutational status

Mutations:
BRAF, MYC, NRAS

Senescence supression
Apoptosis supression
Telomeres alterations

Accumulating mutations



Shain, ***Nature Reviews in Cancer***, 2016

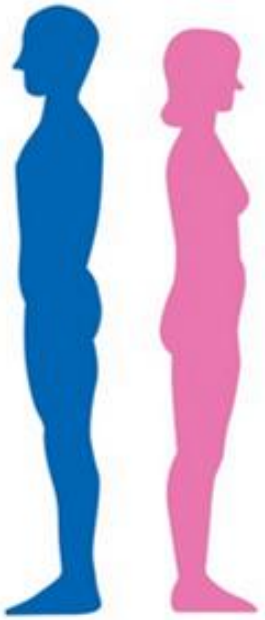
Melanoma skin cancer rates are on the rise

- Skin cancer: **30% of all tumors**

- Melanoma: **4-6% of all cases** but the most aggressive (**80% of skin cancer deaths**)

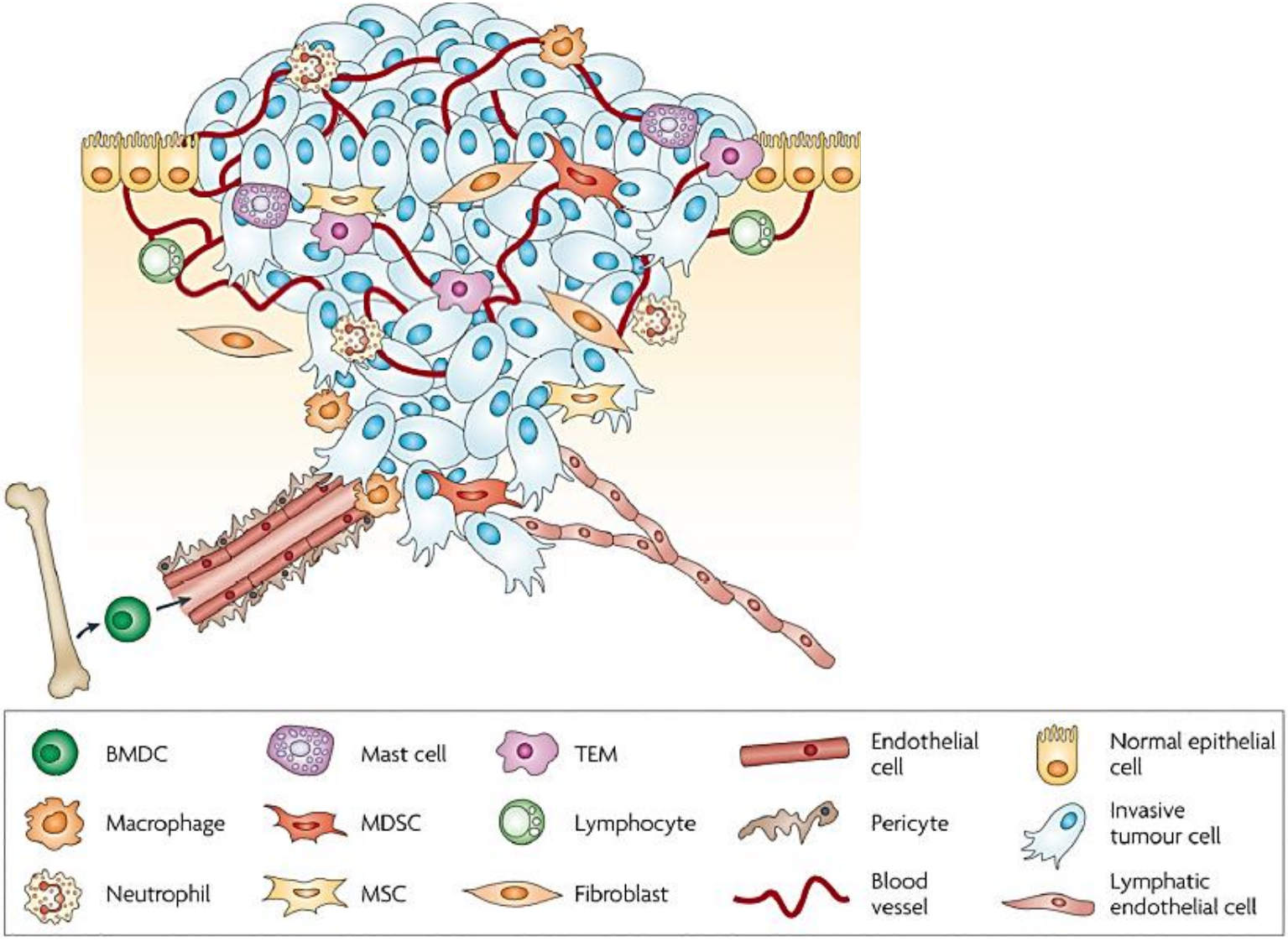
Change in melanoma incidence since 90's **+128%**

Males			Females		
Prostate	164,690	19%	Breast	266,120	30%
Lung & bronchus	121,680	14%	Lung & bronchus	112,350	13%
Colon & rectum	75,610	9%	Colon & rectum	64,640	7%
Urinary bladder	62,380	7%	Uterine corpus	63,230	7%
Melanoma of the skin	55,150	6%	Thyroid	40,900	5%
Kidney & renal pelvis	42,680	5%	Melanoma of the skin	36,120	4%
Non-Hodgkin lymphoma	41,730	5%	Non-Hodgkin lymphoma	32,950	4%
Oral cavity & pharynx	37,160	4%	Pancreas	26,240	3%
Leukemia	35,030	4%	Leukemia	25,270	3%
Liver & intrahepatic bile duct	30,610	4%	Kidney & renal pelvis	22,660	3%
All Sites	**856,370**	**100%**	**All Sites**	**878,980**	**100%**



Cancer statistics, 2018

Tumor microenviroment



Joyce e Pollard, ***Nature Reviews Cancer***, 2008.

Romano et al., ***International Journal of Molecular Science,*** 2017

Modeling melanoma *in vivo*

Genetically engineered mouse

- Fully functional immune system
- Complete microenviroment
- Genetic manipulation

-Mice rarely develop melanoma spontaneously
- Differences in histology
- Mouse cells

