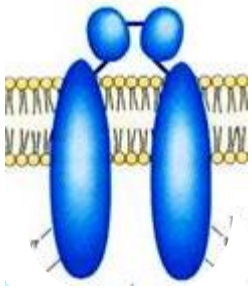
RANK PATHWAY INHIBITORS FOR BREAST CANCER PREVENTION AND TREATMENT

Eva Gonzalez Suarez, PhD
Head of the Transformation and Metastasis group
CNIO (Madrid)/ IDIBELL (Barcelona)
Spain

Clinically relevant factors in breast cancer



Estrogen receptor

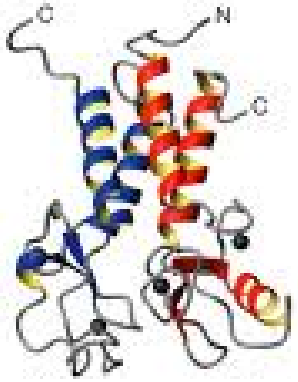
Luminal.
60% of the cases

Aromatase inhibitors.

HER2

30% of the cases.
Homodimerization and ligand-independent activation.
Cell transformation.

Trastuzumab

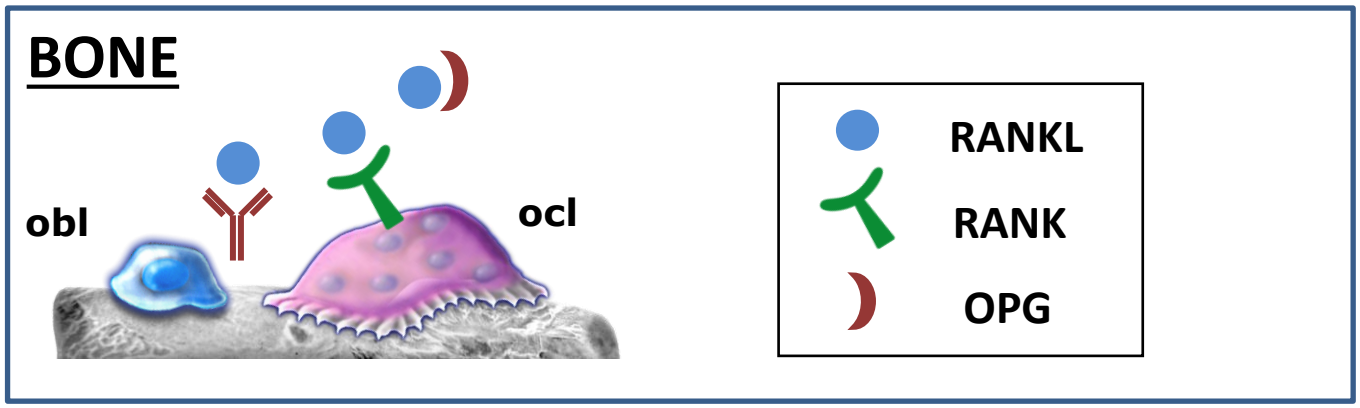


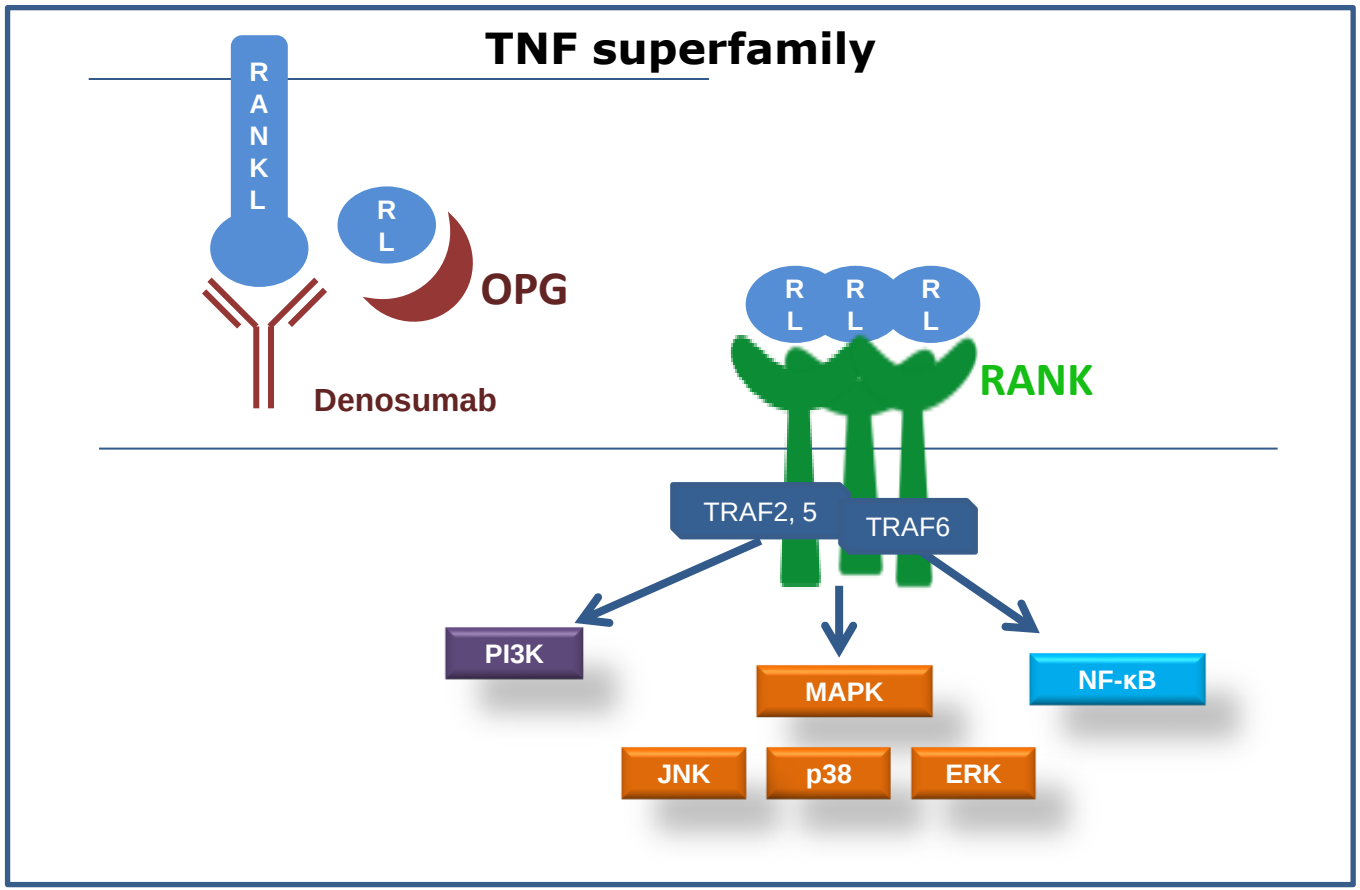
BRCA1

High-penetrance gene
Decrease in sporadic tumors.
Genomic stability.
Mutations induce breast and ovarian cancer.

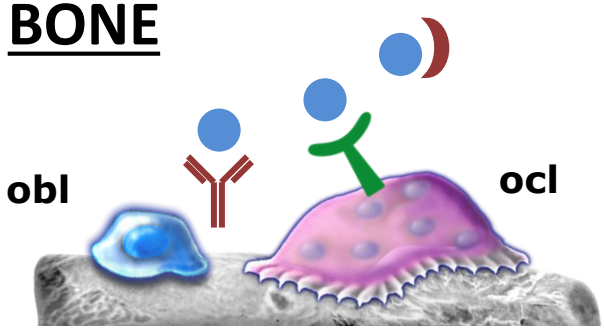
PARP inhibitors

RANK-RANKL pathway inhibition:
a promising novel strategy for breast cancer prevention and treatment

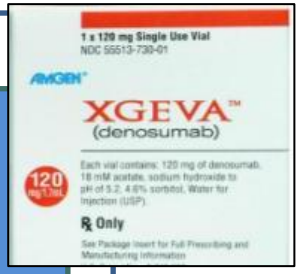

BONE
obl
ocl
RANKL
RANK
OPG

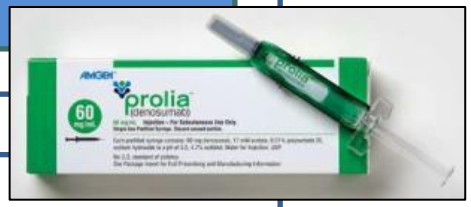

TNF superfamily
RANKL
RL
OPG
Denosumab
RL RL RL
RANK
TRAF2, 5
TRAF6
PI3K
MAPK
NF-κB
JNK
p38
ERK

