

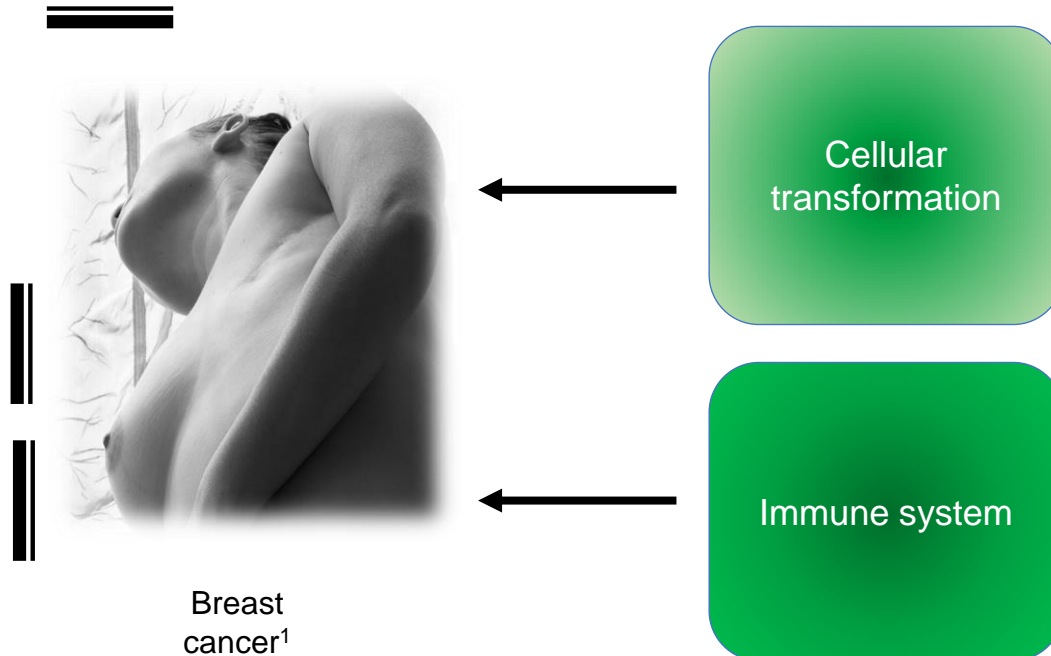


The role of Toll Like Receptor 9 (TLR9) in breast cancer

Uzma Hasan (CIRI Lyon) & Nathalie Bendriss-Vermare (CRCL, Lyon)



Immunity and Cell death



FACTS on TLR9

Highly expressed on immune cells
(pDC and B cells human)

Weakly expressed on epithelial cells
(skin and cervix human)

Activated by dsDNA

Expressed in the ER - shifts to endosome

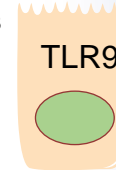
TLR9 pivotal in immune responses and cell cycle control

Immune response ^{1, 2, 3, 4}

Type I IFN by pDC



Oncogenic stress ^{5, 6, 7, 8}



¹ Kadowaki, N. et al. J. Exp. Med 2001

² Krug A et al., Eur J Immunol 2001

³ Lund J et al., J Exp Med 2003

⁴ Lande et al, Nature 2007

⁵ **Proliferation**, Colorectal cancer, Si Ming Man *et al.*, Cell 2015

⁶ **Senescence**, Gluck *et al.*, Nature Cell Biology 2017

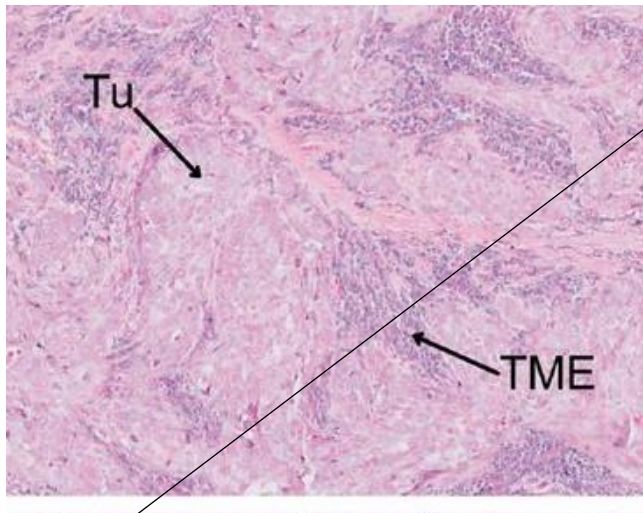
⁷ **Proliferation**, Pacini *et al.*, J Virology 2015

⁸ **p16 up regulation**, Parroche *et al.*, Oncogenesis 2016

Question

Role of TLR9 in Breast Cancer?

TLR9 tumor
Team 1 UH
KCL



TLR9 pDC
TEAM 2 NVB
TEAM 3 OT

Methods

5 WPs

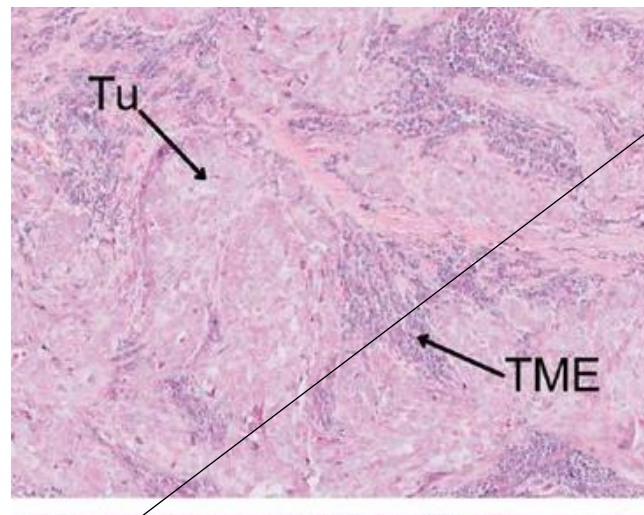
Objective 1.
TLR9 expression
Cohort (Kings College London)

Objective 2.
Mechanism
BC human *in vitro and ex vivo* models

Objective 3.
Mechanism
BC murine *in vivo* models

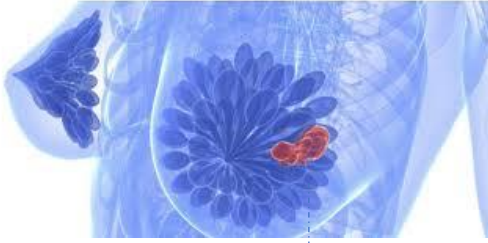
TLR9 in BC tumour cells

TLR9 tumor
Team 1 UH
KCL

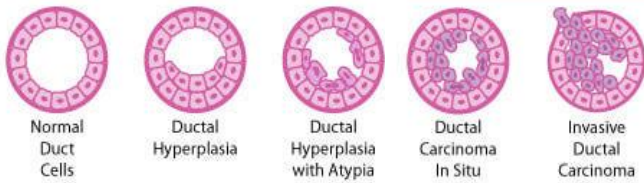


TLR9 pDC
TEAM 2 NVB
TEAM 3 OT

Objective 1. TLR9 expression Cohort (Kings College London)



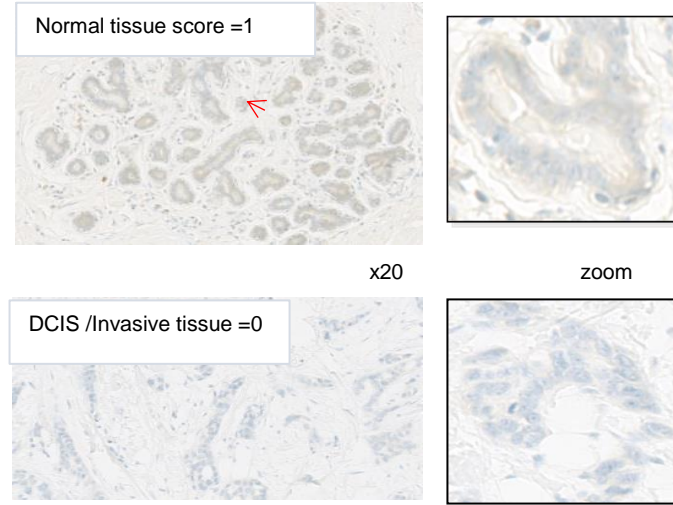
EXPRESSION TLR9?