Xenograft

- Human cells
- Complete microenviroment
- Study of spontaneous metastasis

- Lacks the immune system
- Differences in physiology
- Mouse microenviroment

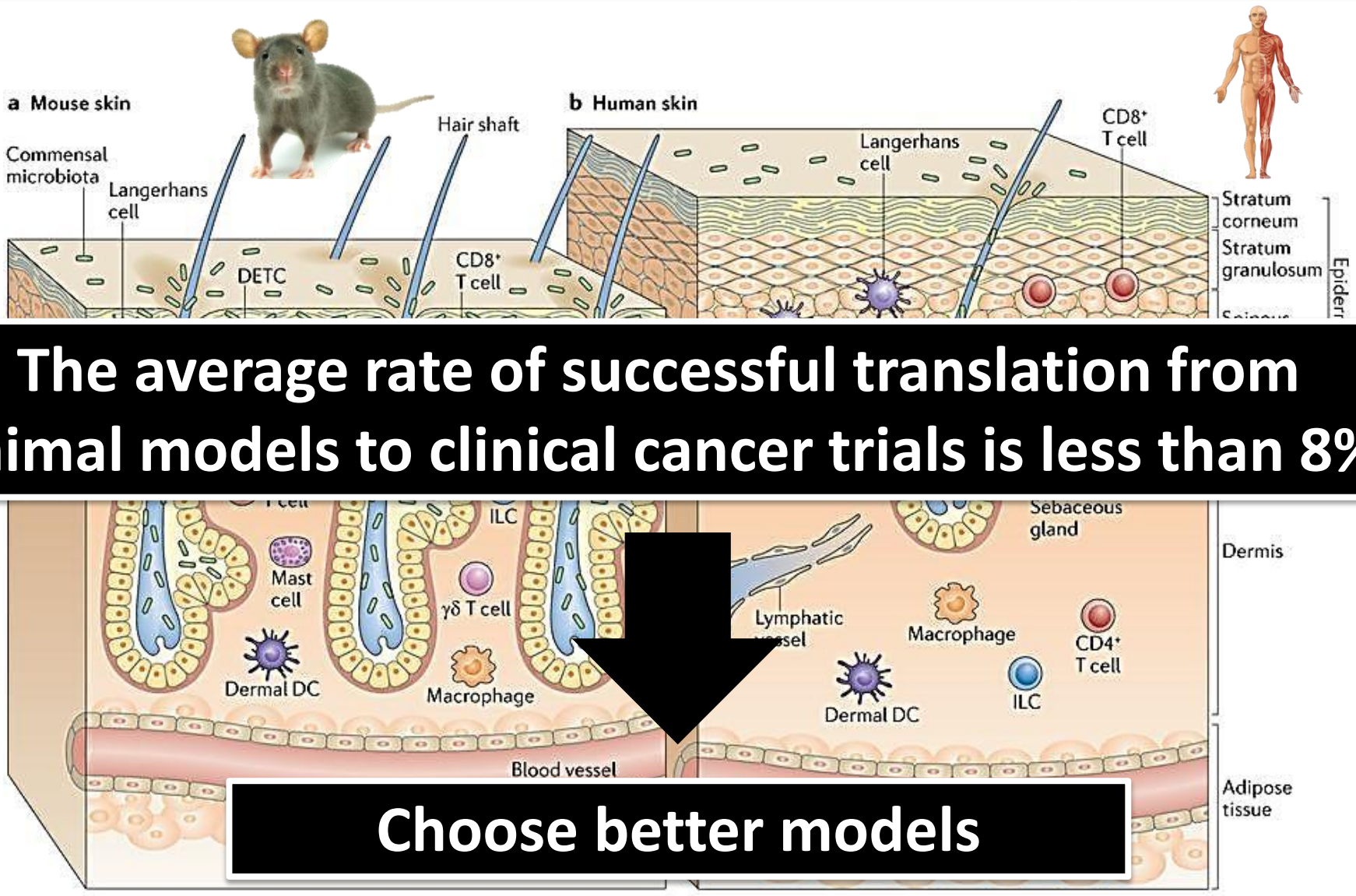
Zebrafish

- Shorter generation time, large number of progeny
- Develop spontaneous melanoma
- Simple genetic manipulation

- Differences in physiology

Beaumont *et al.*, ***Healthcare***, 2014

Human and mouse skin are different

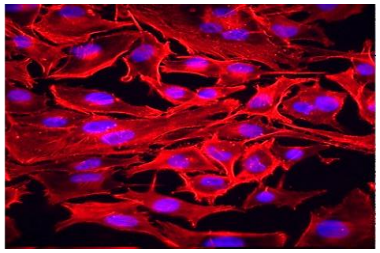


The average rate of successful translation from animal models to clinical cancer trials is less than 8%

Choose better models

Nature Reviews | Immunology

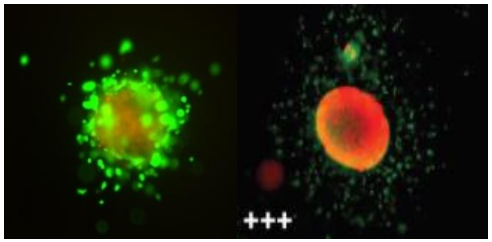
Modeling melanoma *in vitro*



2D cell culture

- Patient cells or cell line
- Simplicity, convenience
- Preliminary drug screening: drugs that do not work in 2D cultures have no effect in more realistic models

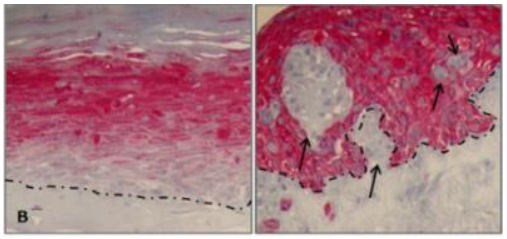
- Lacks the microenviroment



3D spheroid

- Oxygen/nutrient gradient with a hypoxic zone and a central necrosis
- Interaction between melanoma cells and their stroma

- Lacks the whole system



Organotypic skin reconstructs

- Closely resembles histologically human skin
- Cells from patients keep their properties
- Drug screening