BONE

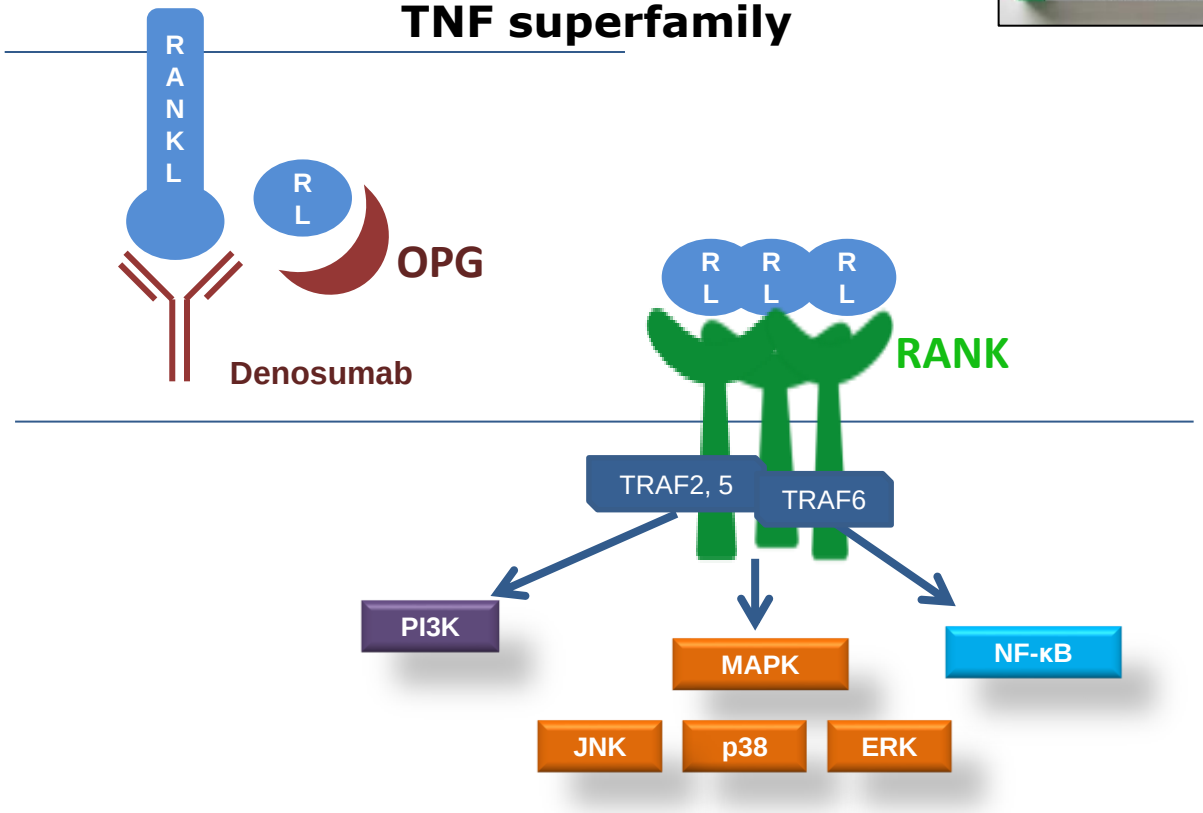


RANKL inhibitor: DENOSUMAB osteoporosis, bone metastasis

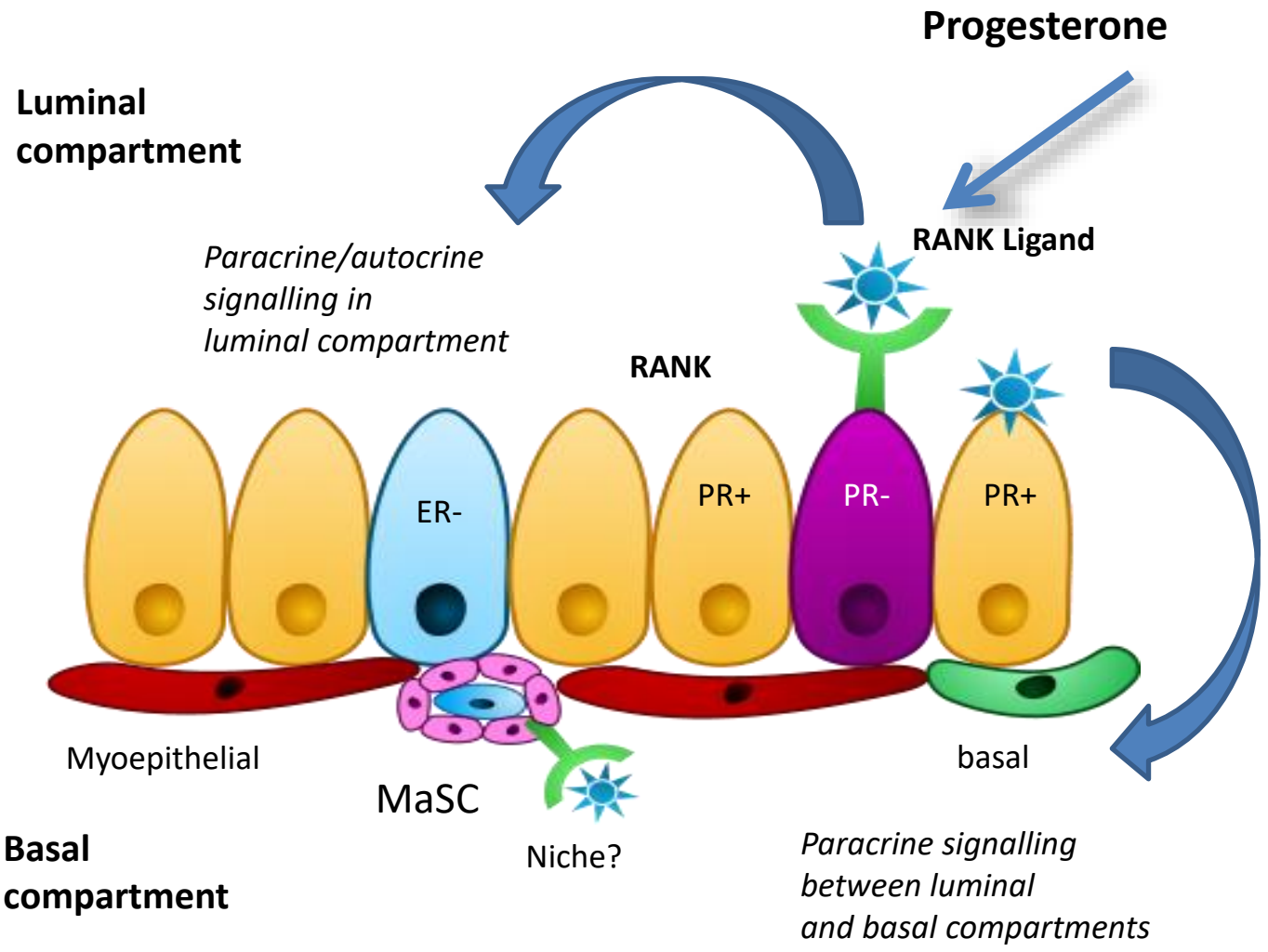




TNF superfamily



RANKL IS AS A PARACRINE MEDIATOR OF PROGESTERONE IN THE MAMMARY GLAND

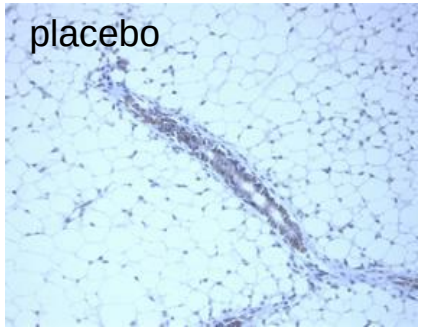


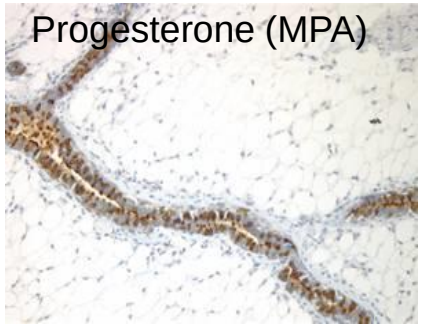
Adapted from Watson CJ and Khaled WT, et al. Development 2008;135: 995–1003.

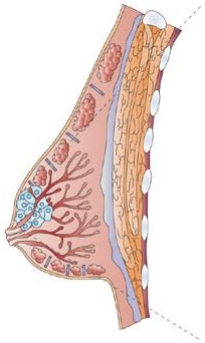
MaSC, mammary stem cell.

Progesterone induces RANKL expression in mouse and human mammary epithelia

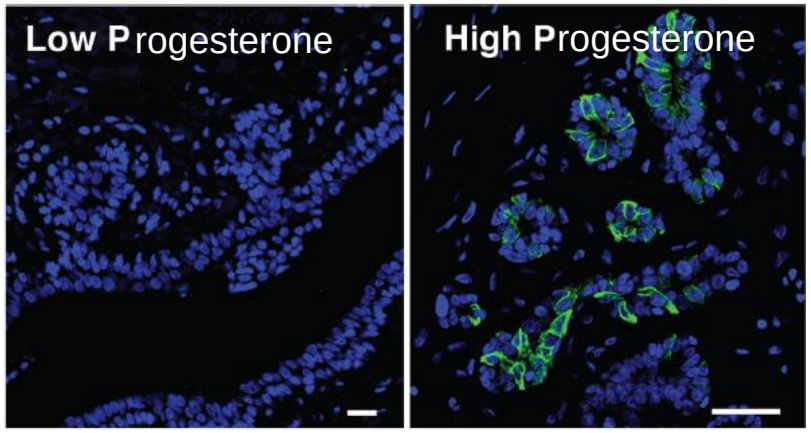
RANKL







RANKL



Tanos T, et al. Sci Transl Med 2013; Gonzalez Suarez et al., Nature 2010

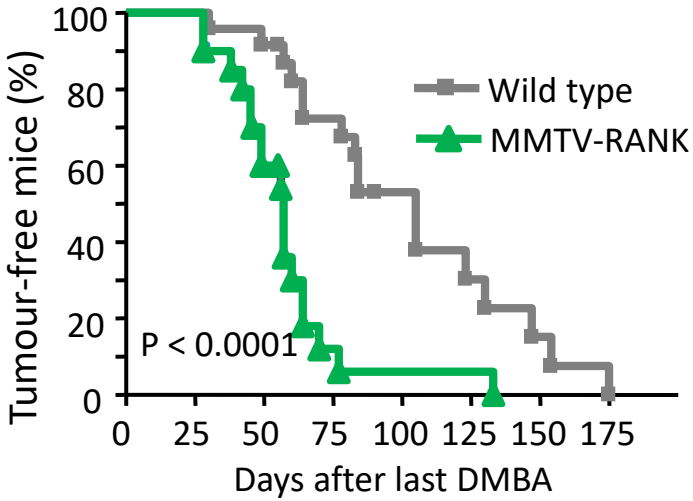
RANK pathway is the main mediator of the pro-tumorigenic role of progesterone in the mammary gland