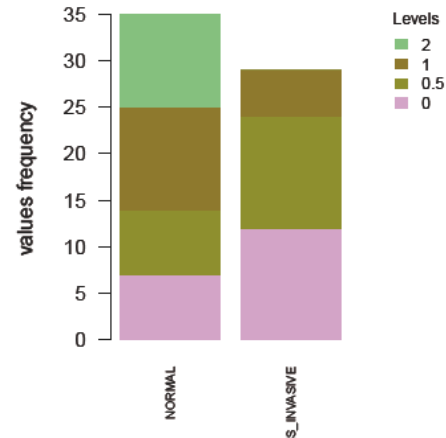
30 sections

30 sections 220 TMA

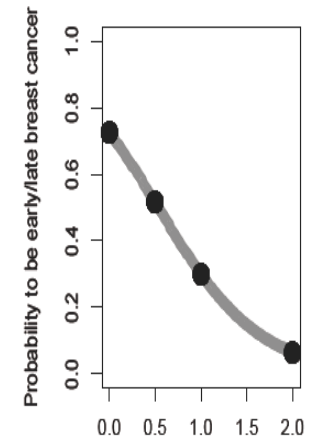
RESULTS



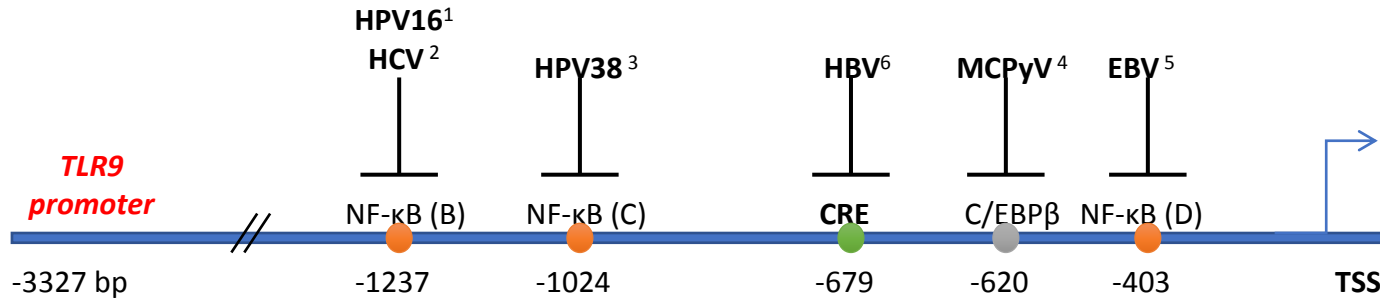
Levels of TLR9 expression



NORMAL Vs. DCIS_INVASIVE logistic regression model
odds ratio = 0.16 (normal is the reference)



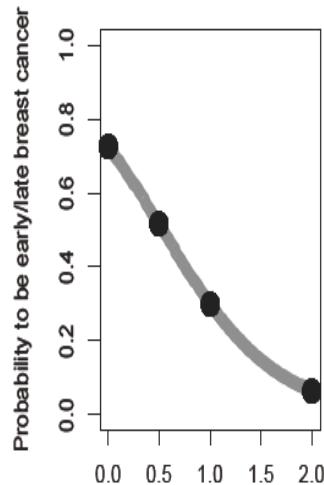
TLR9 expression is blocked in several virus-induced cancers



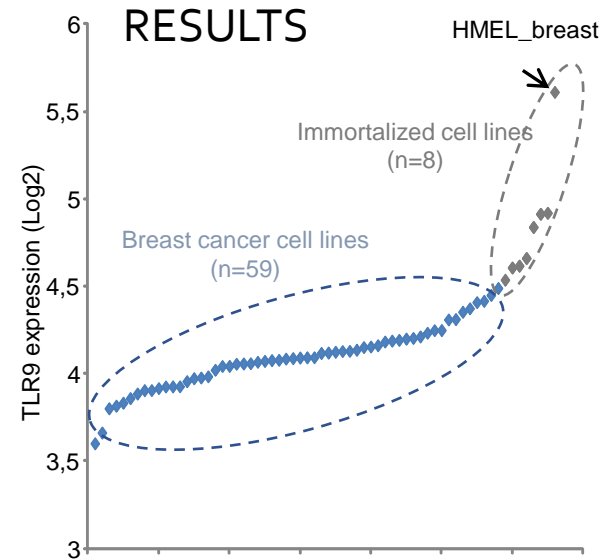
1. Hasan UA *et al.* *J Exp Med* 2013
2. Fischer J *et al.* *Gut*, 2017
3. Pacini L *et al.* *J Virol*, 2015b
4. Shahzad N *et al.* *J Virol*, 2013
5. Fathallah I *et al.* *J Immunol*, 2010
6. Tout *et al.* *J I* 2018

Ex vivo

NORMAL Vs. DCIS_INVASIVE logistic regression model
odds ratio = 0.16 (normal is the reference)



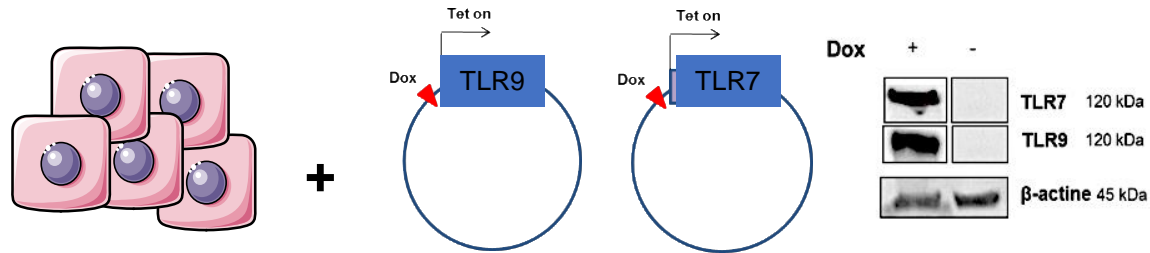
In vitro



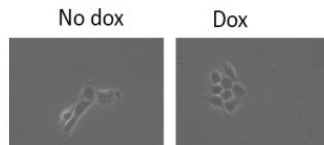
<http://www.broadinstitute.org/ccle/home>

<http://brainarray.mbni.med.umich.edu/Brainarray/Database/>

Objective 2. Mechanism BC human *in vitro* models

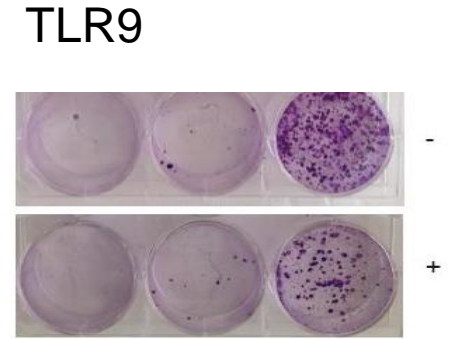
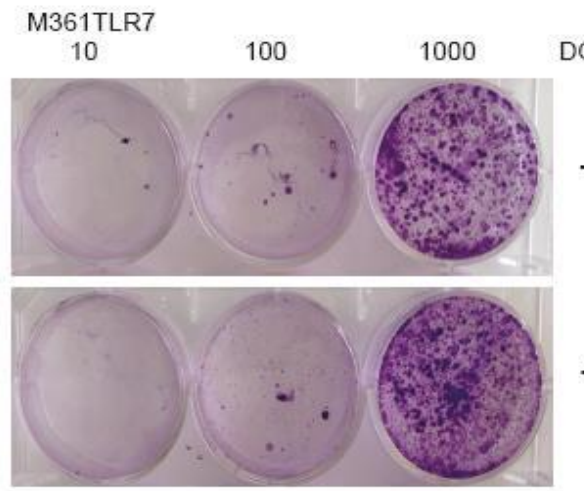
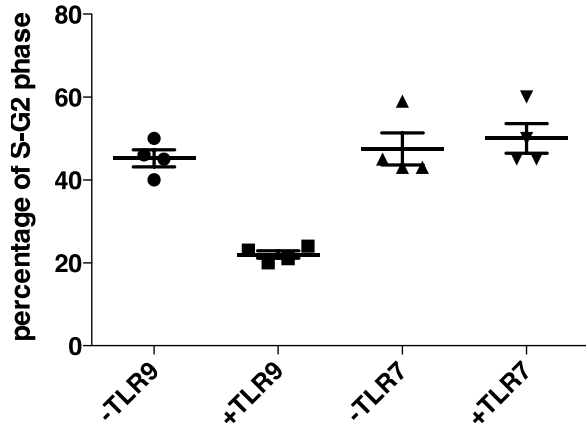
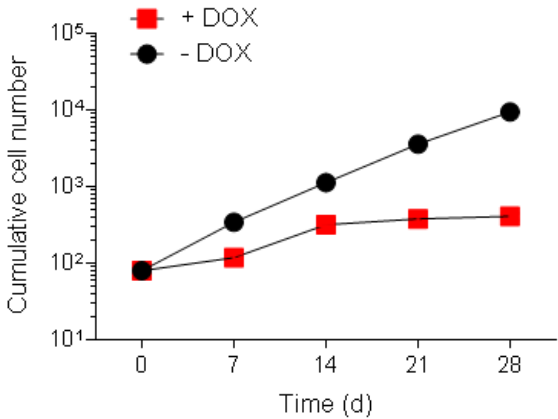
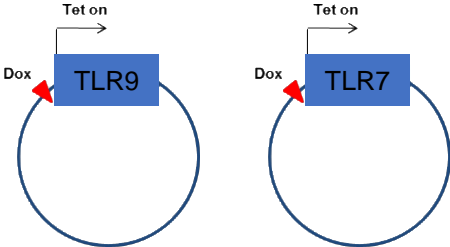


- Proliferation: colony assay
- SASP (Senescence-Associated Secretory Phenotype)