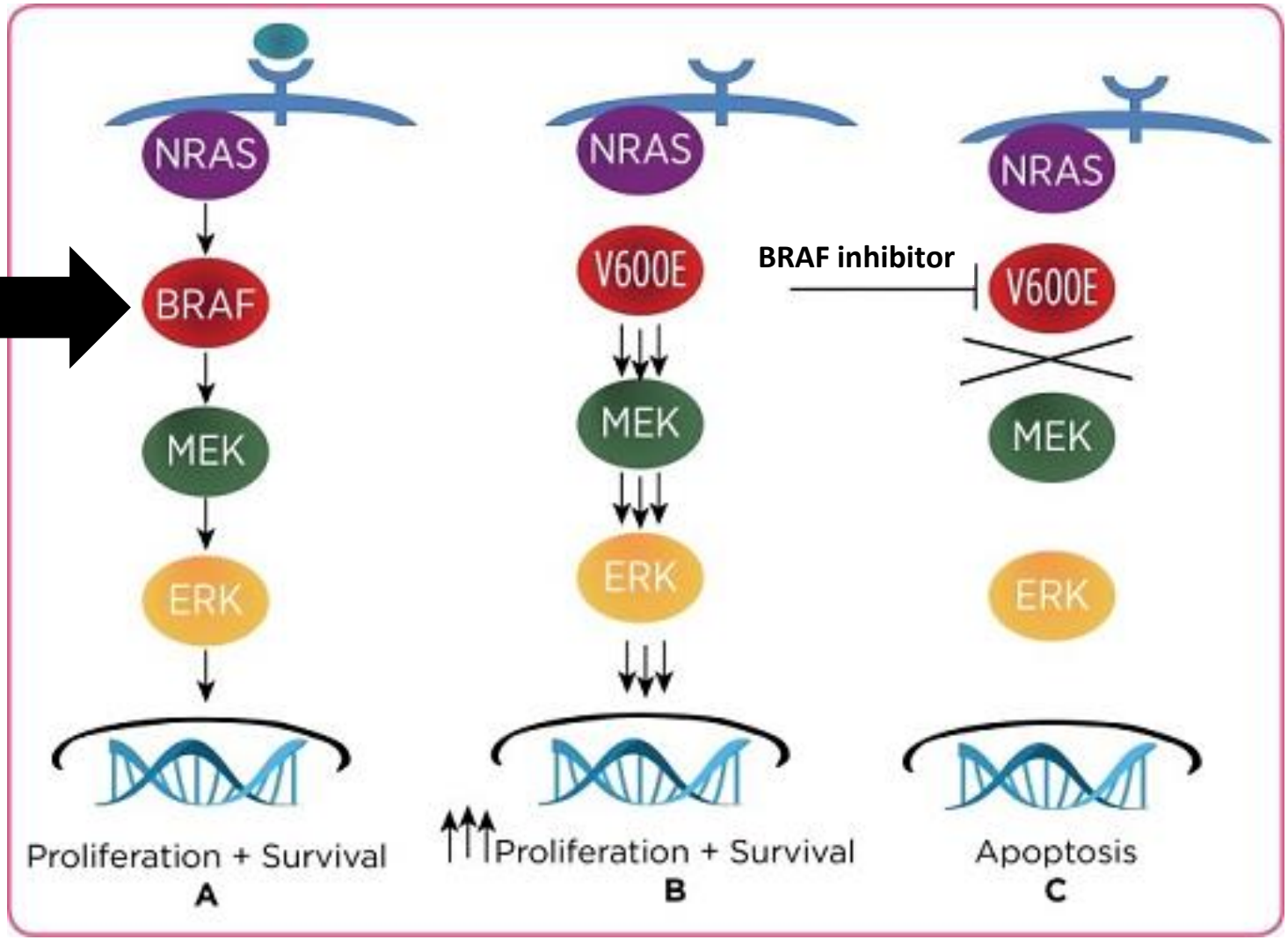
- Long-term development

Brohem *et al.*, ***Pigment Cell Melanoma***, 2011

Oncogene targeted therapy: BRAF inhibitor

MAPKinase pathway

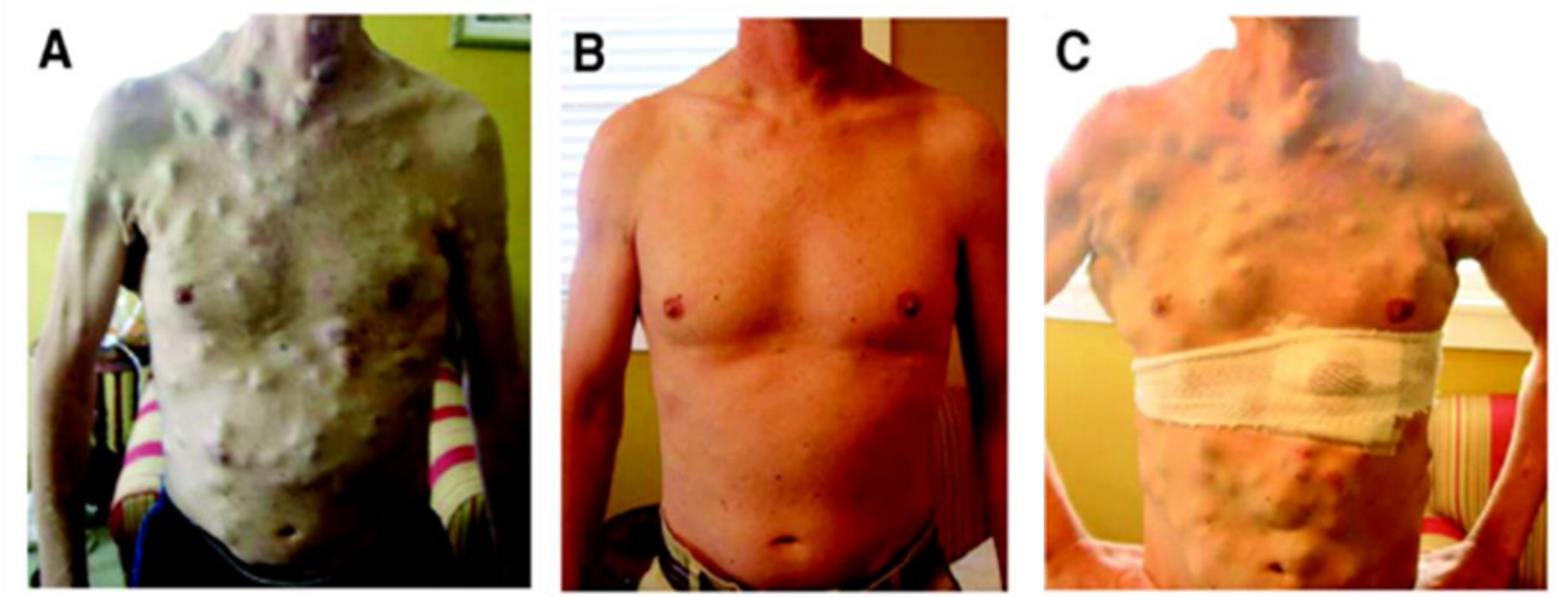
Mutated in 60% of melanomas



Hagen *et al*., ***Journal of Advanced Practitioner Oncology ,*** 2014

Melanoma treatment – Resistance

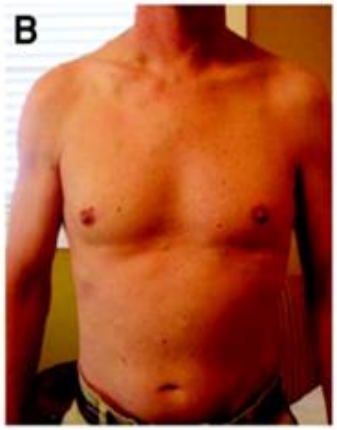
No treatment **15 weeks - Remission** **23 weeks - Relapse**

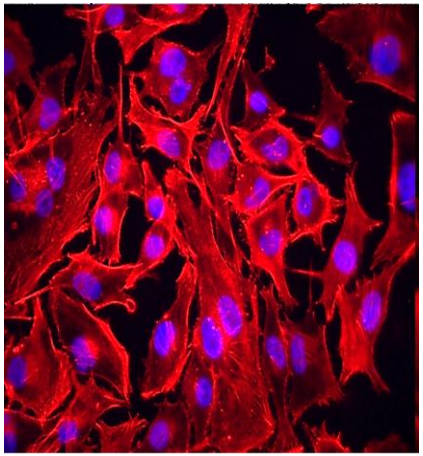


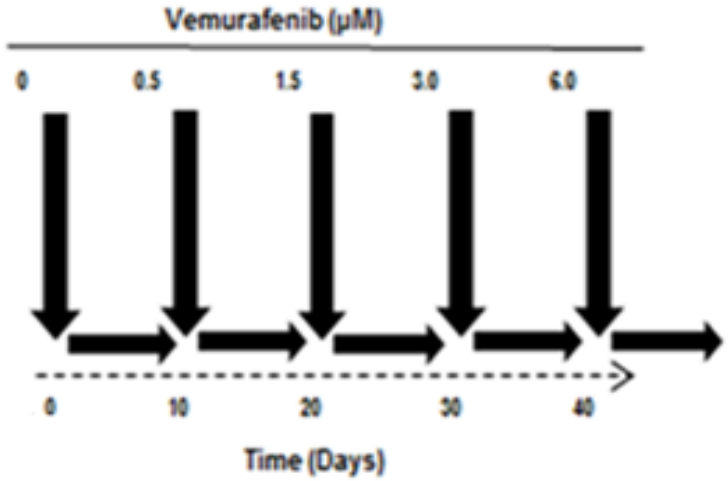
Wagle et al., ***Journal of Clinical Oncology***, 2011

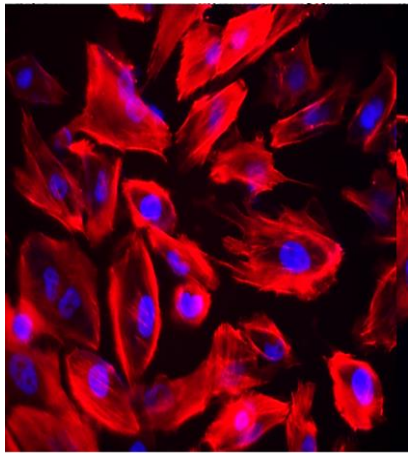
How to model resistance *in vitro*?

Generation of BRAFi resistant cells









ANAKA, 2013; LOSCALZO, 2012; FLAHERTY et al., 2010; SOSMAN et al., 2012

Why does melanoma resistance occur?

Acquired resistance

- Activation of different pathways to overcome the drug

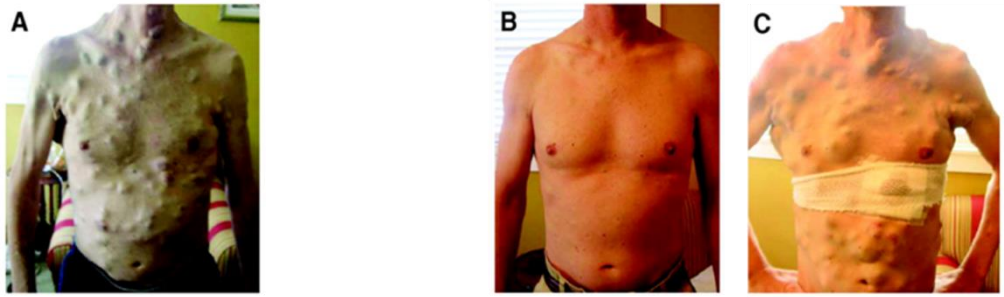
Intrinsic resistance

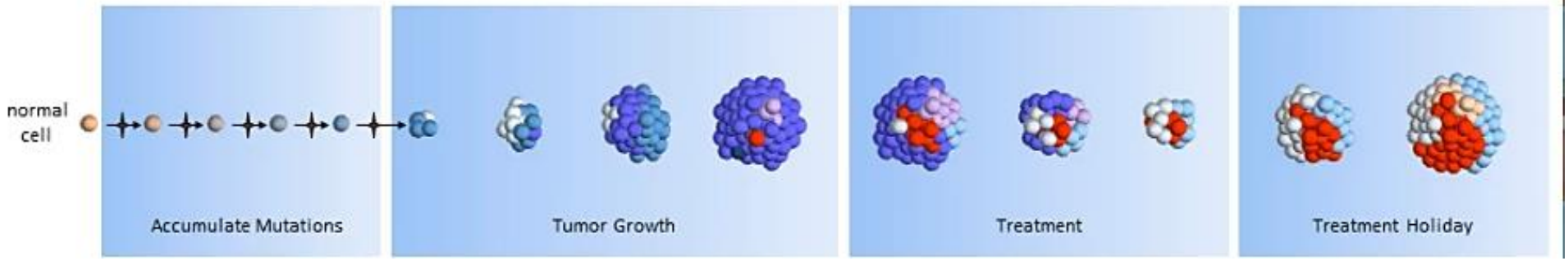
- Cells are resistant to the drug before the use of it