GOF

RANK $^{tg/+}$

MPA+
DMBA

DECREASED TUMOR LATENCY
INCREASED INCIDENCE



Gonzalez-Suarez et al. Nature, 2010; *Schramek et al., Nature 2010*

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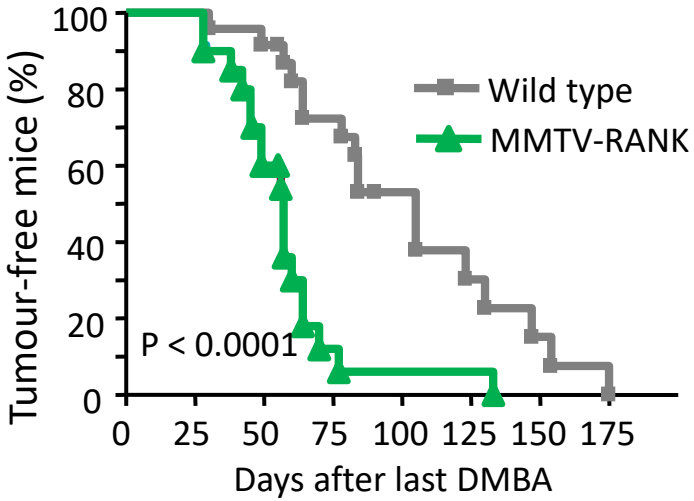


RANK PROMOTES MAMMARY TUMORIGENESIS DRIVEN BY PROGESTERONE

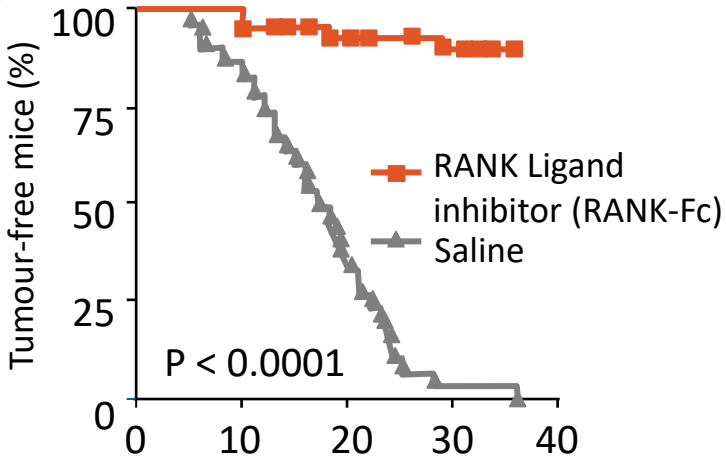
LOF

WT

anti-RANKL

MPA+
DMBA

NO TUMOR FORMATION



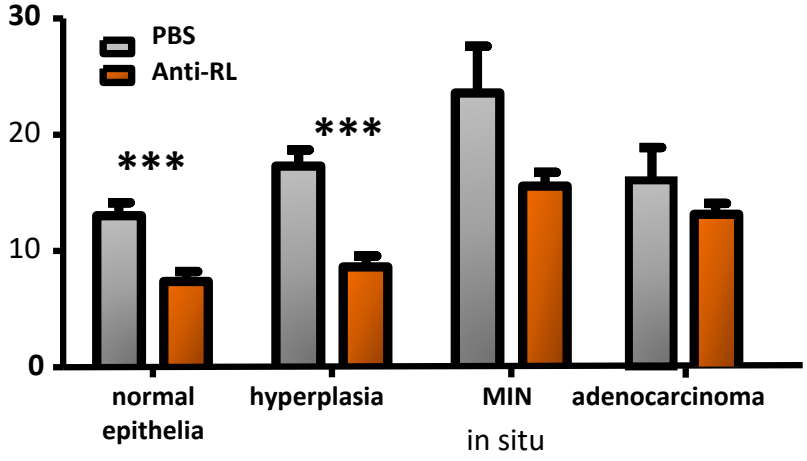
DMBA, 2,4-dimethoxybenzaldehyde (carcinogen); MPA, medroxyprogesterone acetate (progesterone derivative).

Gonzalez-Suarez et al. Nature, 2010; *Schramek et al., Nature 2010*

Inhibition of RANKL decreases proliferation and survival in mammary preneoplasic lesions

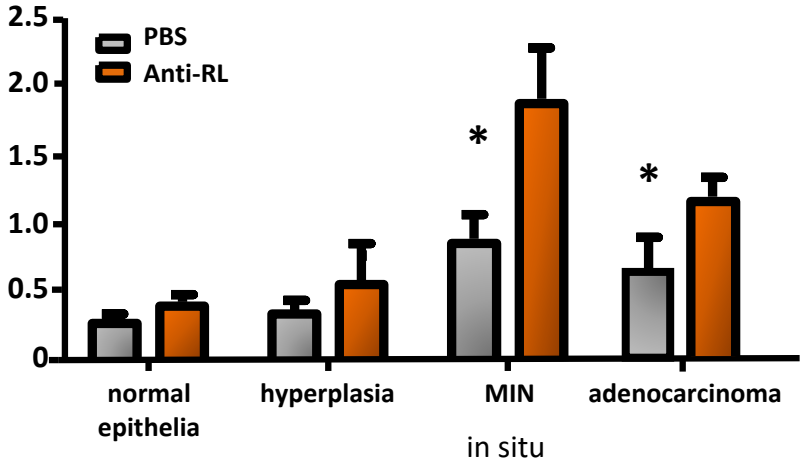
Proliferation

% BrdU positive nuclei



Apoptosis

% cleaved caspase 3 positive nuclei



MIN: mammary intraepithelial neoplasia

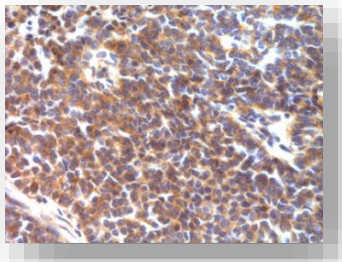
- González-Suárez E, et al. Nature 2010;468:103–7.

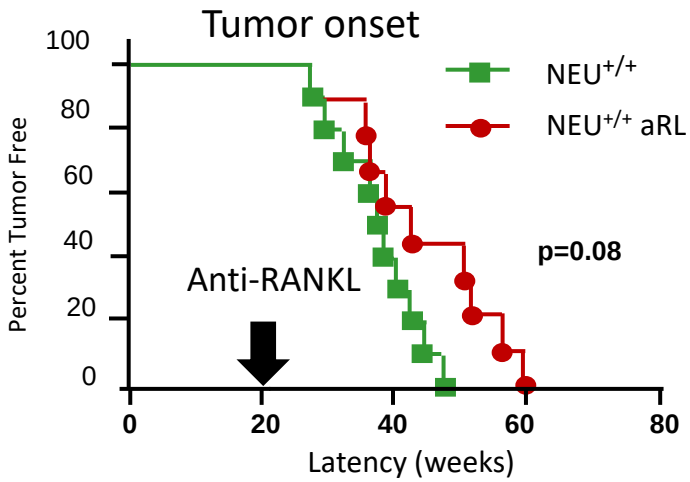
Preventive inhibition of RANK signaling decreases mammary tumor incidence and lung metástasis in oncogene driven models

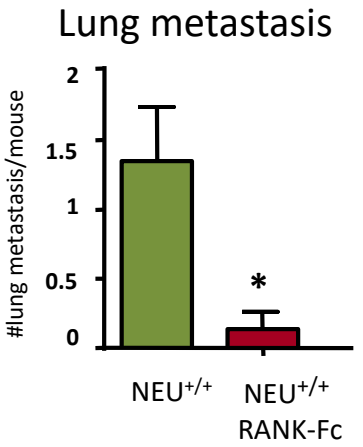
LOF: pharmacologic anti-RANKL

MMTV-Neu

RANK







Gonzalez-Suarez et al., Nature, 2010

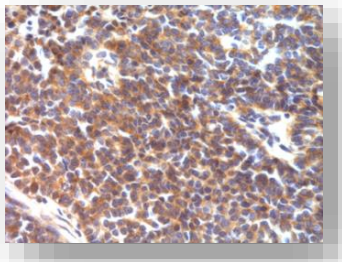
Yoldi G et al ., Cancer Research, 2016

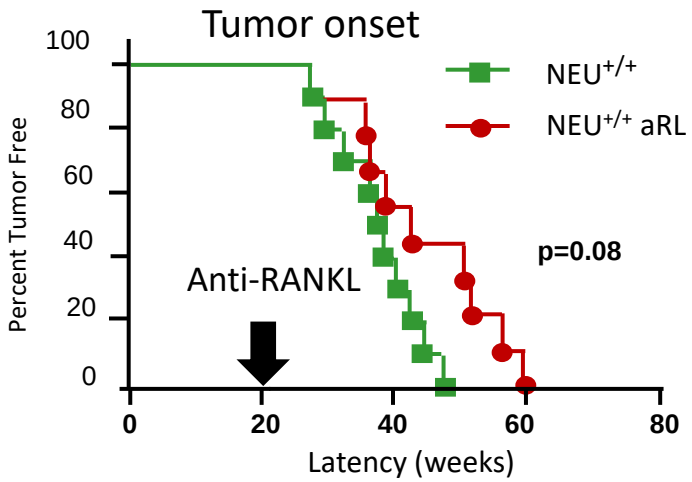
Preventive inhibition of RANK signaling decreases mammary tumor incidence and lung metastasis