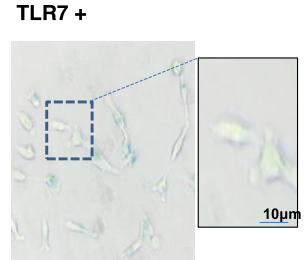
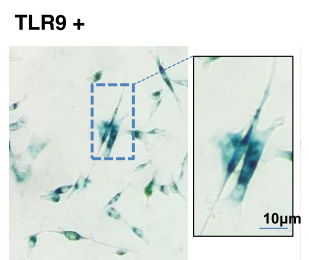
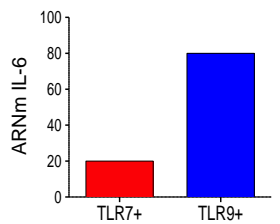
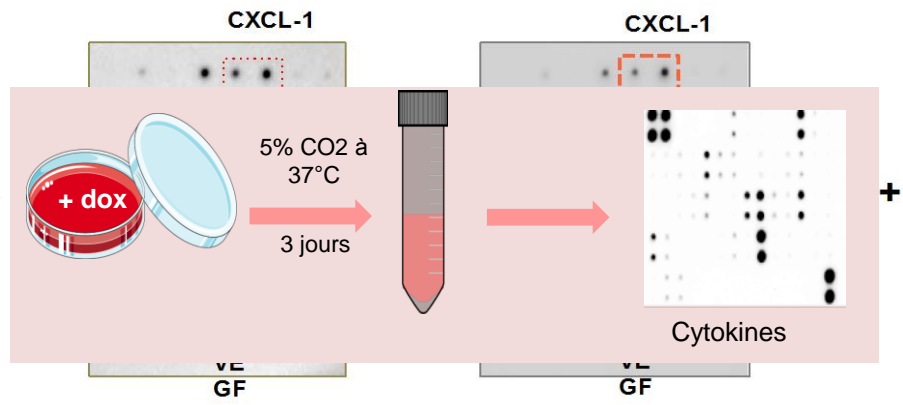
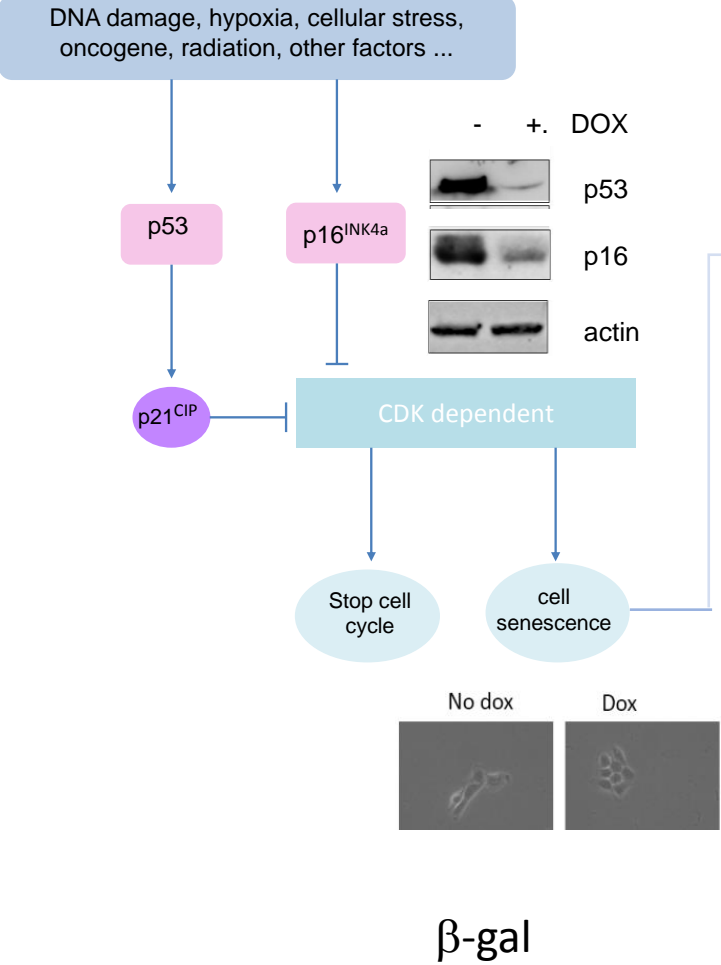