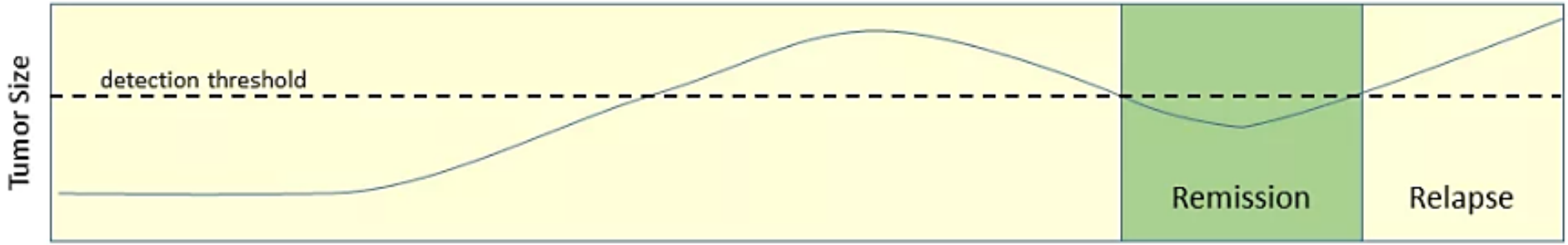
Source: clipground

Intratumor heterogeneity

Tumors comprises heterogeneous subpopulations

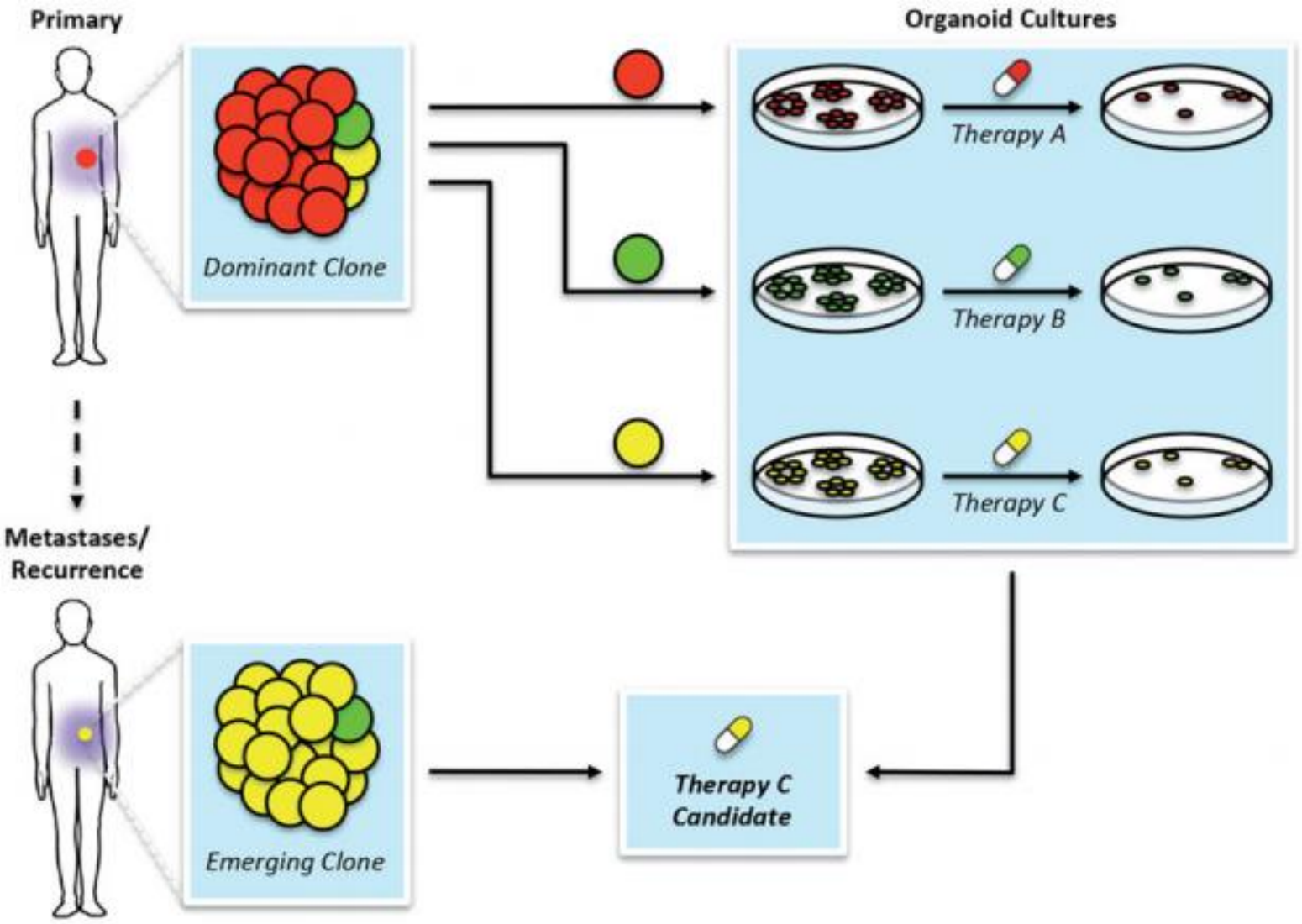






Riveiro-Falkenbach et al., ***Journal of Investigative Dermatology***, 2015

Clinical impact: from *in vitro* to patient

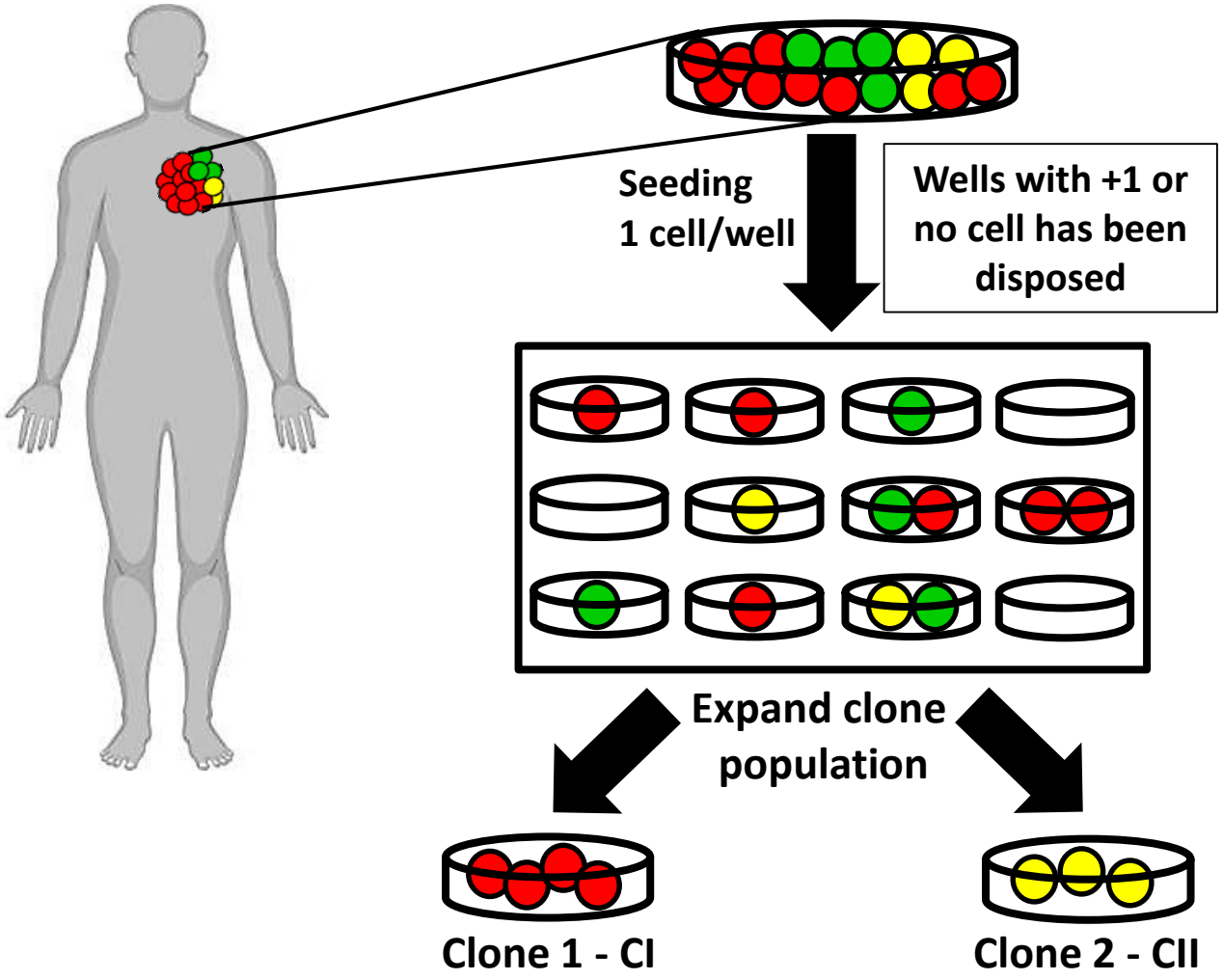


Apostoli and Ailles, ***Critical Reviews in Clinical Laboratory Sciences***, 2015

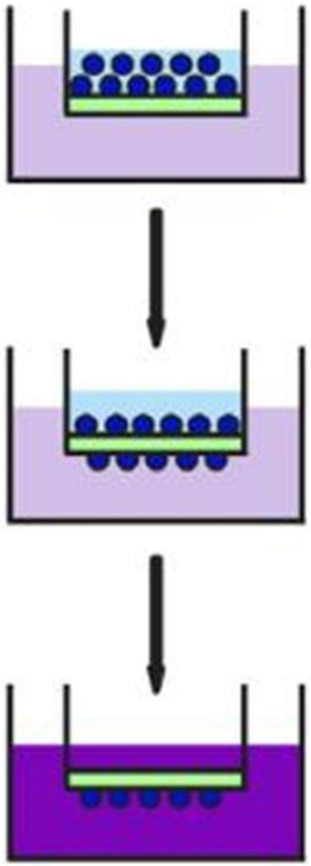
How to model heterogeneity *in vitro*?