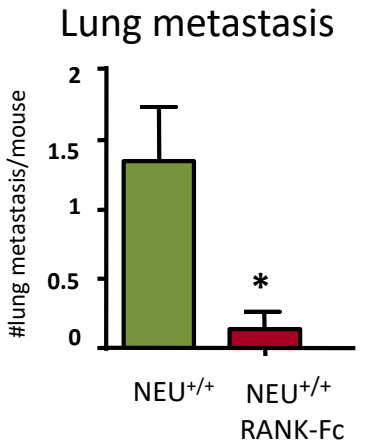
LOF: pharmacologic anti-RANKL

MMTV-Neu

RANK



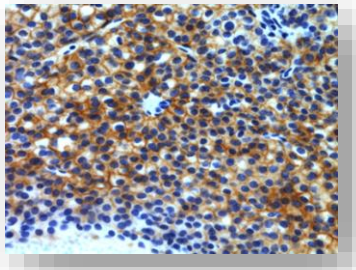


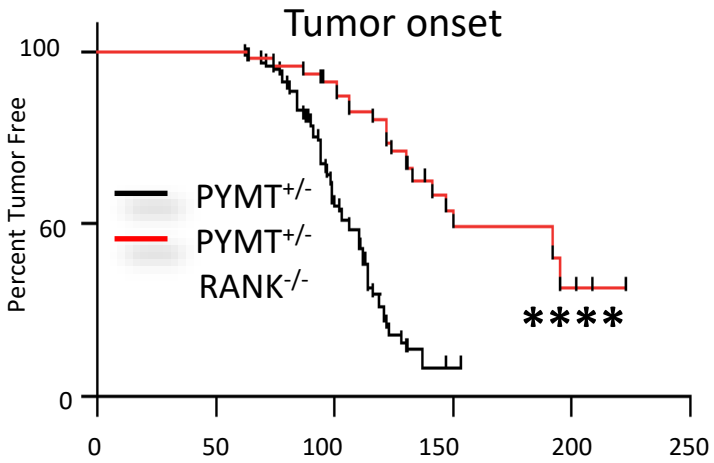


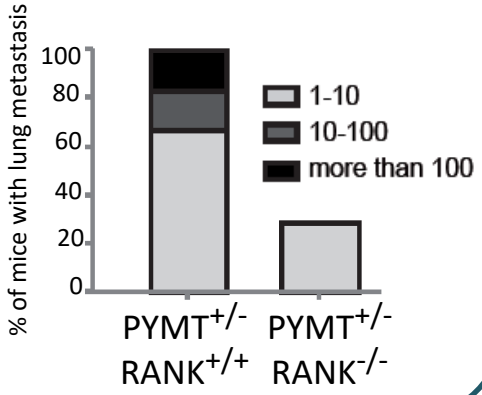
LOF: genetic RANK-/-

MMTV-PyMT

RANK





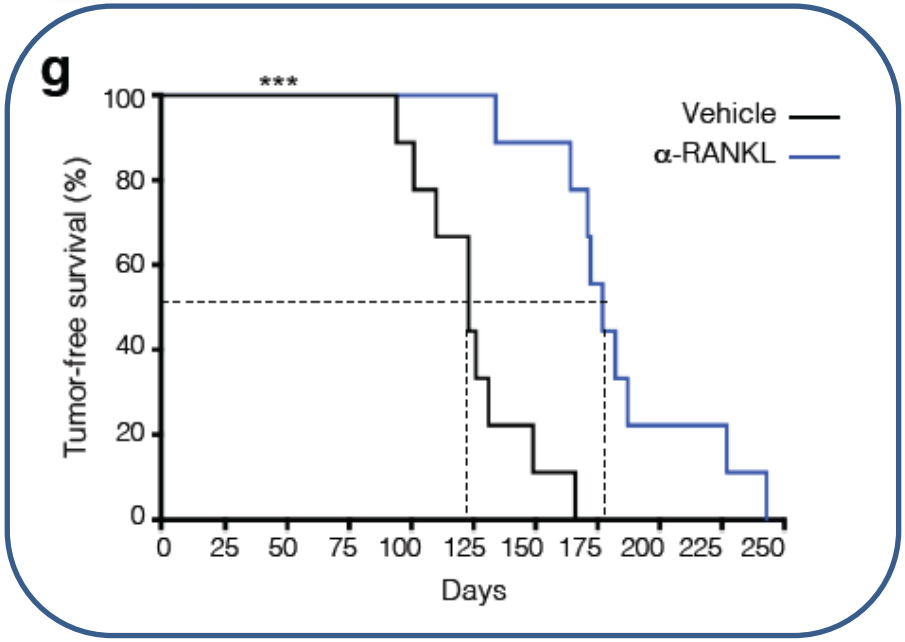


Gonzalez-Suarez et al., Nature, 2010

Yoldi G et al ., Cancer Research, 2016

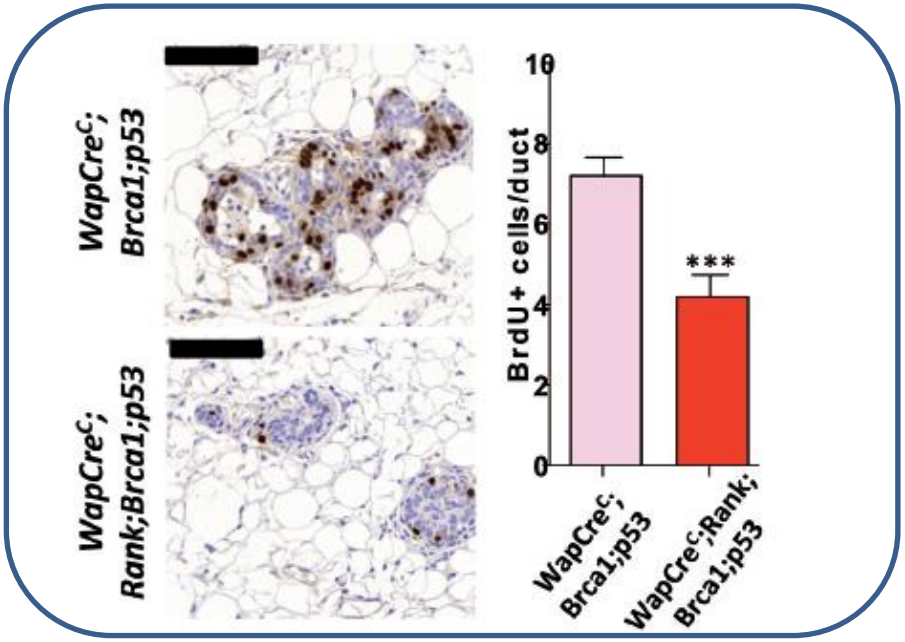
Preventive inhibition of RANK signaling delays mammary tumor onset and mediates mammary epithelial proliferation in Brca1 mutant models

Tumor onset



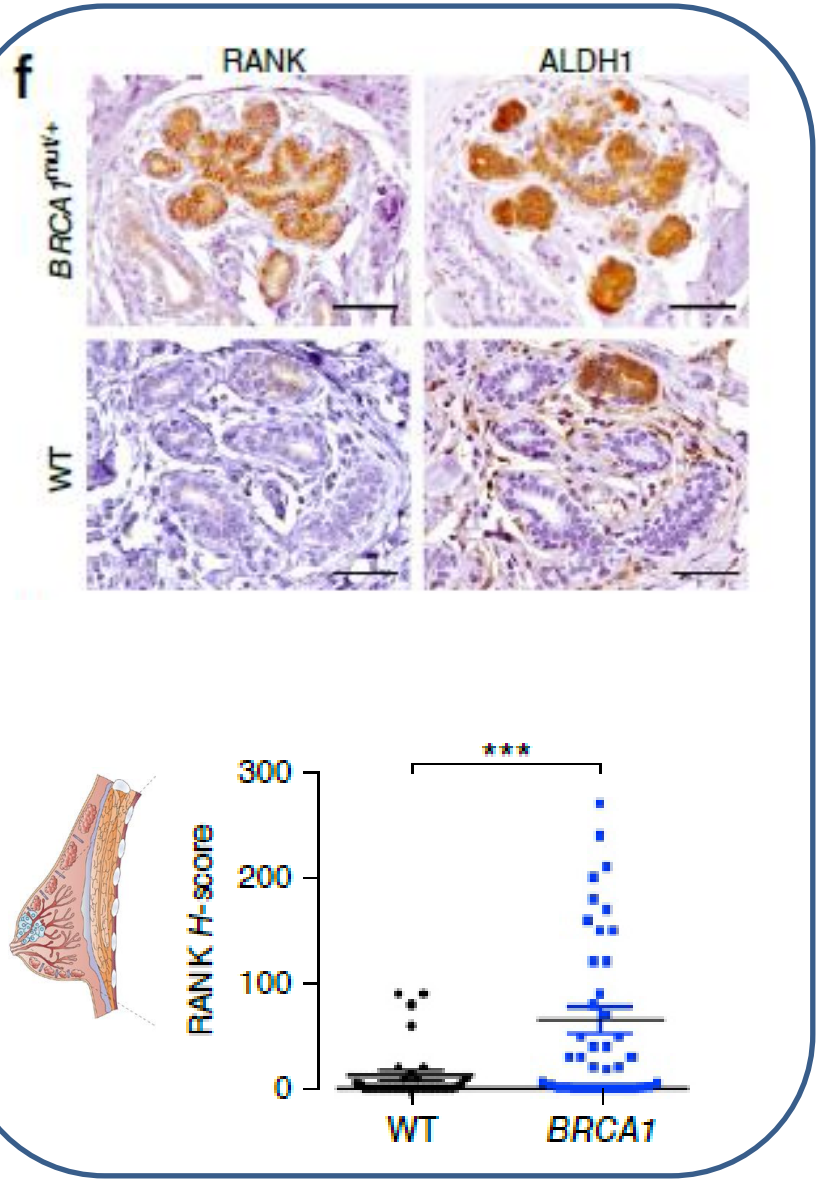
MMTV-cre BRCA1 floxed p53+/-

Proliferation



Nolan et al., Nat med 2016; *Sigl et al, Cell Res 2016*

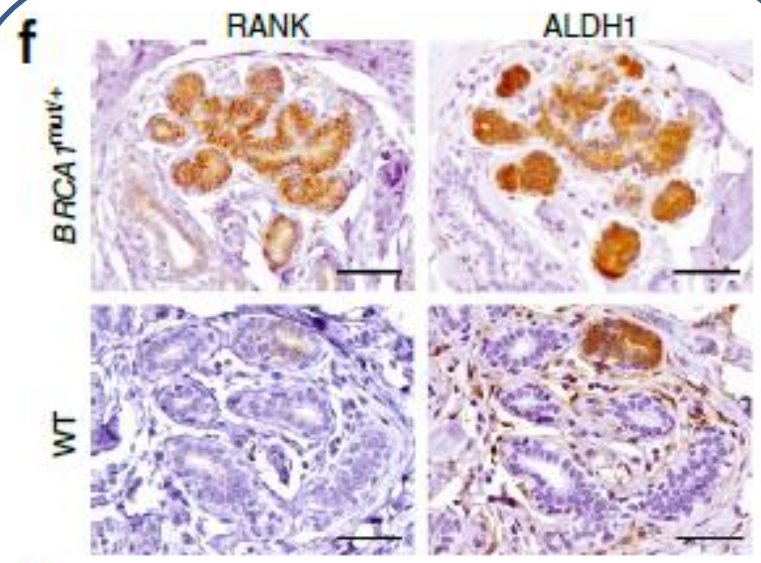
RANK is expressed in the mammary epithelia of BRCA1 mutation carriers and mediates progesterone-induced proliferation