- Tumour suppressor

RESULTS: Proliferation is blocked by TLR9 in breast cancer cells

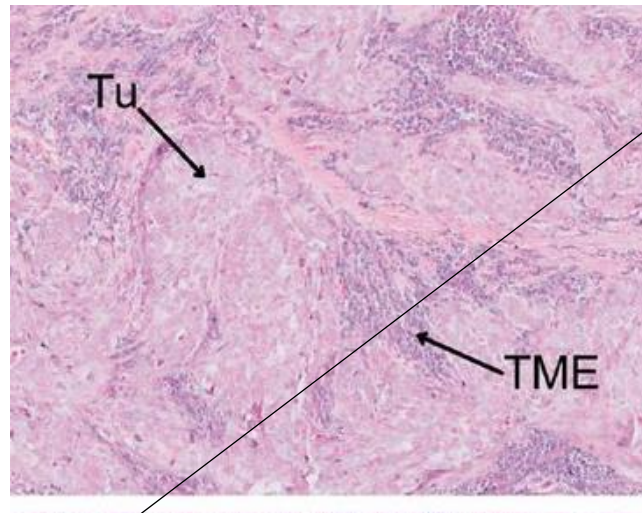


RESULTS: TLR9 induces tumour suppressors and SASP



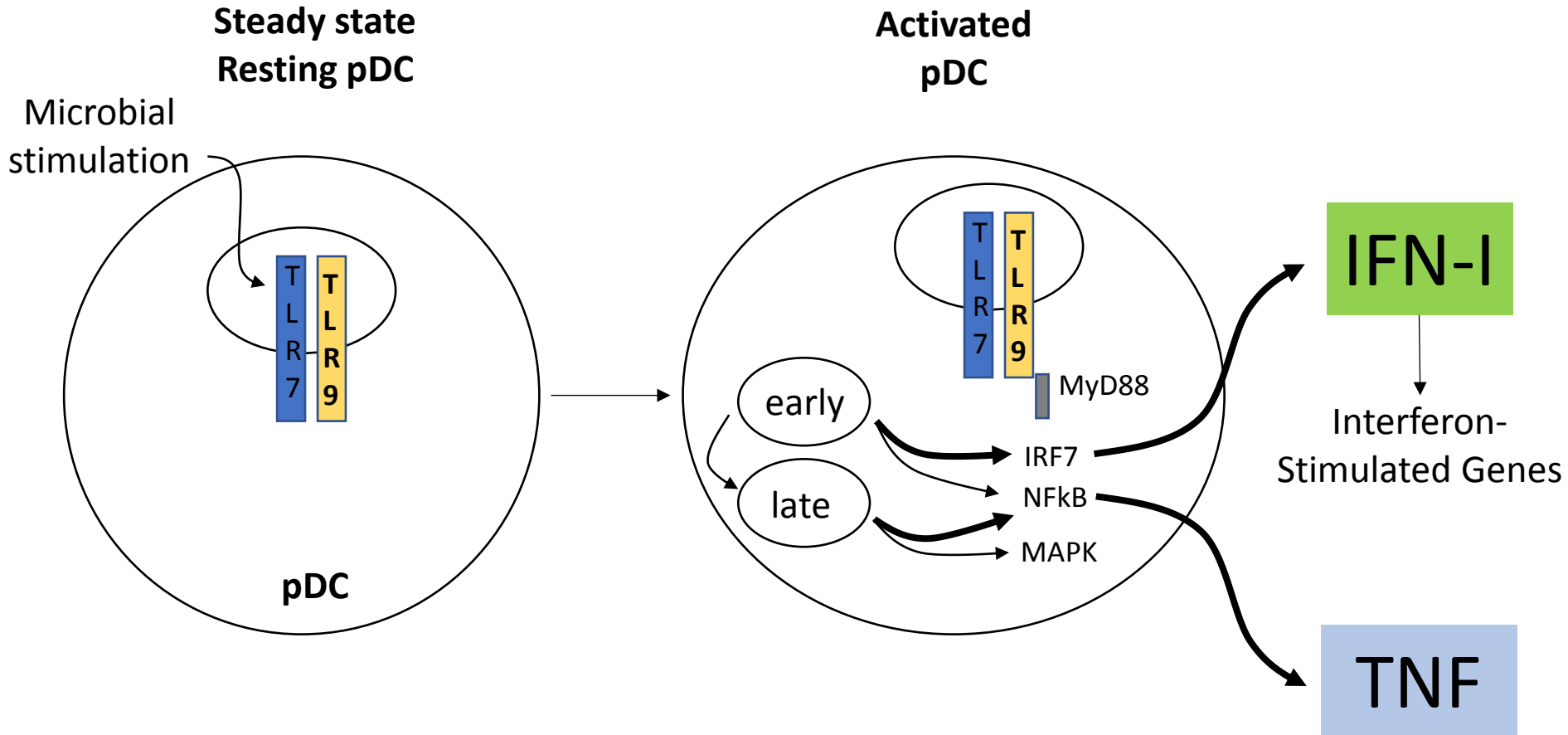
TLR9 immunity in BC

TLR9 tumor
Team 1 UH
KCL

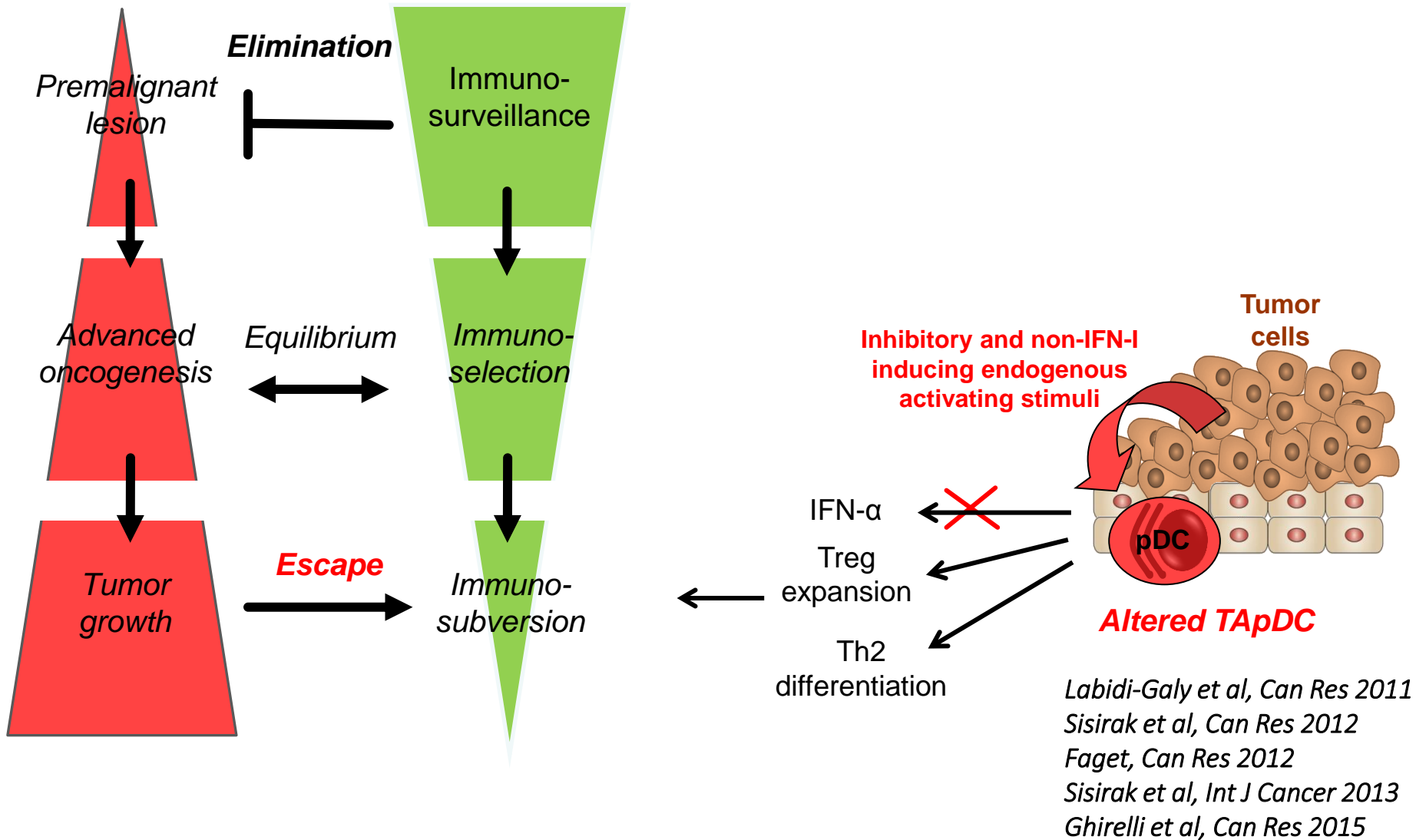


TLR9 pDC
TEAM 2 NVB
TEAM 3 OT

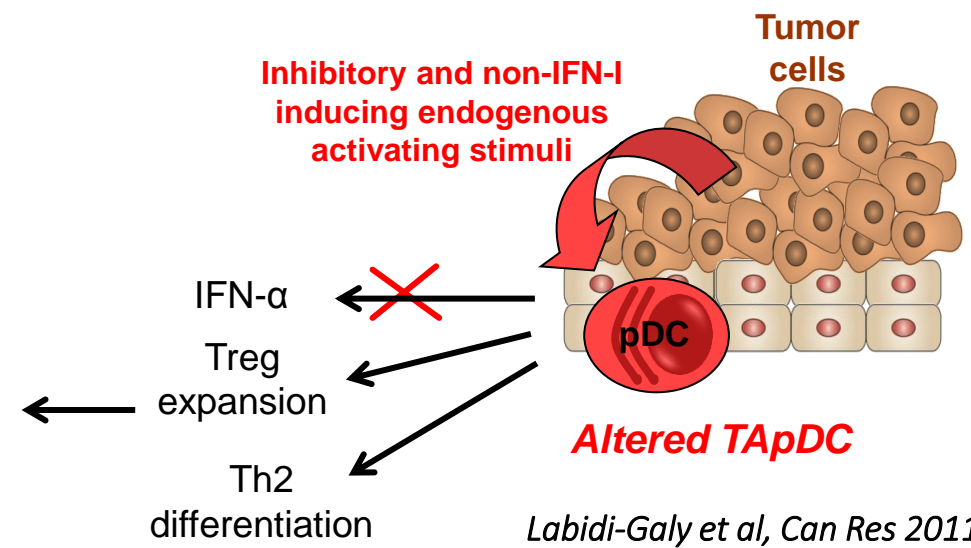
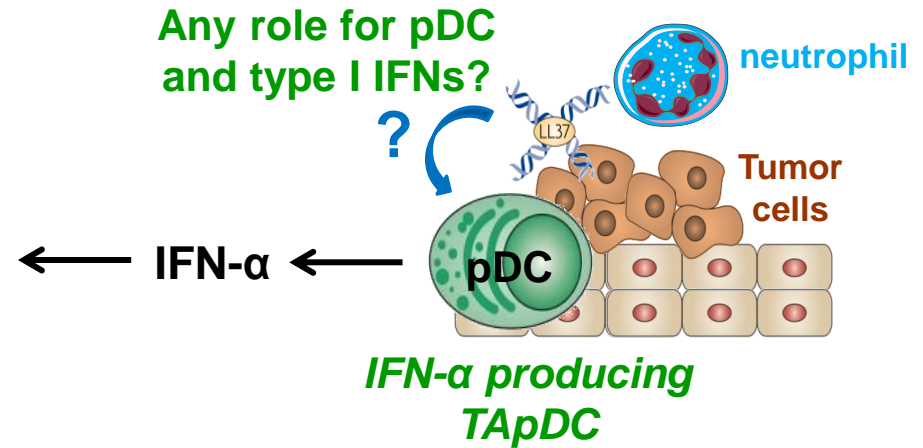
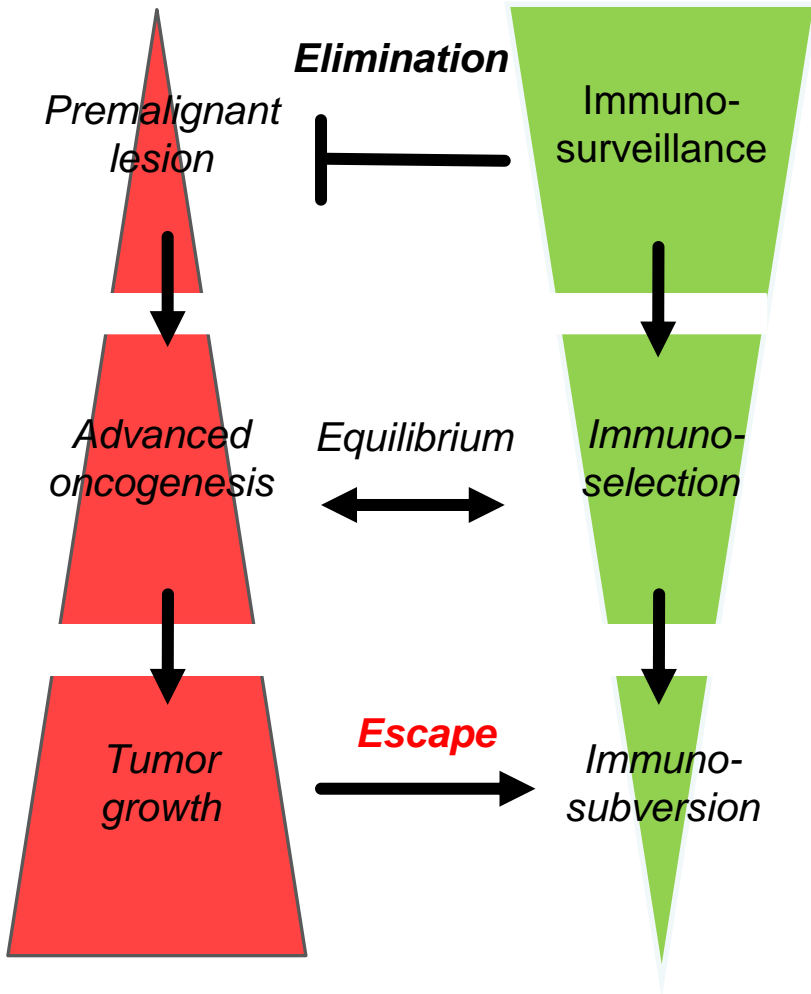
pDC are specialized in antiviral responses via type I IFN production



pDC at the center of an immunosuppressive microenvironment in breast and ovarian cancers