Parental heterogeneous population with no drug treatment –

WM164 cell line



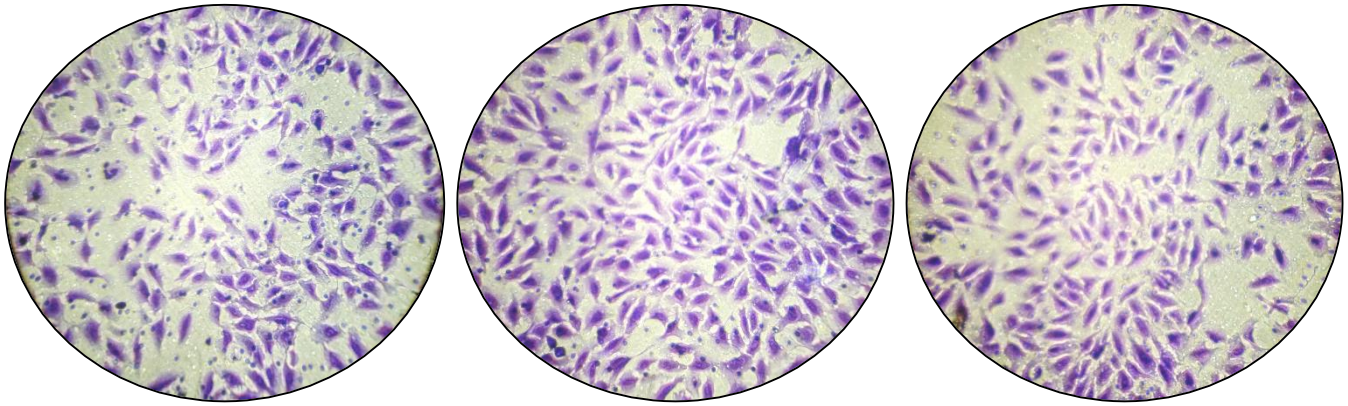
Characterization of the clones: Invasion