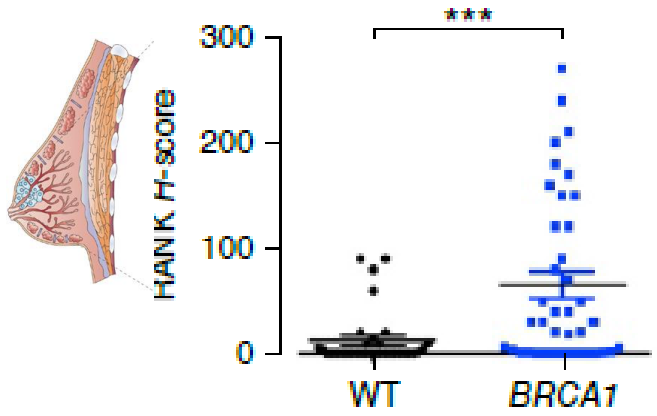


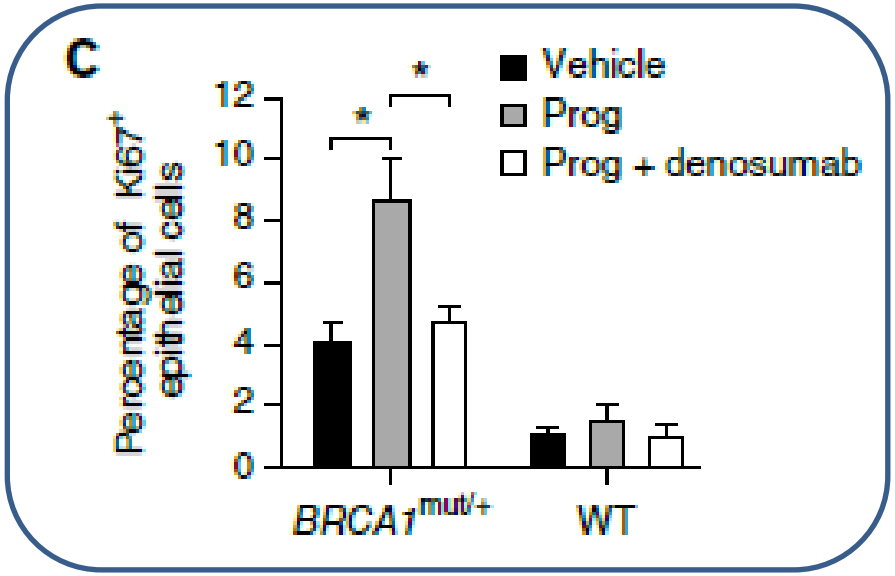
Nolan et al., Nat med 2016

RANK is expressed in the mammary epithelia of BRCA1 mutation carriers and mediates progesterone-induced proliferation

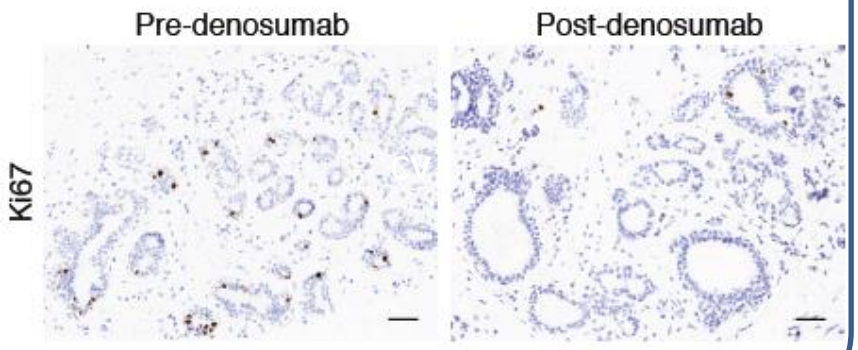
organoids







BRCA-D study



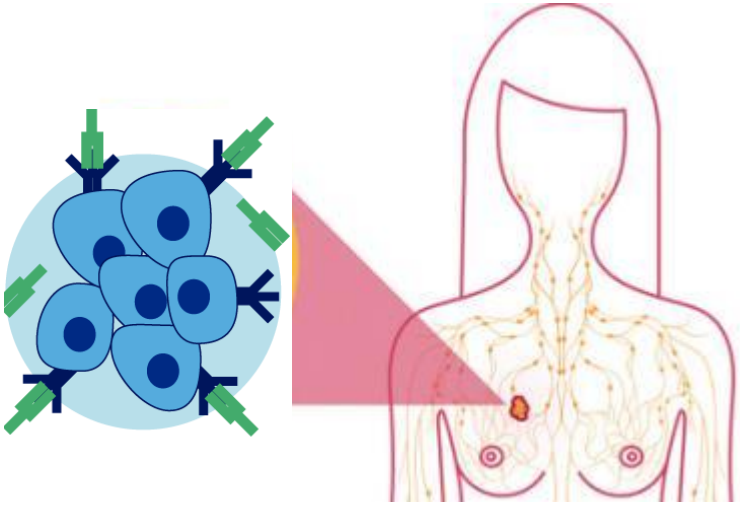
Nolan et al., Nat med 2016

RANK PATHWAY INHIBITORS FOR BREAST CANCER PREVENTION

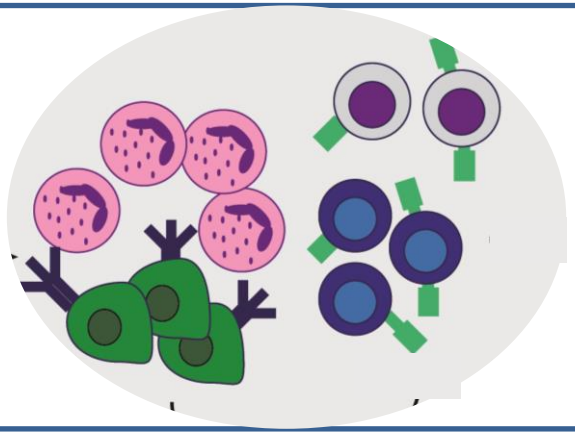
…but TREATMENT?

RANK in breast cancer and immunesurveillance

Breast Cancer



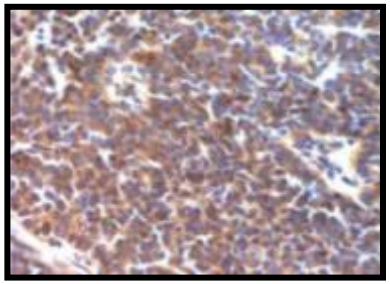
Immune system



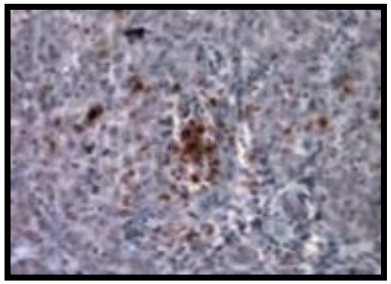
RANK and RANKL expression follow similar patterns in MMTV_PyMT mouse model and in breast cancer patients

MMTV-PYMT mouse model

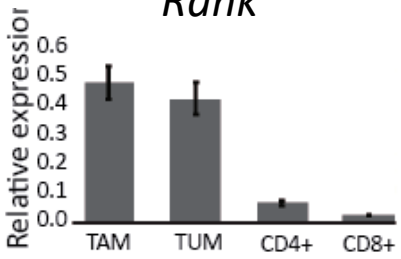
RANK



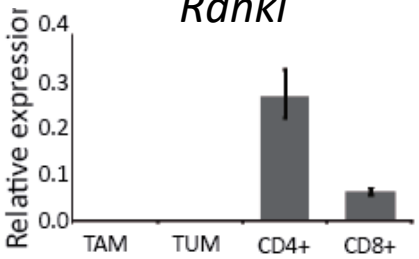
RANKL



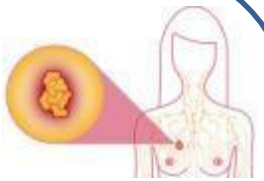
Rank



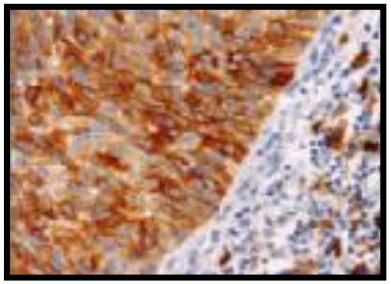
Rankl



Breast Cancer Patient sample

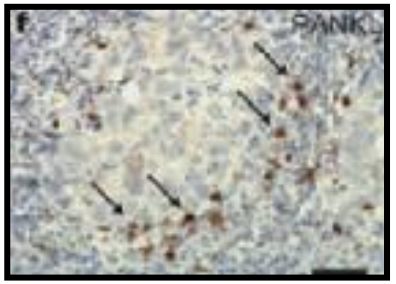


RANK



Tumor cells and TAMs

RANKL



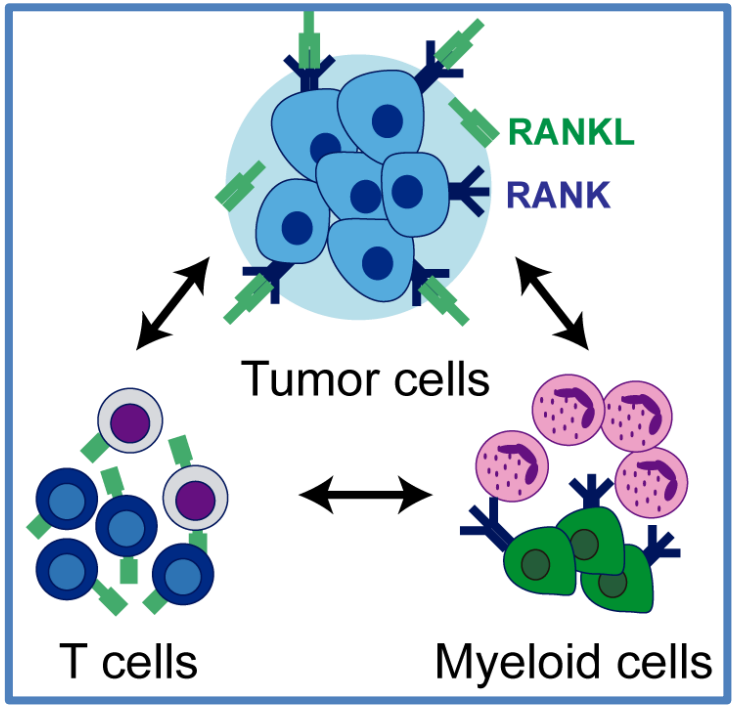
Lymphocytes

TUM: Tumor cells, TAM: Tumor associated macrophages

Yoldi et al., Cancer Res, 2016

Palafox et al., Cancer Res, 2012

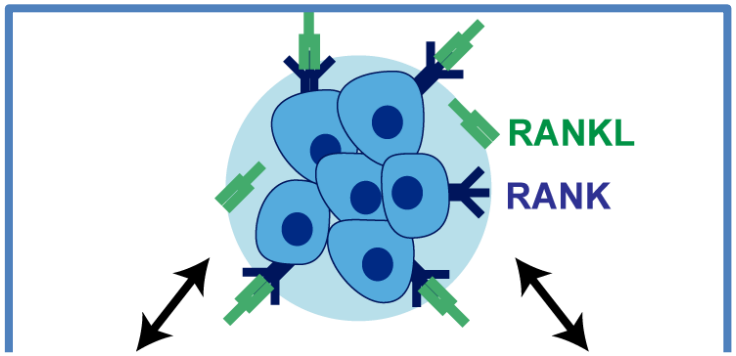
Therapeutic RANK inhibition?