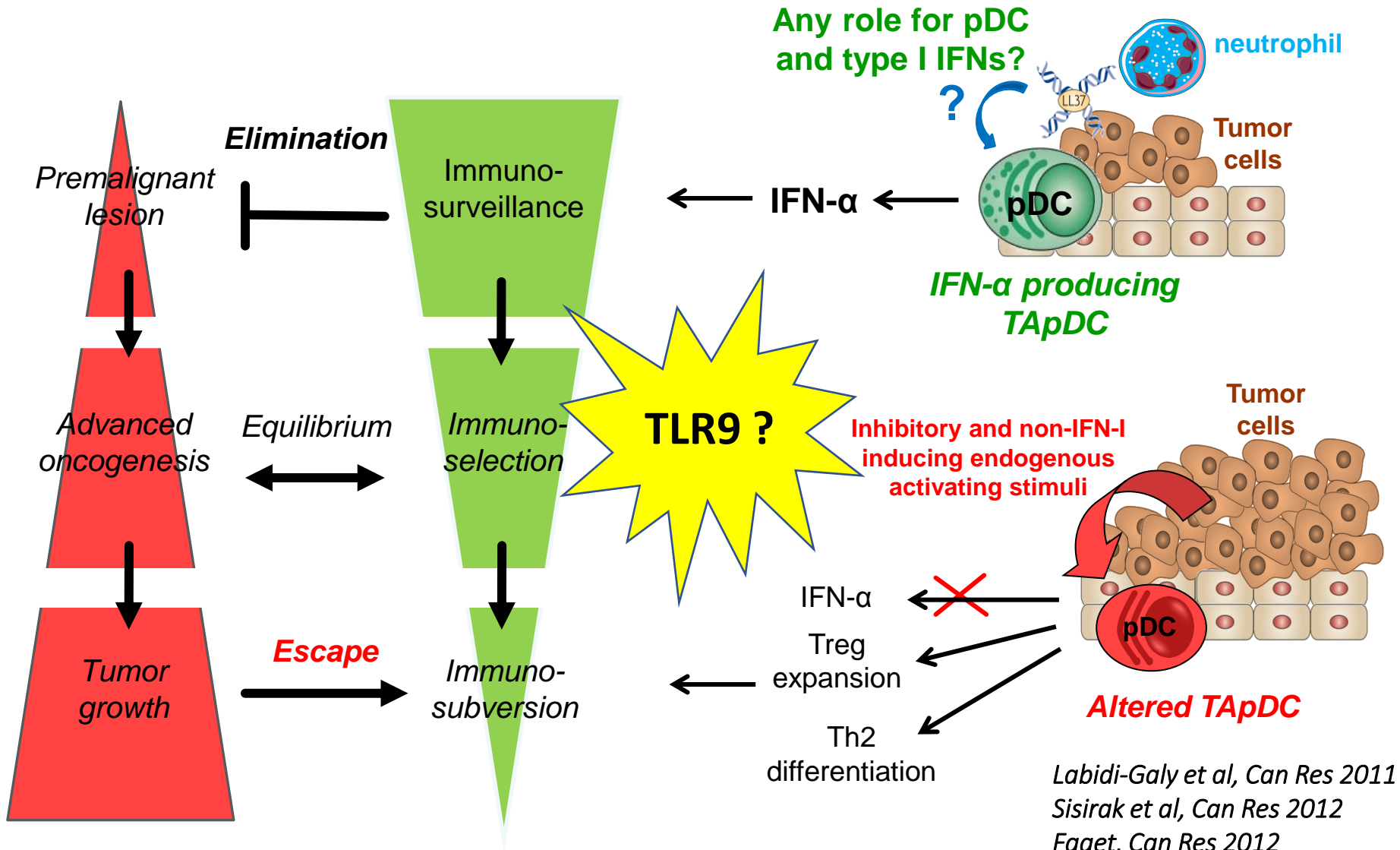
Do pDC play a dual role in breast cancer immunity?



Labidi-Galy et al, Can Res 2011
Sisirak et al, Can Res 2012
Faget, Can Res 2012
Sisirak et al, Int J Cancer 2013
Ghirelli et al, Can Res 2015

Do pDC play a dual role in breast cancer immunity ?

What is the role for TLR9 ?



Labidi-Galy et al, Can Res 2011

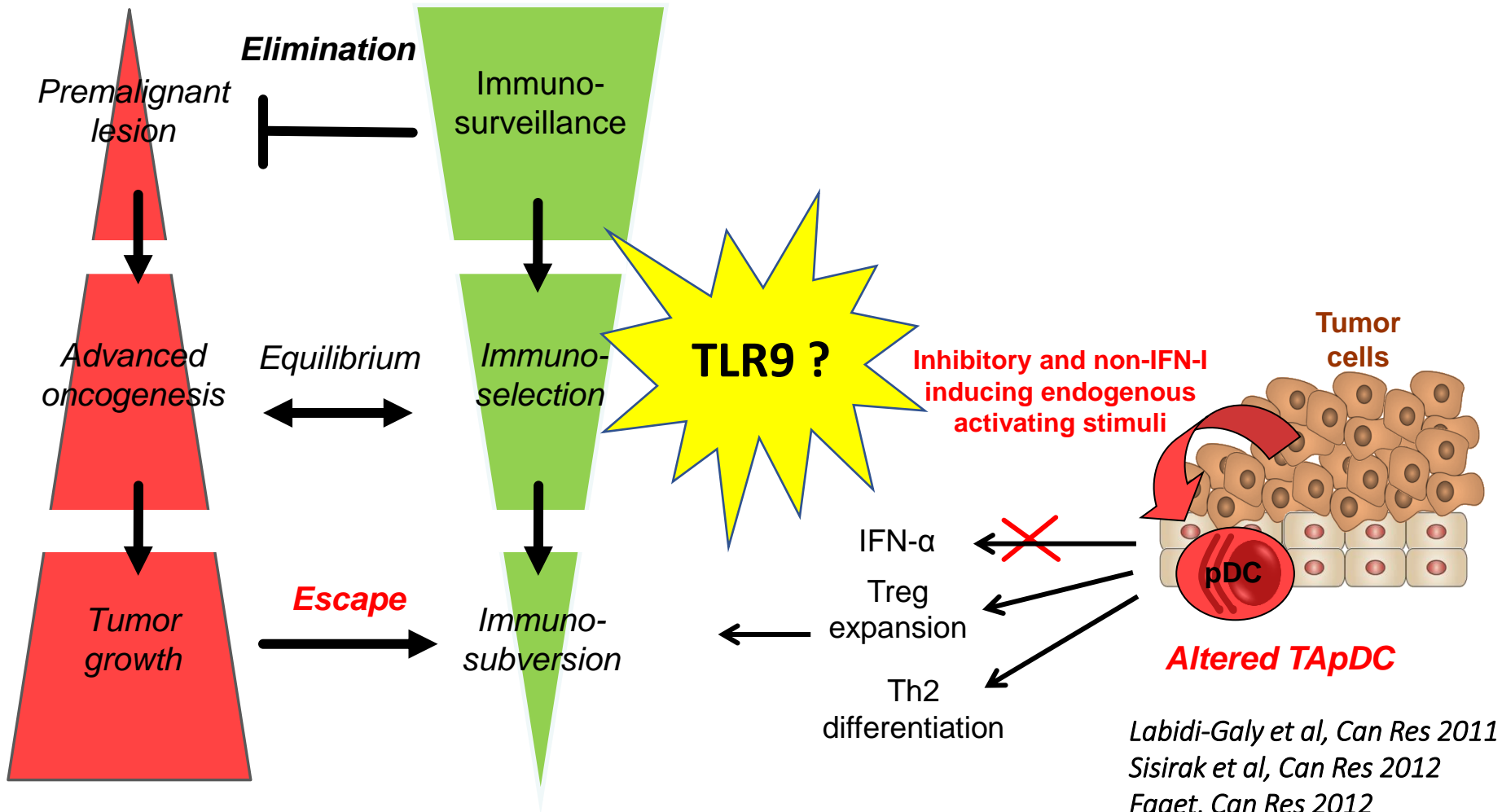
Sisirak et al, Can Res 2012

Faget, Can Res 2012

Sisirak et al, Int J Cancer 2013

Ghirelli et al, Can Res 2015

Do pDC play a dual role in breast cancer immunity ?
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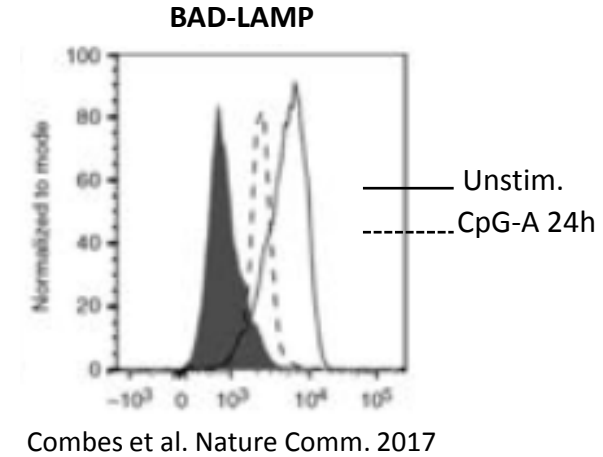
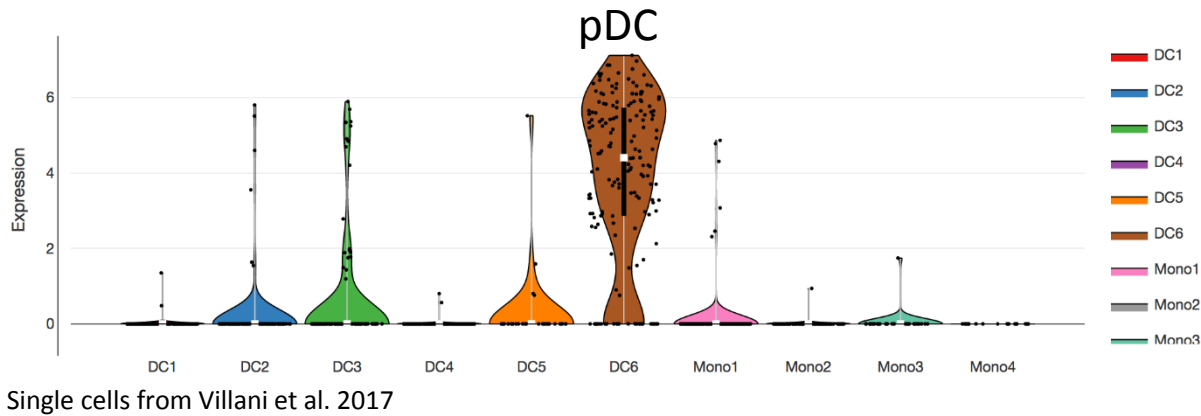