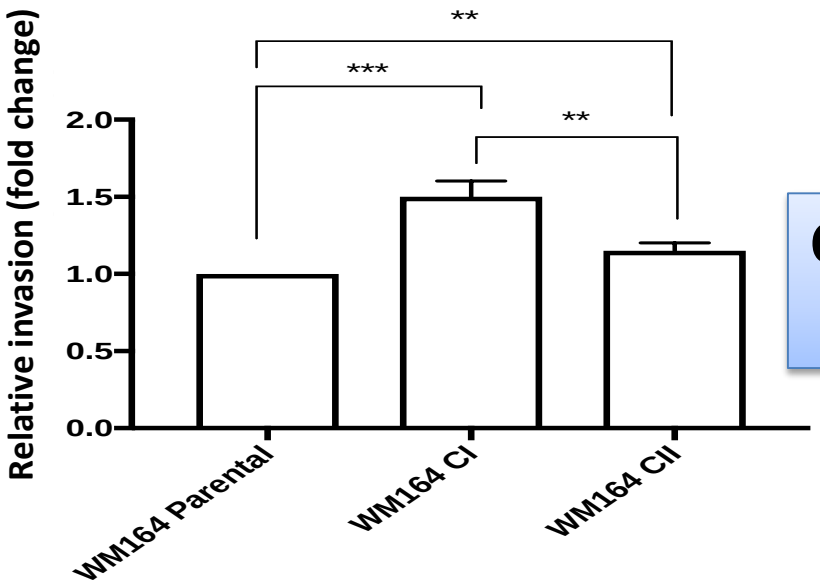


WM164 parental

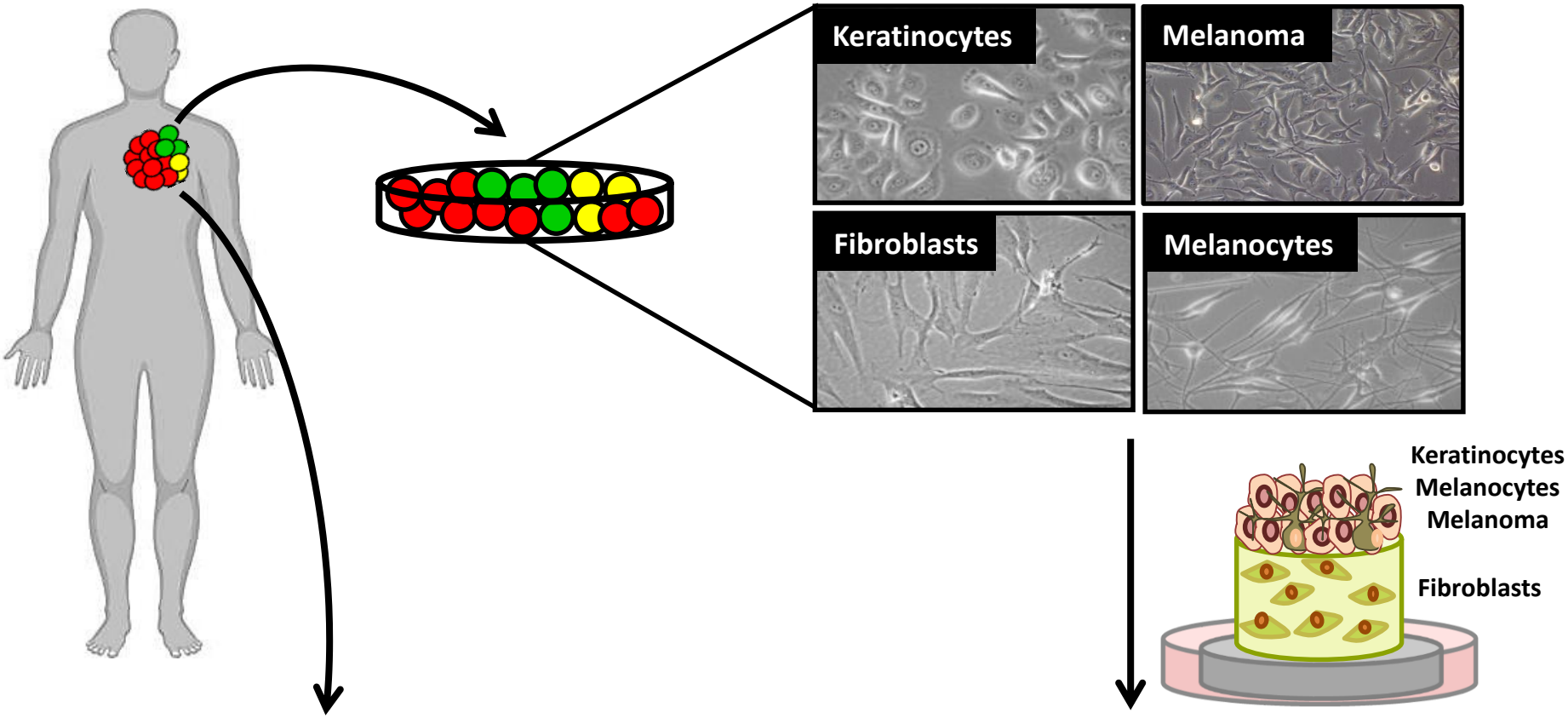
WM164 CI

WM164 CII

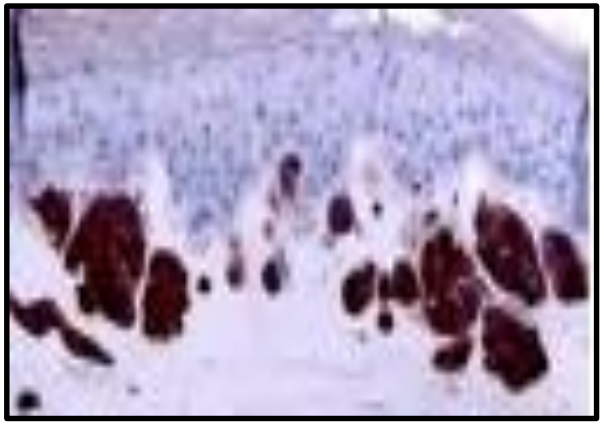




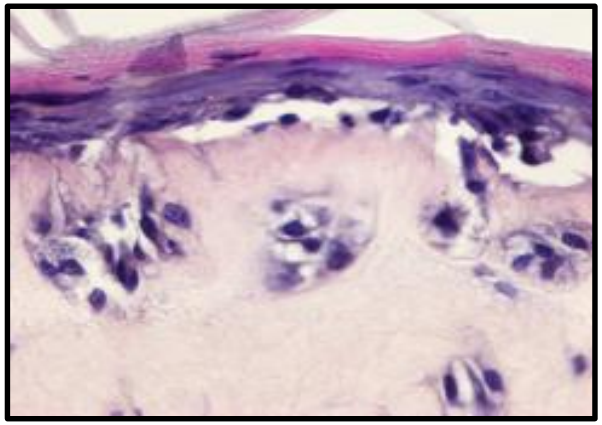
CI is more invasive than CII



Melanoma patient biopsy

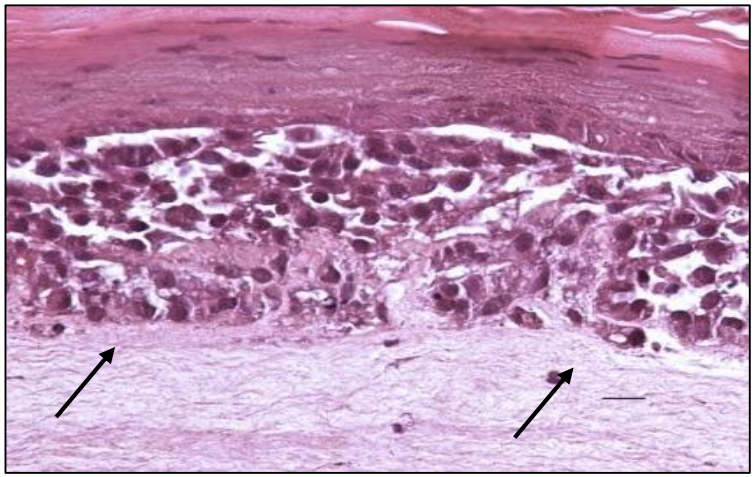


Melanoma invasion *in vitro*

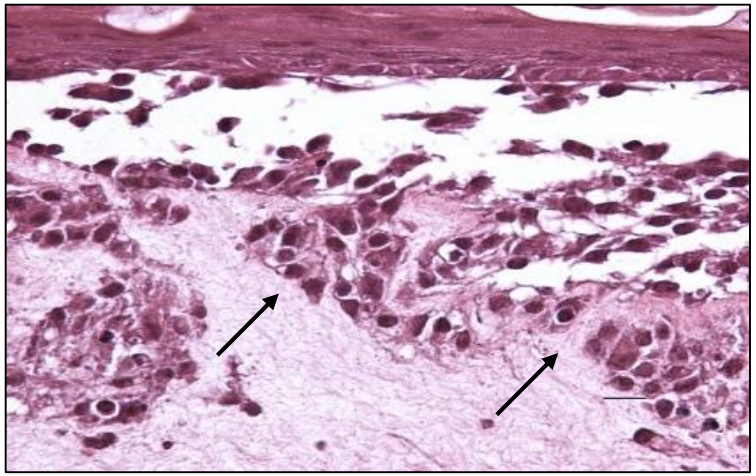


Reconstructed human skin: Invasion

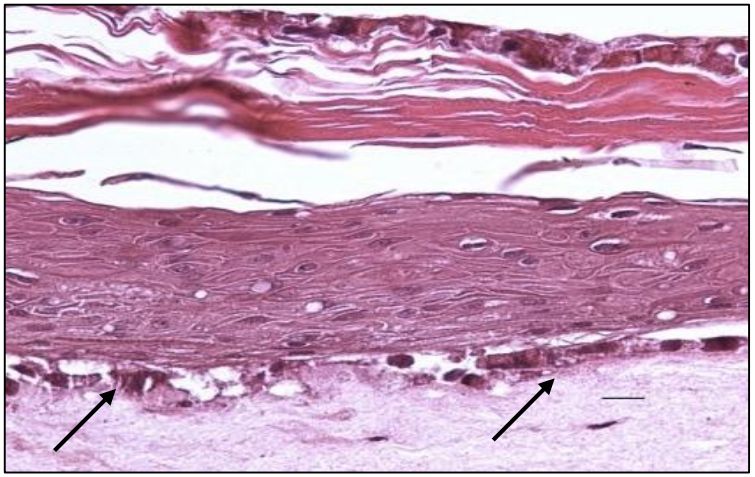