Tumor cell intrinsic

Tumor cell extrinsic

Therapeutic RANK inhibition?



Tumor cell intrinsic

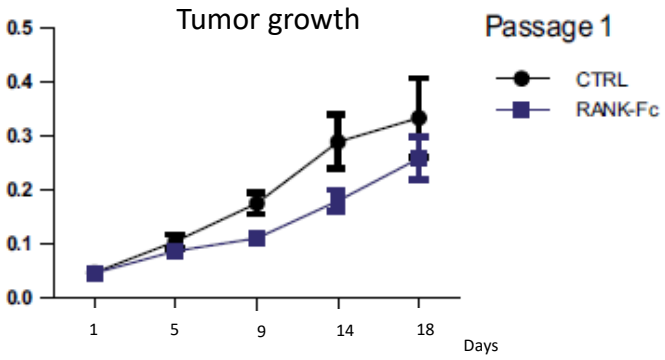
RANKL inhibition decreases cancer stemness and induces tumor cell differentiation

MMTV-PyMT

Surgery
Occult disease

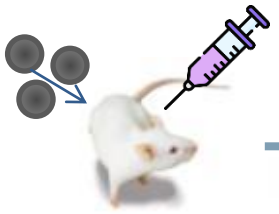
MMTV-PyMT

Anti RANKL treatments in mice



Yoldi, Pellegrini, Trinidad *et al. Can Res* 2016

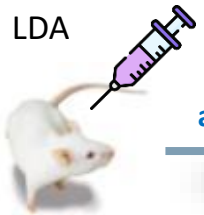
RANKL inhibition decreases cancer stemness and induces tumor cell differentiation



MMTV-PyMT

neoadjuvant anti-RANKL

LDA



Surgery
Occult disease

adjuvant anti-RANKL

TICS

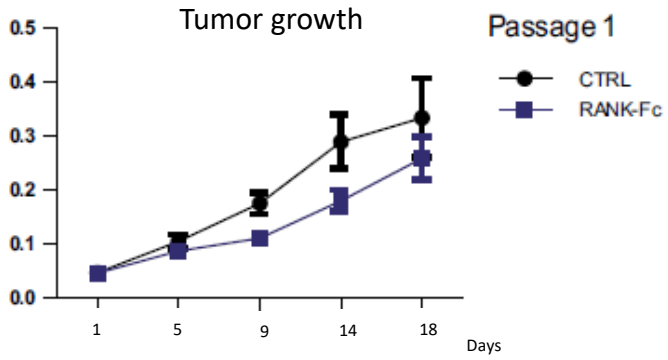
Recurrence

CTRL: 1/ 206
neoad anti-RL: 1/ 1929*
adjuv anti-RL: 1/ 466
neoad + adjuv anti-RL: 1/2353*

LDA: Limiting Dilutions Assays

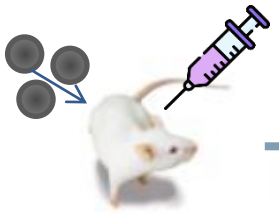
MMTV-PyMT

Anti RANKL treatments in mice



Yoldi, Pellegrini, Trinidad *et al. Can Res* 2016

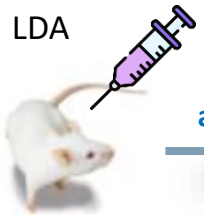
RANKL inhibition decreases cancer stemness and induces tumor cell differentiation



MMTV-PyMT

neoadjuvant anti-RANKL

LDA



Surgery
Occult disease

adjuvant anti-RANKL

TICS

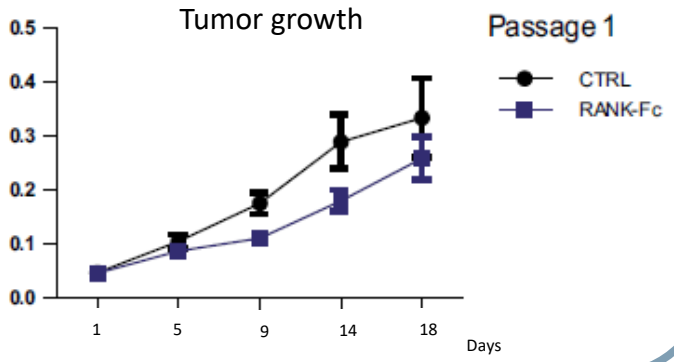
Recurrence

CTRL: 1/ 206
neoad anti-RL: 1/ 1929*
adjuv anti-RL: 1/ 466
neoad + adjuv anti-RL: 1/2353*

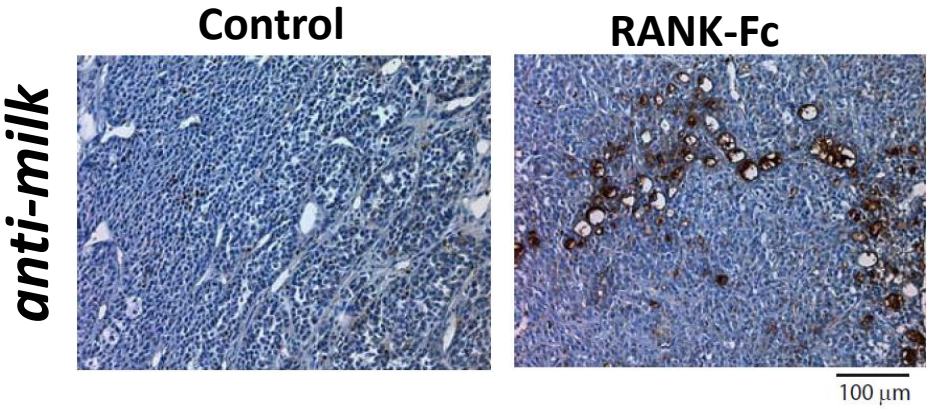
LDA: Limiting Dilutions Assays

MMTV-PyMT

Anti RANKL treatments in mice

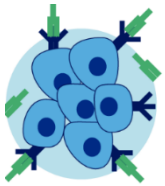


Tumor Cell differentiation

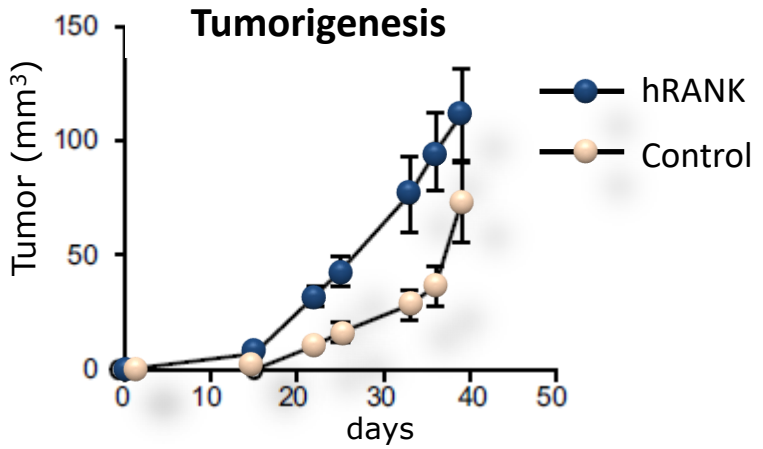


Yoldi, Pellegrini, Trinidad *et al. Can Res* 2016

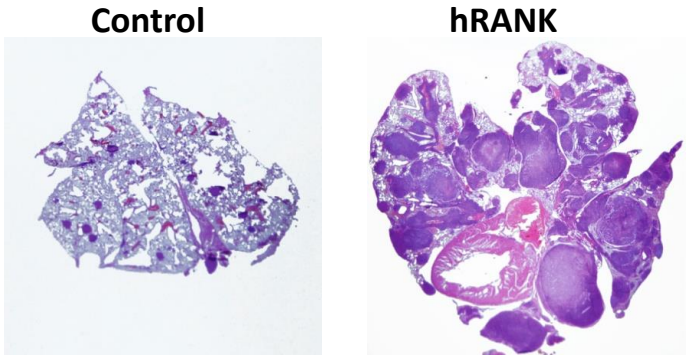
RANK overexpression induces stemness & increases tumorigenesis & metastasis in BRCA1 mutant breast cancer cell lines



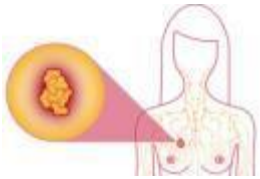
Cancer cell lines (BRCA1)