Labidi-Galy et al, Can Res 2011
Sisirak et al, Can Res 2012
Faget, Can Res 2012
Sisirak et al, Int J Cancer 2013
Ghirelli et al, Can Res 2015

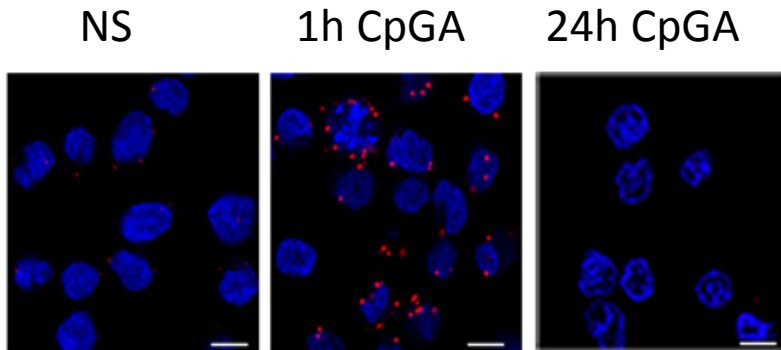
Objective 2.
Mechanism

BC human *in vitro* and *ex vivo* models

BAD-LAMP controls TLR9 trafficking and signaling in human pDC



BAD-LAMP & TLR9



BAD-LAMP downmodulation



BAD-LAMP overexpression



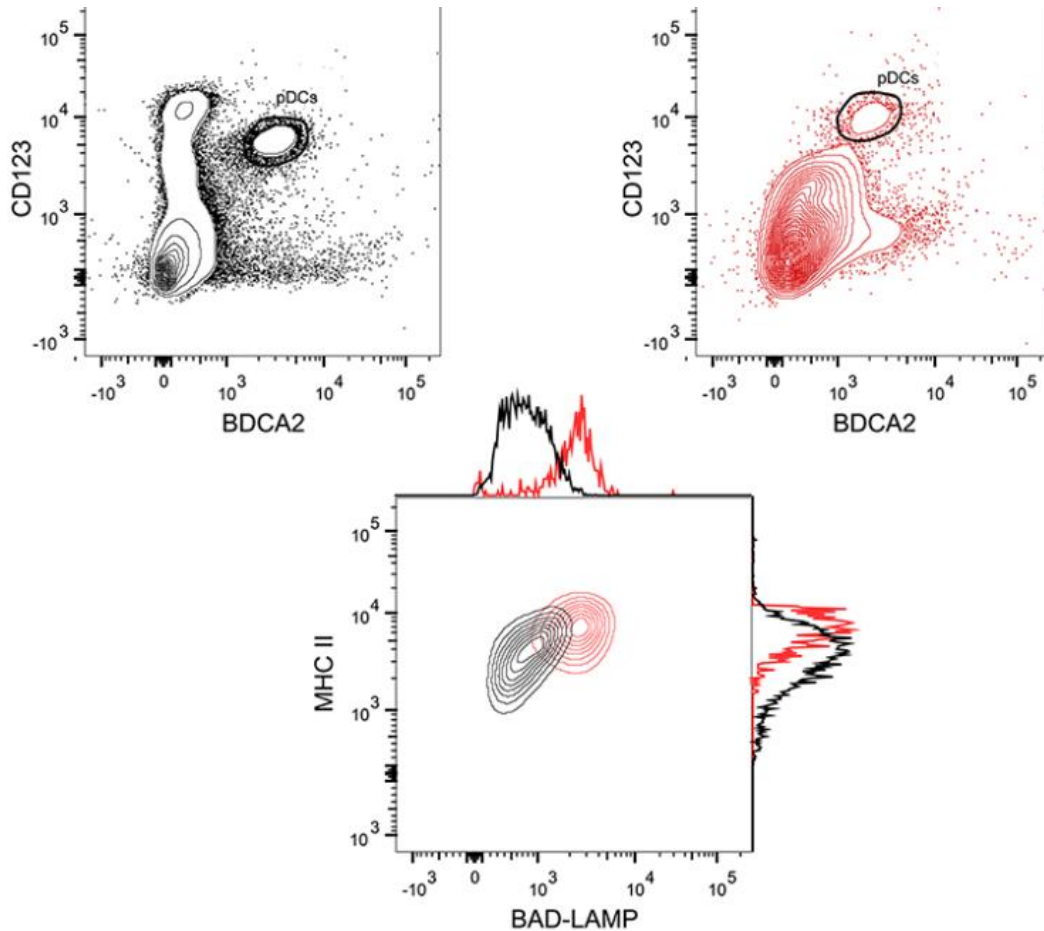
Objective 2.
Mechanism

BC human *in vitro* and *ex vivo* models

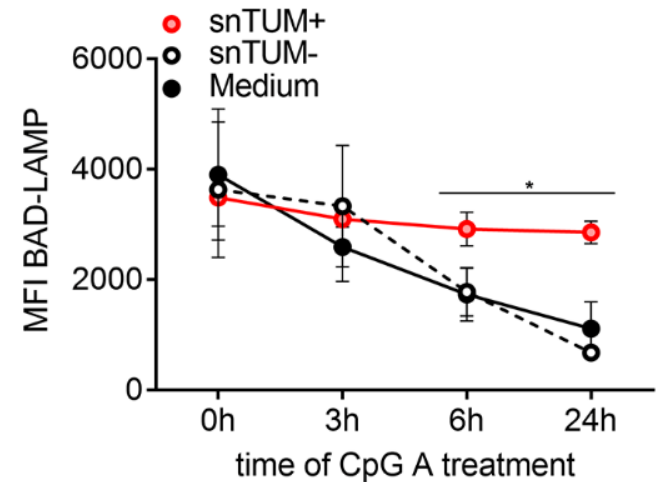
BAD-LAMP expression is enhanced in breast tumor pDCs and its downregulation is prevented by inhibitory tumor supernatants

A Blood patients' pDC

Breast Tumor pDC

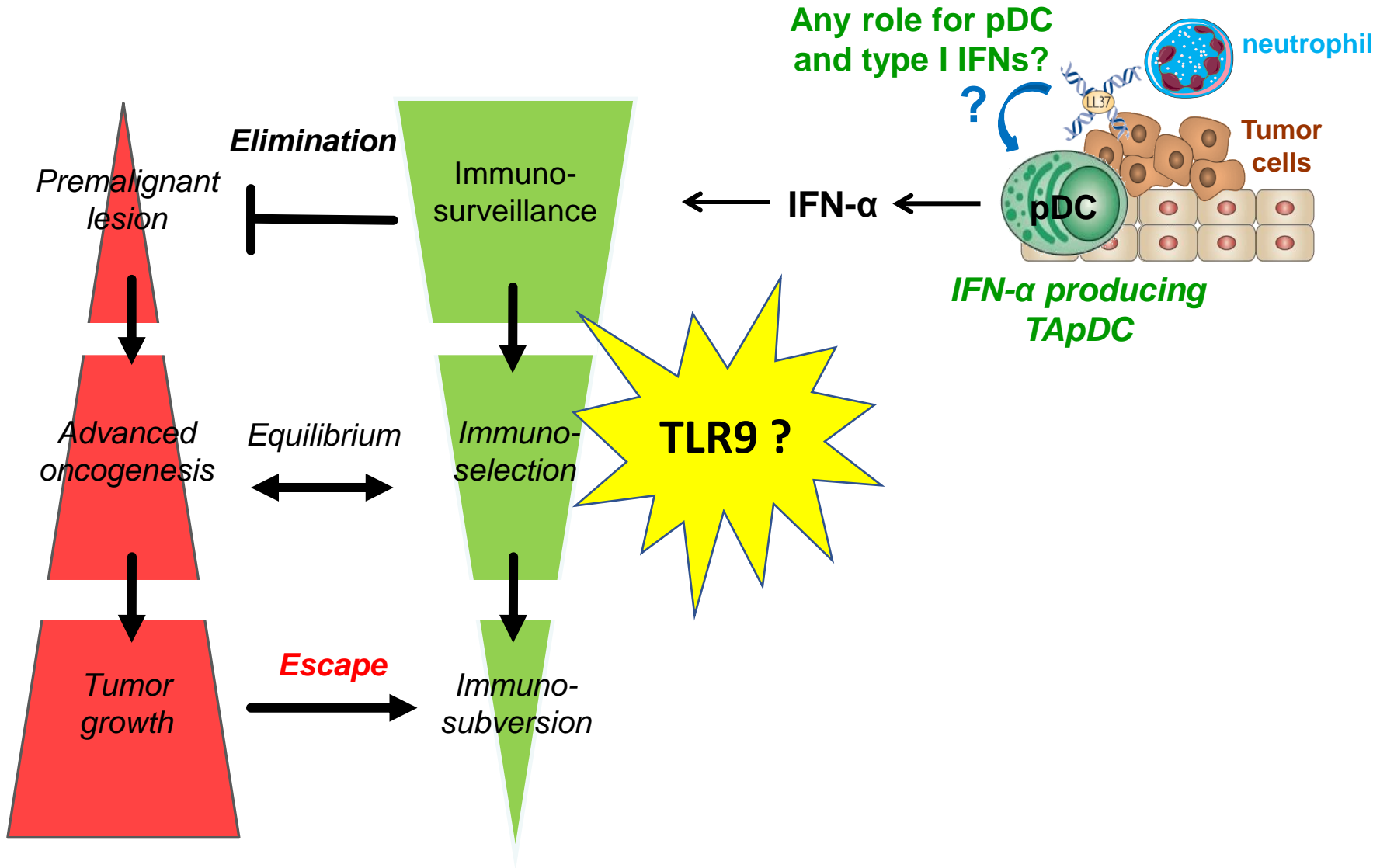


B BAD-LAMP PROTEIN EXPRESSION



Do pDC play a dual role in breast cancer immunity ?