WM164 Parental



WM164 CI

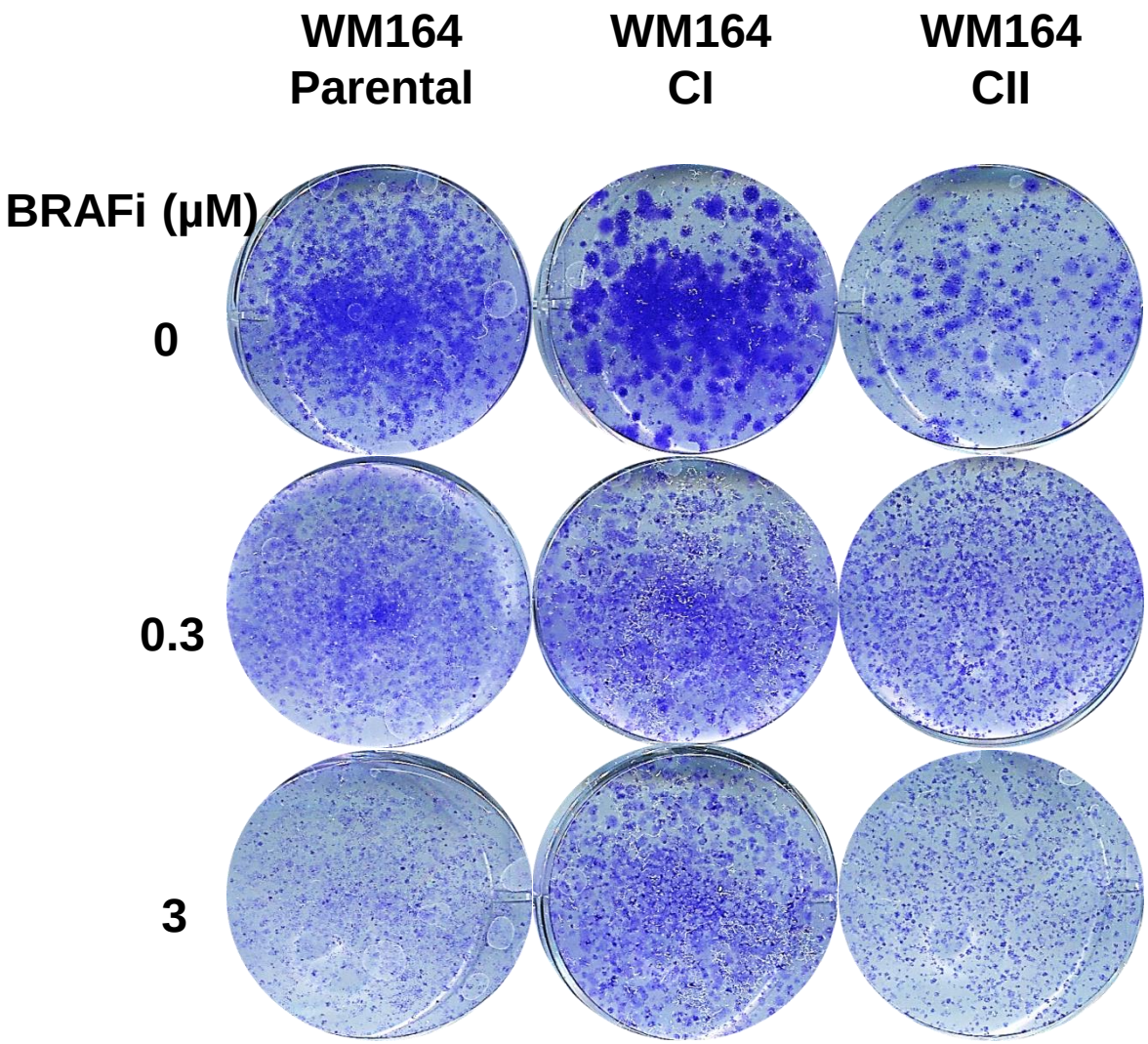


WM164 CII

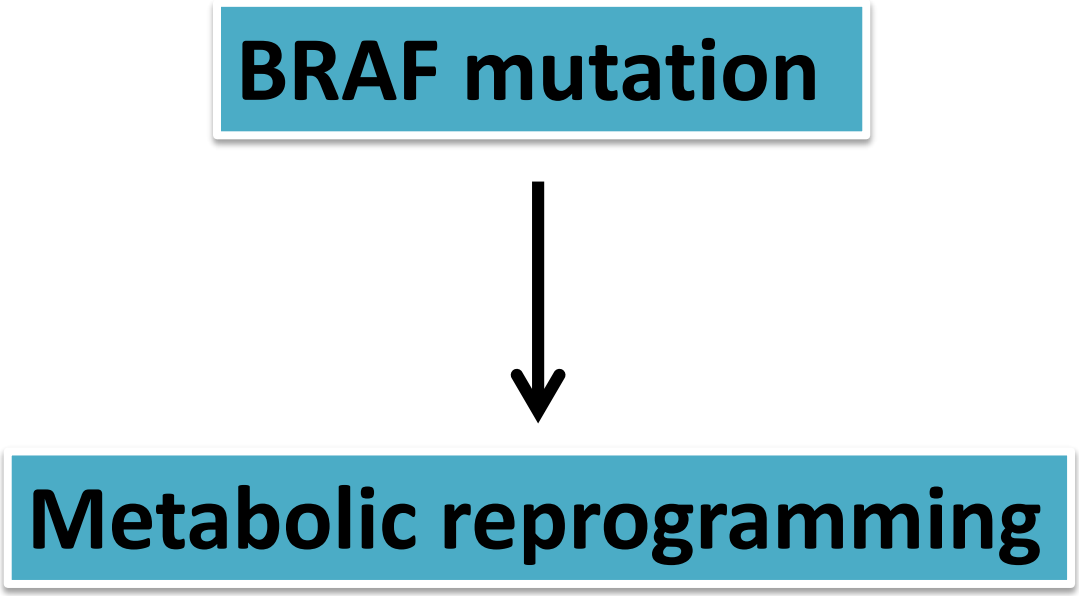


CI is more invasive than CII

Characterization of the clones: intrinsic resistance to BRAFi

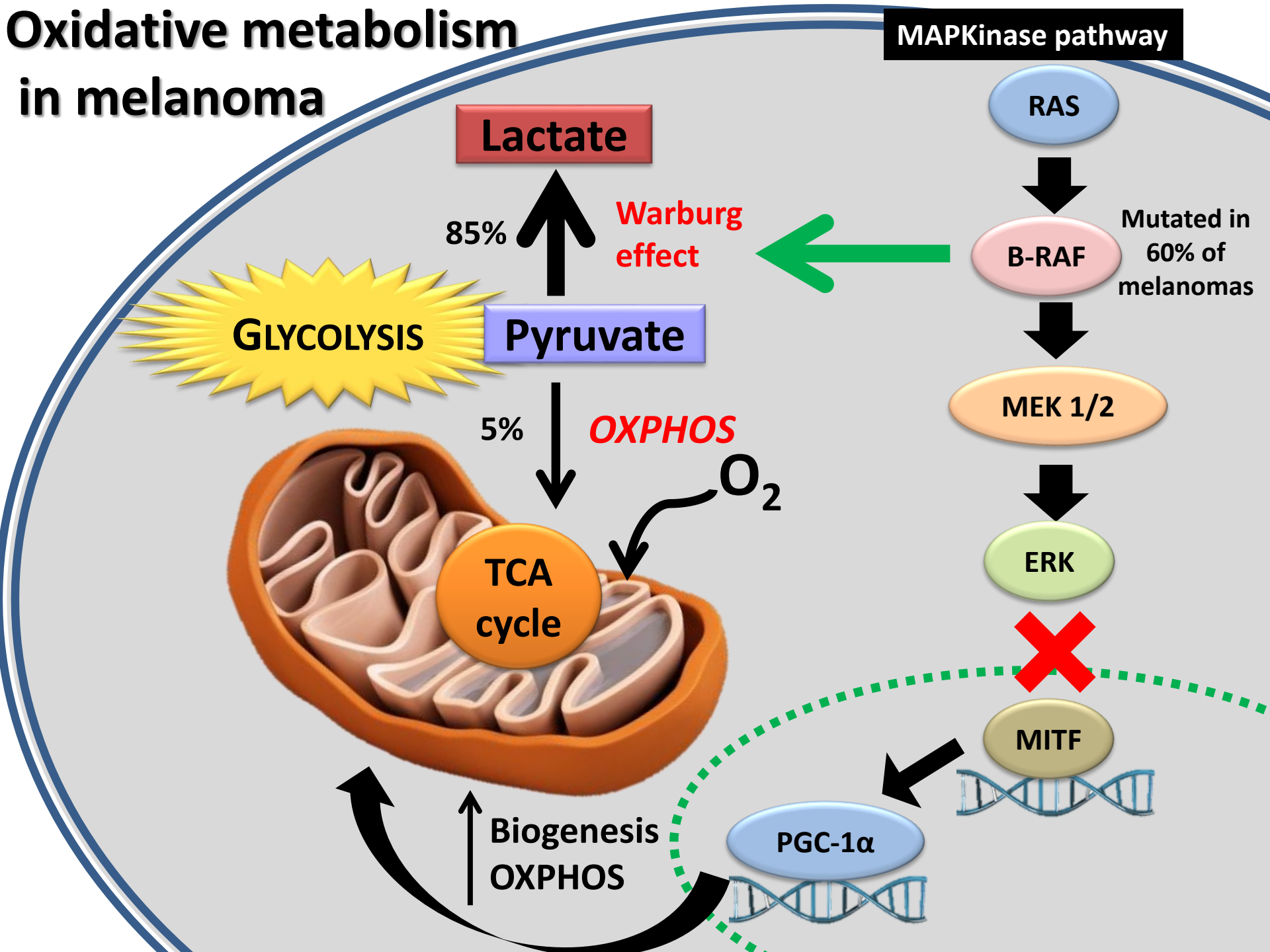


CI is more resistant than CII

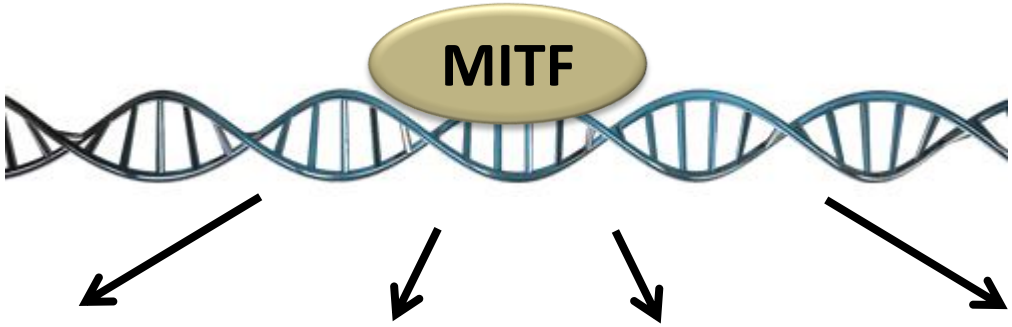


Can mitochondria be the main responsable for the tumor heterogeneity and resistance?