Metastasis

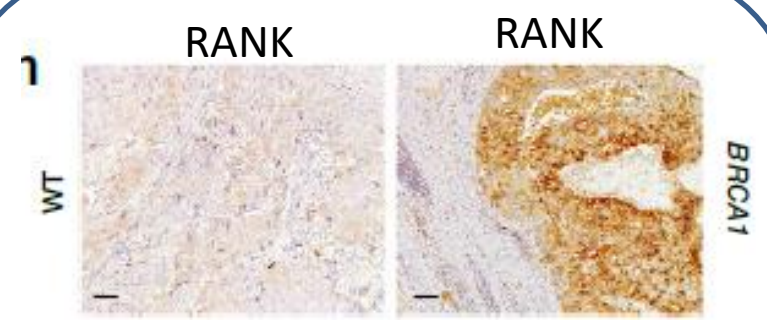


MDA-MB-436

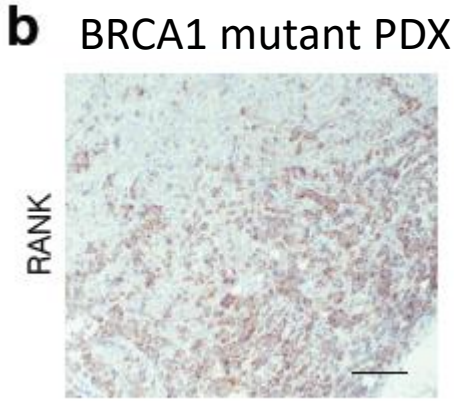


Palafox et al, Cancer Res 2012

RANK is expressed in a subset of BRCA1 mutant breast cancer PDX & RANKL inhibition enhances the response to docetaxel

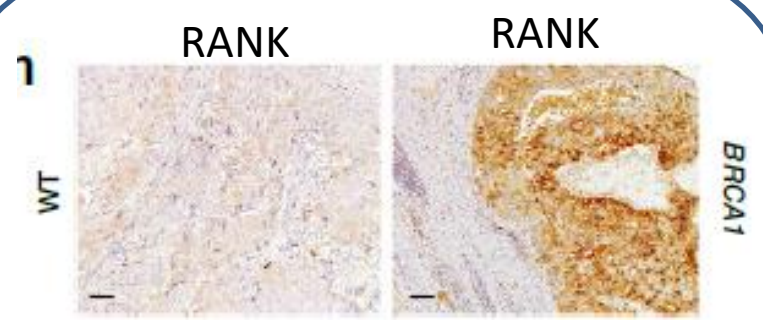


RANK+ (40% of BRCA1 mutated tumors)



Nolan et al., Nat med 2016

RANK is expressed in a subset of BRCA1 mutant breast cancer PDX & RANKL inhibition enhances the response to docetaxel



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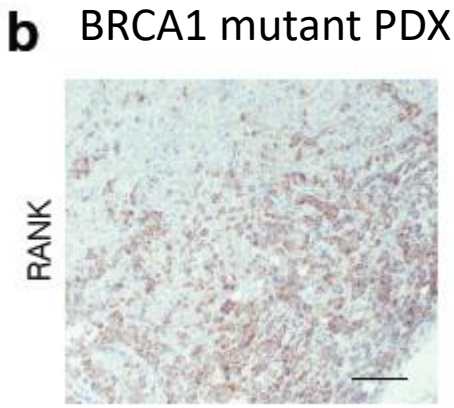
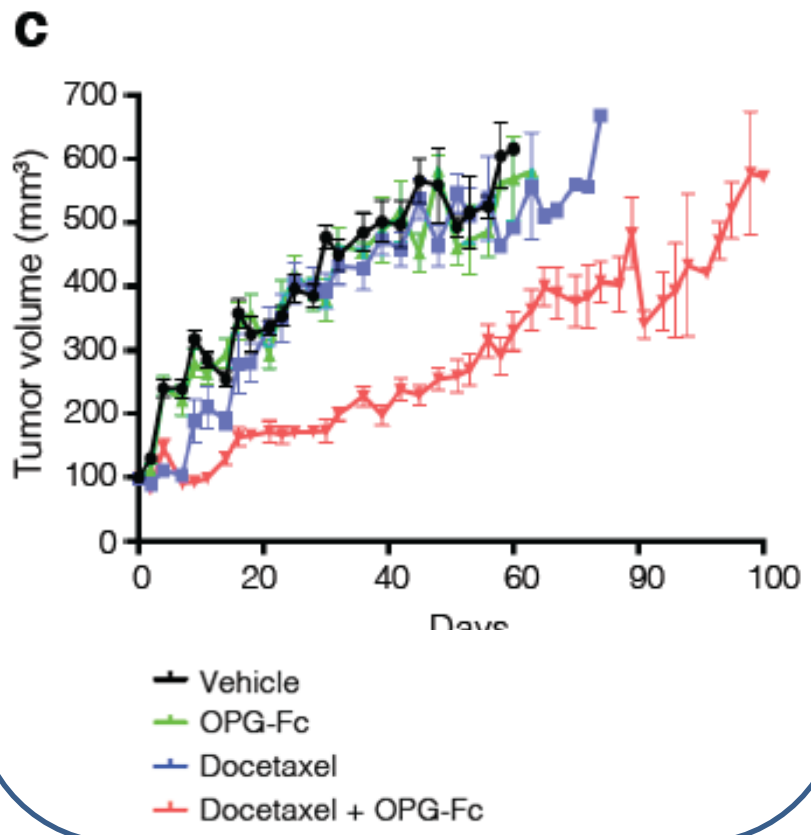
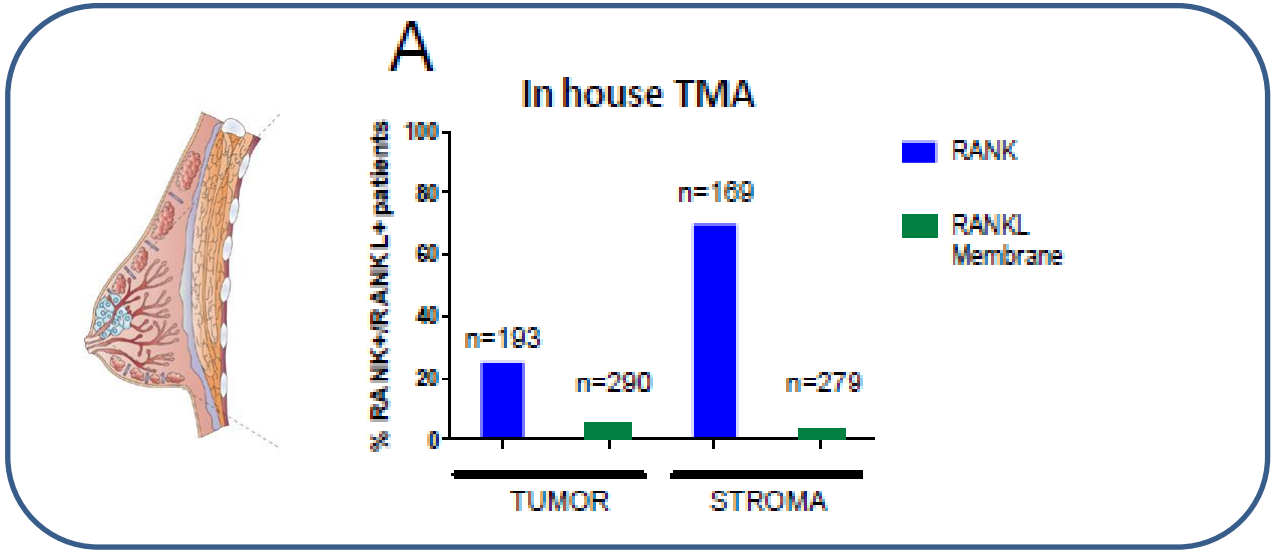




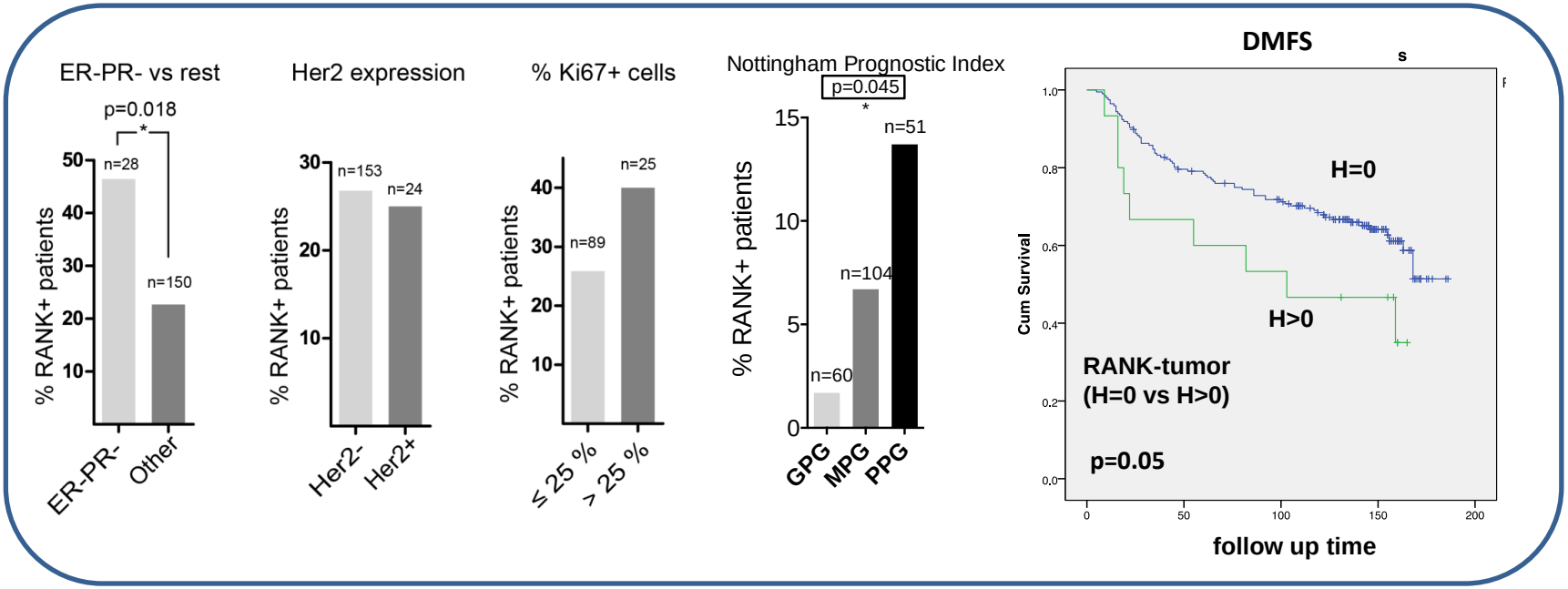
Nolan et al., Nat med 2016

RANK is expressed in a subset of ER+ and ER- tumors and associates with TNBC disease