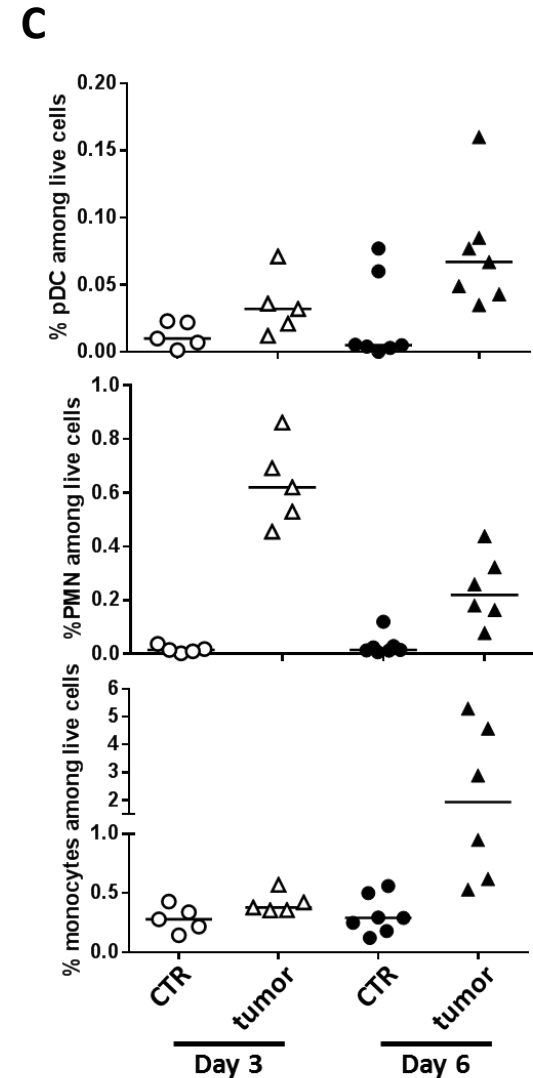
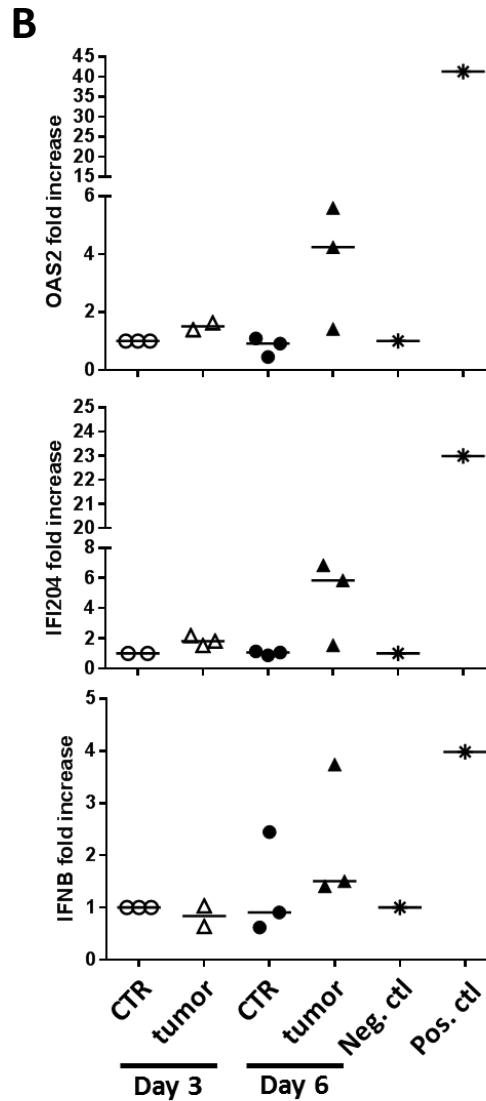
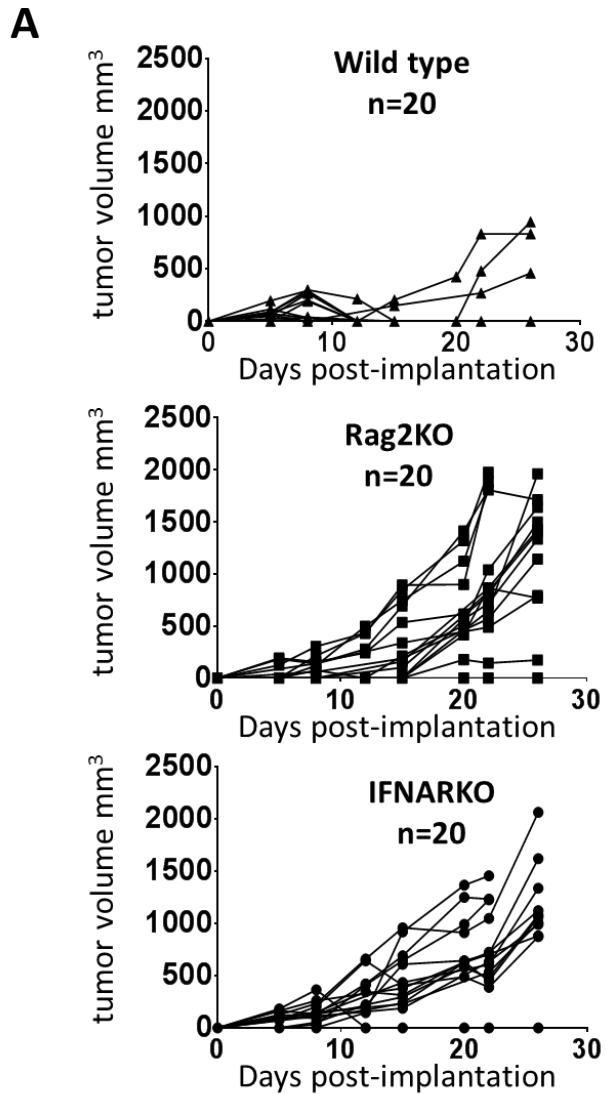
What is the role for TLR9 ?



Objective 2.
Mechanism

BC human *in vitro* and *ex vivo* models

Type 1 IFN pathway contributes to breast
tumor immunosurveillance in mice



Objective 2.

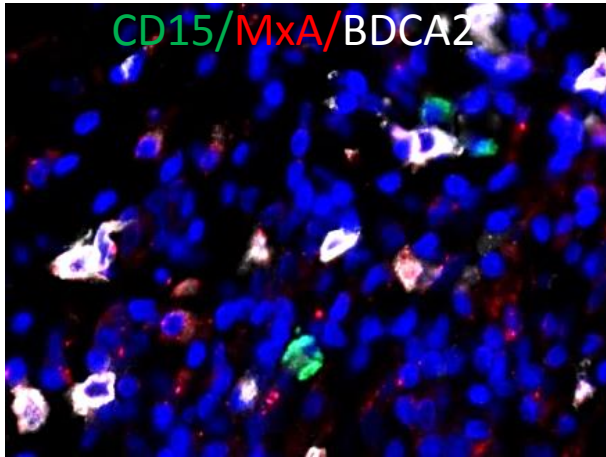
Mechanism

BC human *in vitro* and *ex vivo* models

Evidence for the activation of type I IFN pathway in human breast tumors

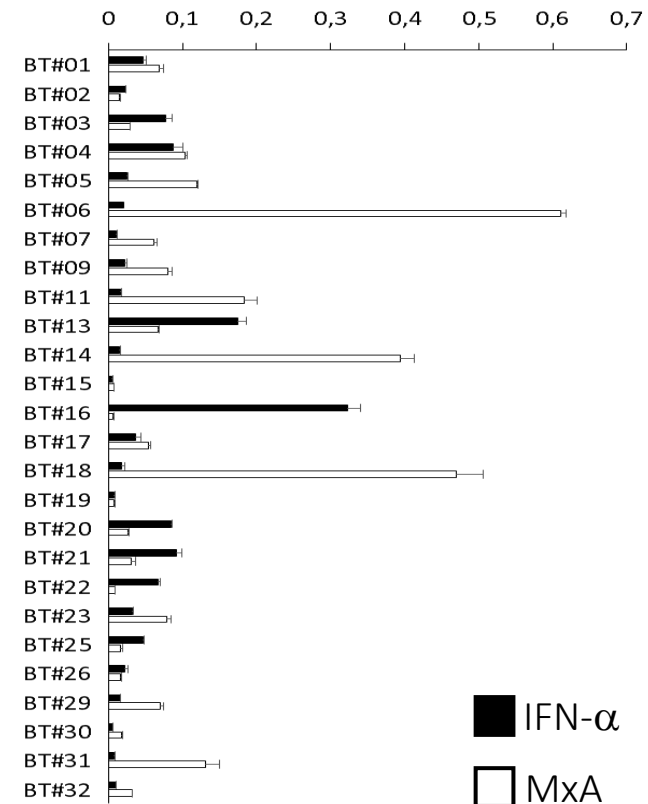
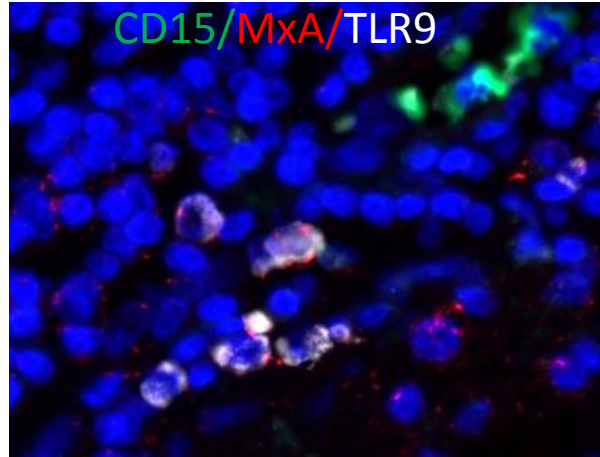
Invasive tumor