Oxidative metabolism in melanoma

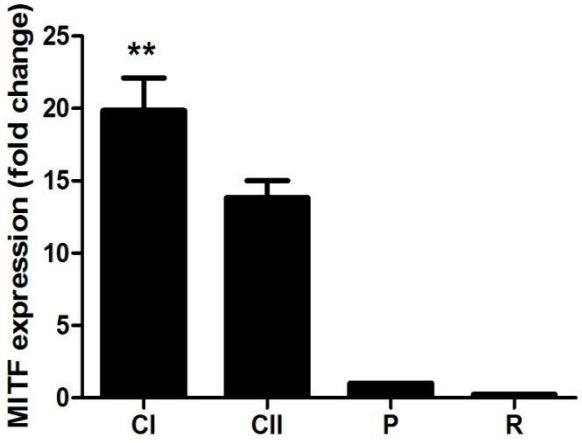


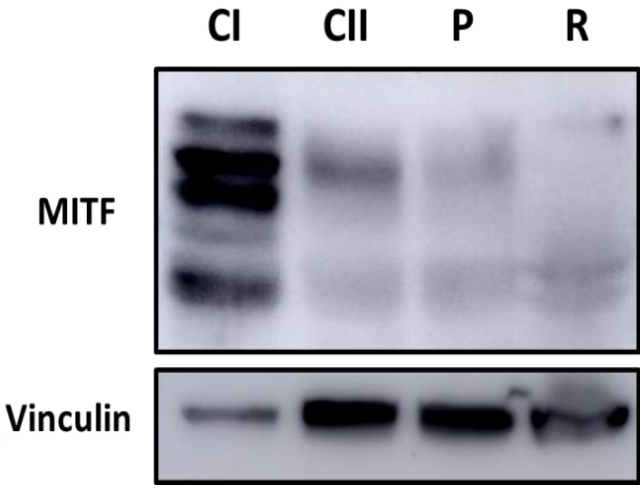
The major transcriptional melanocyte regulator



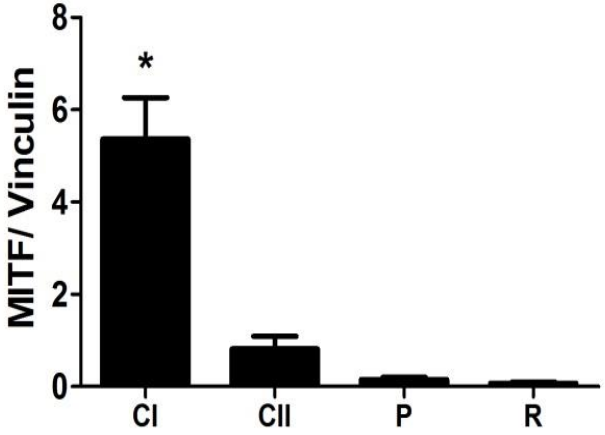
Differentiation Cell cycle Survival **Mitochondrial metabolism**

Gene expression





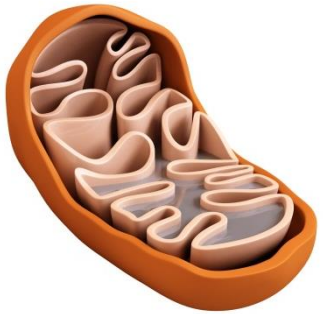
Protein expression

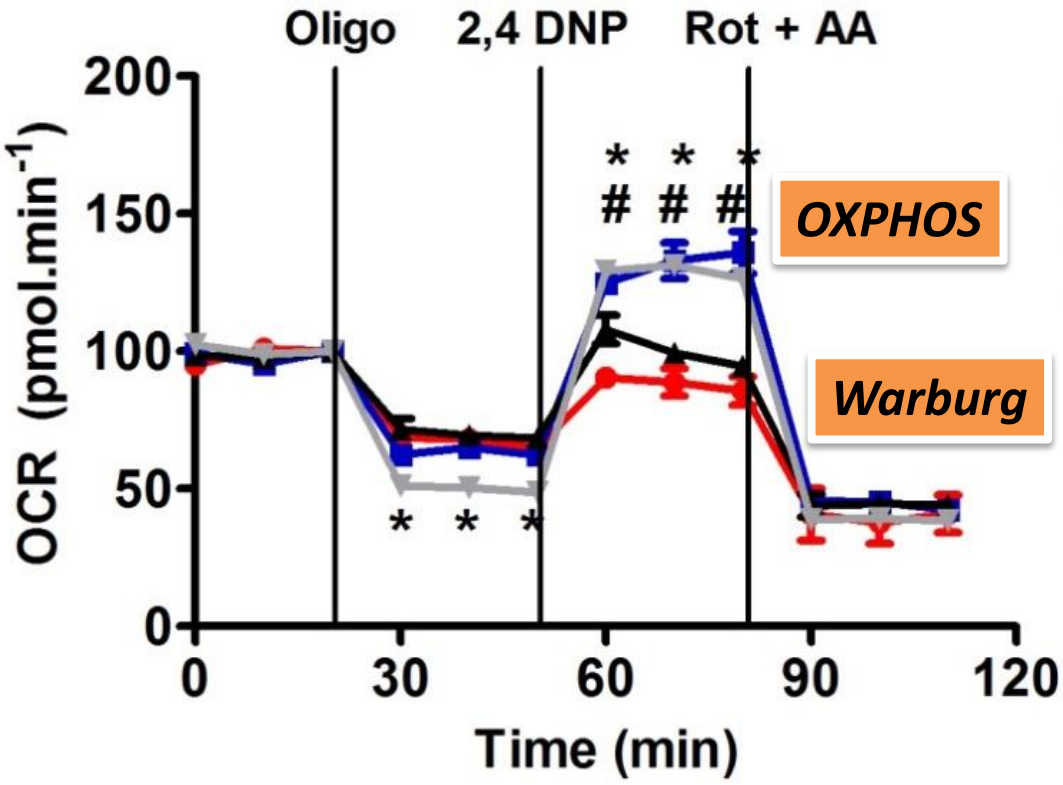


CI showed higher expression of MITF

Mitochondrial metabolism

Oxygen consumption rate





CI = Resistant
CII = Parental

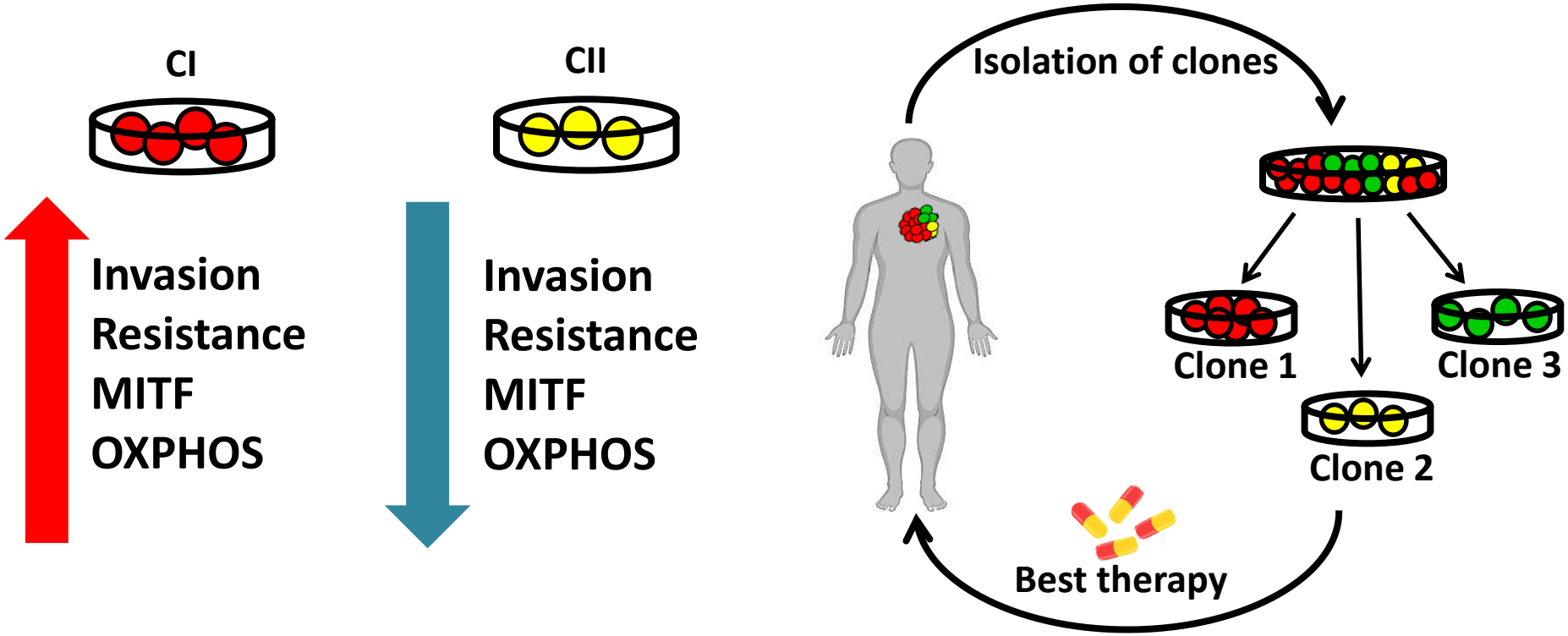
CI and Resistant showed identical profiles

Can we develop better tools to detect melanoma earlier?

Will identification of melanoma clones dictate therapy?

In vitro — non-animal model → *Patient*

Predict metastasis and resistance → **Choose the best therapy**



Acknowledgments

Skin Biology Group – School of Pharmaceutical Sciences

Dr. Manoela Tiago

MOFFITT CANCER CENTER

Prof. Dr. Keiran Smalley
Dr. Inna Smalley

Universidade de São Paulo
Instituto de Química

Prof. Dr. Nadja C. Souza-Pinto
Dr. Mateus P. Mori

Financial support:

FAPESP