CD15/MxA/BDCA2



Invasive tumor

CD15/MxA/TLR9

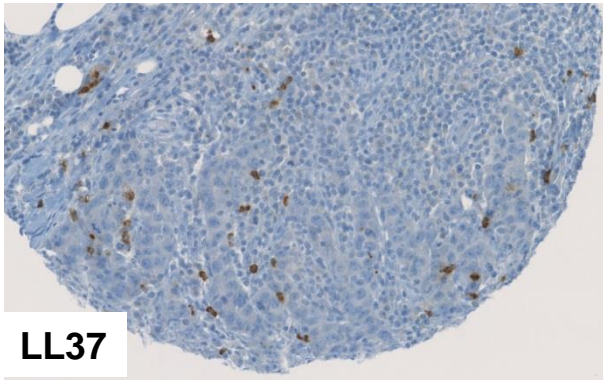


■ IFN- α
□ MxA

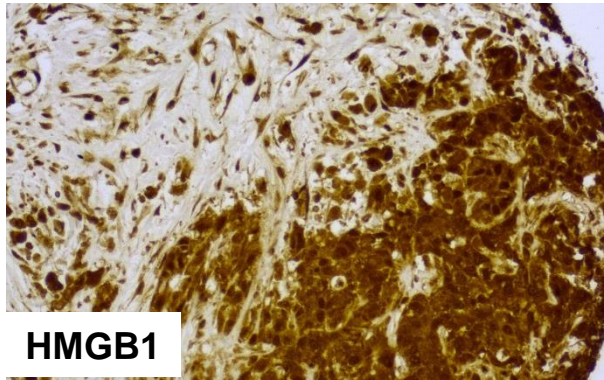
Objective 2.
Mechanism

BC human *in vitro* and *ex vivo* models

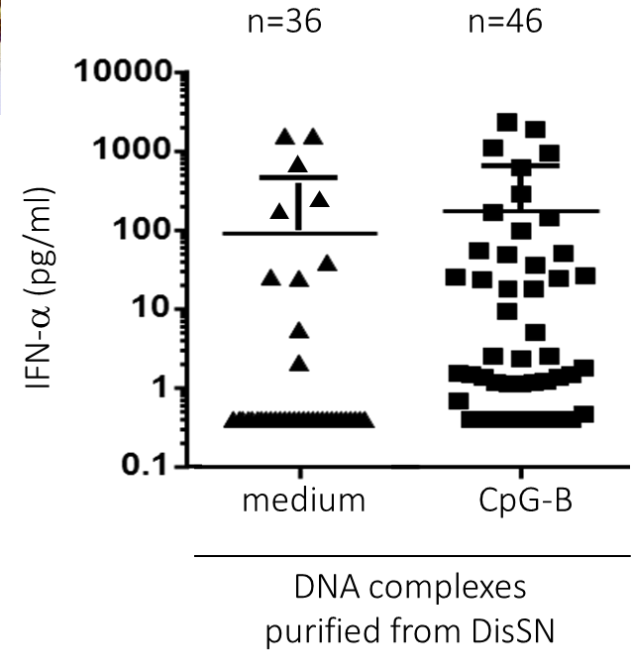
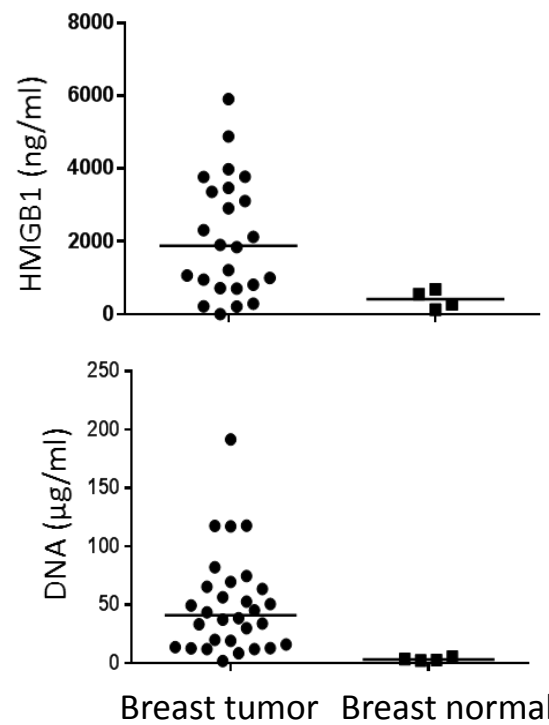
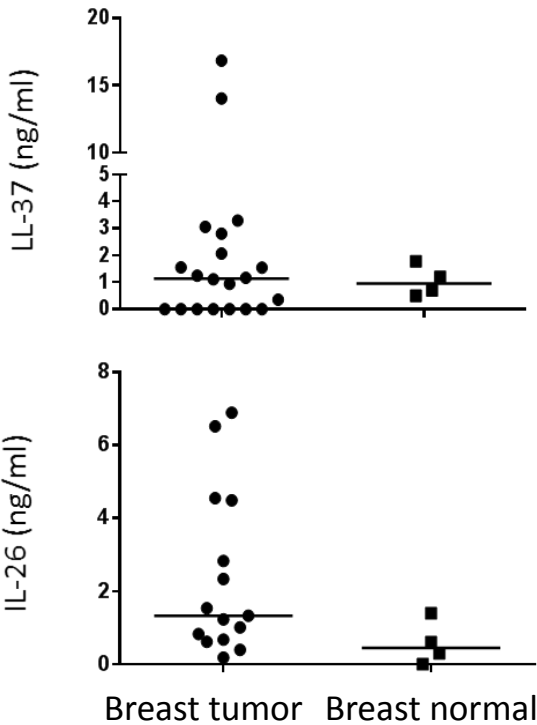
Endogenous TLR agonists are present in breast tumors and are able to activate or potentiate pDC activation in vitro



LL37



HMGB1



Conclusion and next steps

- **Further characterize the intratumor endogenous TLR ligands**
 - Mitochondrial DNA vs Genomic DNA
 - Demonstrate the role of TLR
- **Further characterize immune infiltrate and immune pathways (TLR9, IFN) dominating in early breast cancers versus invasive breast cancers in patients**
- **Demonstrate the role of pDC and neutrophils in breast tumor immunosurveillance in vivo by depleting experiments**
- **Establish a new model of spontaneous mammary tumor model in B6 mice (BRCA1 Δ 22-24, p53null - Drost RM et al, Jonkers Lab. Br J Cancer 2009)**

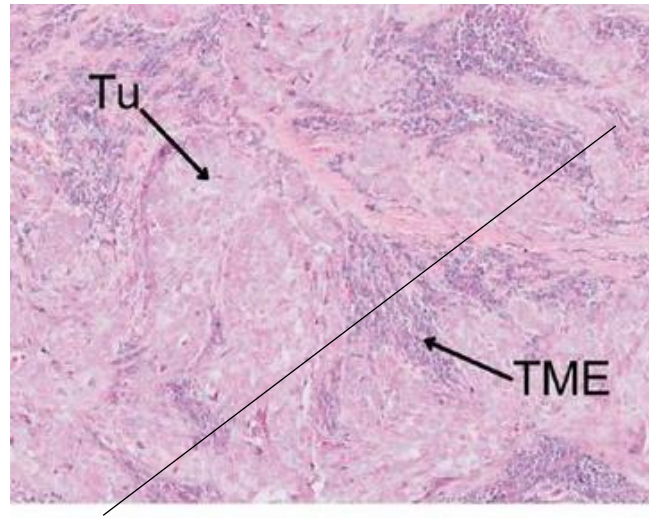
**TLR9 tumor
Team 1 UH
KCL**

Expression
is lost
tumour cells

Slows down
proliferation

Induces
senescence

TLR9 in BC



**TLR9 TME
TEAM 2 NVB
TEAM 3 OT**

Strong
expression in
tumor pDC

Activation
blocked by
BAD-LAMP

Endogenous
ligands are
present in BC



Maire Marotel
Michelle Ainouze
Guillaume Roblot
Kyohei Yamanaka
Salome Amouyal
Omran Allatif



Christophe Caux
Nelly Vey
Julie Mussard
Aurélien Voissière

CLB

Clinicians and patients
Biological resources center
Platforms



Philippe Pierre
Evelina Gatti
Alexis Combes



Tony Ng
Greg Weitsmann
Trupti Pai
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Pr. Ruslan Medzhitov, USA

Ido Amit, Israel

Esteban Ballestar, Spain

Antonio Bertoletti, Singapore

Menna Clatworthy, UK

Julie Dechanet-Merville, France

Sandra Diebold, UK

Eran Elinav, Israel

Nelson Gekara, Sweden

Thomas Gajewski, USA

Ping-Chih Ho, Switzerland

Jonathan Kagan, USA

Mansun Law, USA

Dan Littman, USA

Mala Maini, UK

Frederica M-Berg, UK

Anne Puel, France

John Wherry, USA

Thematic sessions

1. Innate Immunity

2. Immuno-metabolism

3. Immunotherapy and clinics

4. Microbiota

5. Genetics and Epigenetics

6. Oncopathogens and immune responses

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