DO NOT POST

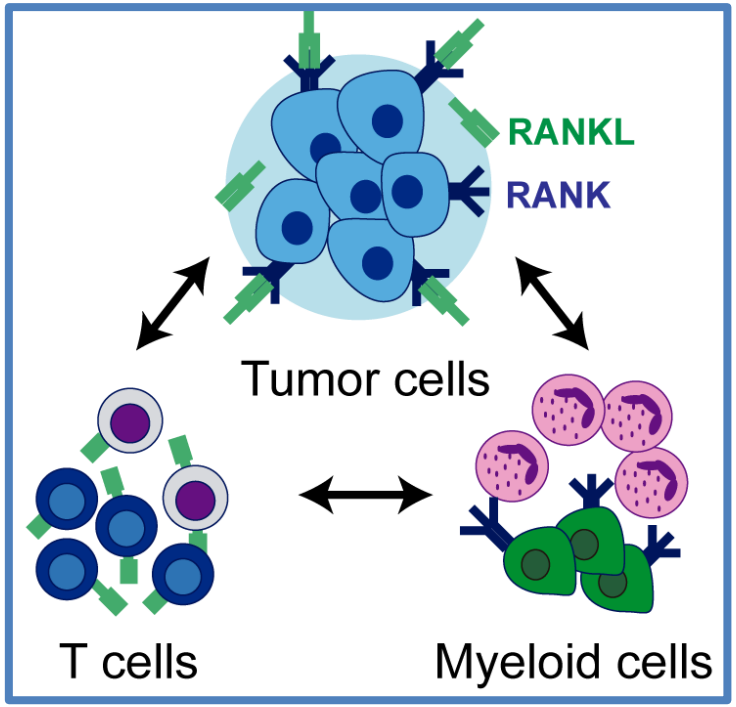


IN HOUSE/METABRIC TMA



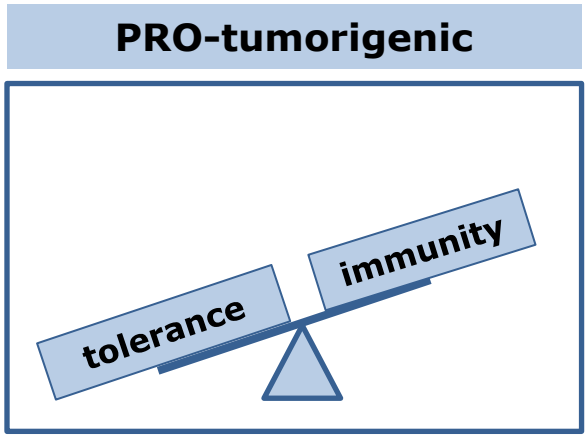
Ciscar et al., in preparation

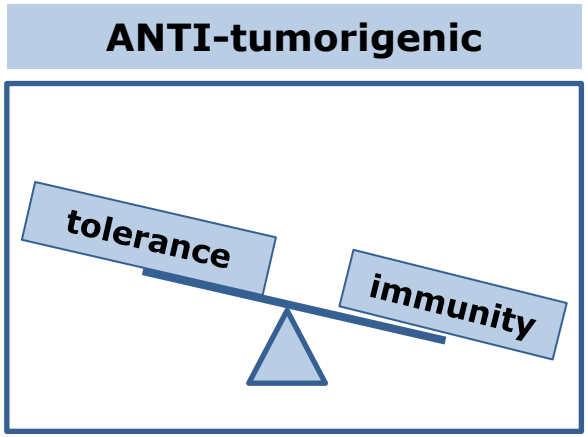
RANK in tumor immune-surveillance



Tumor cell intrinsic

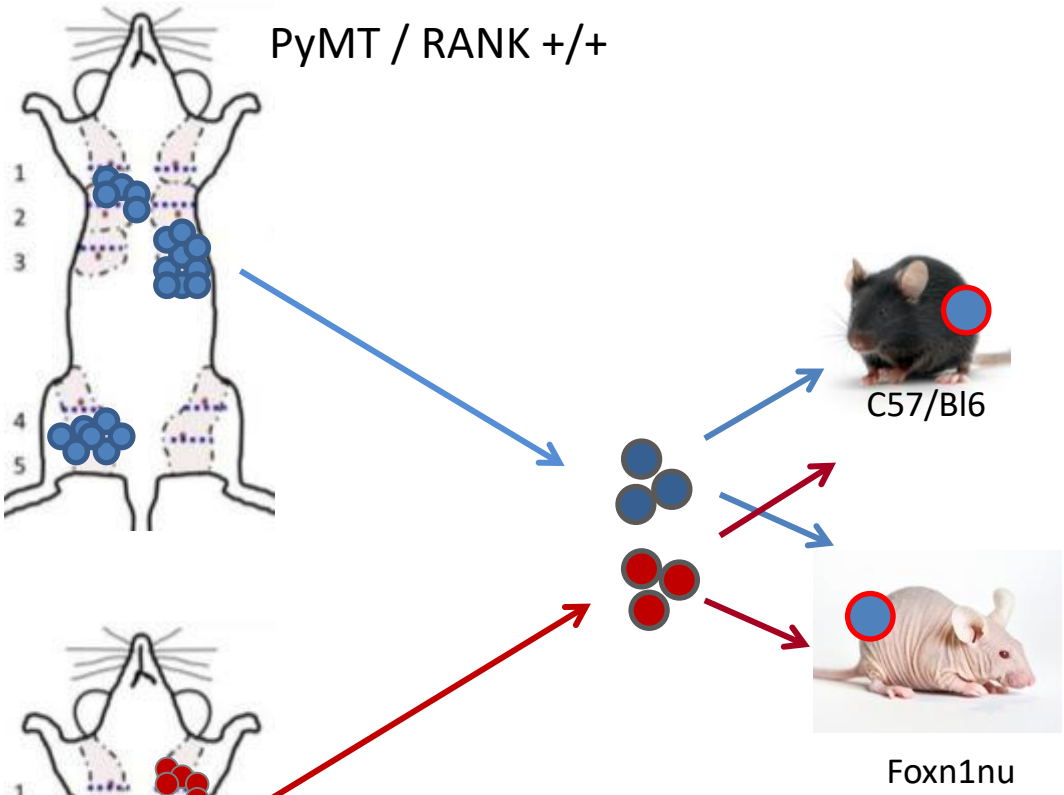
Tumor cell extrinsic

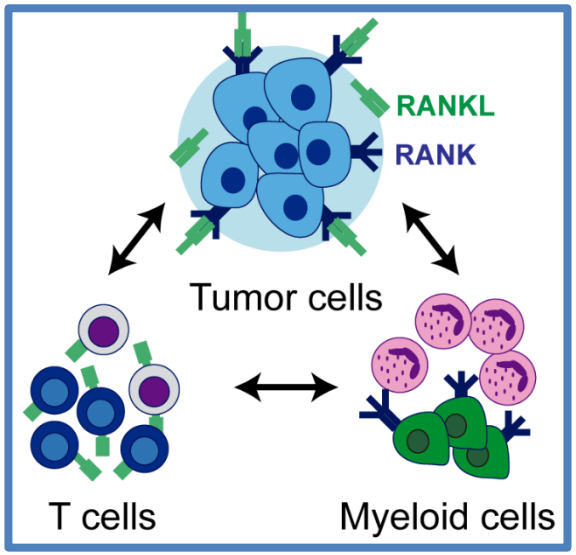




Denosumab

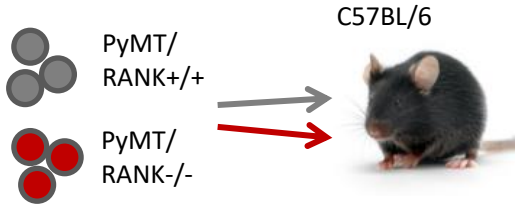
MMTV_PYMT tumor transplants

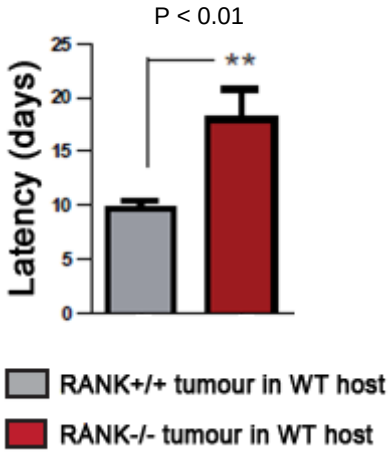




RANK-/- tumour cells show delayed latency and reduced tumour-initiating ability in syngenic hosts
RANK-/- tumours are infiltrated by more TILs and CD8+ T cells

LOF approach





TICs

Tumour-initiating ability:
limiting dilution assays

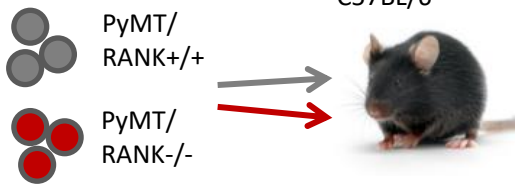
RANK+/+: 1/ 285
RANK -/-: 1/ 1078*

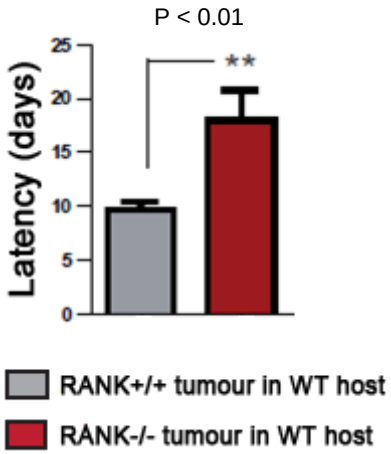
TICs: Tumour-initiating cells

Yoldi et al., Can Res 2016;

RANK-/- tumour cells show delayed latency and reduced tumour-initiating ability in syngenic hosts
RANK-/- tumours are infiltrated by more TILs and CD8+ T cells

LOF approach





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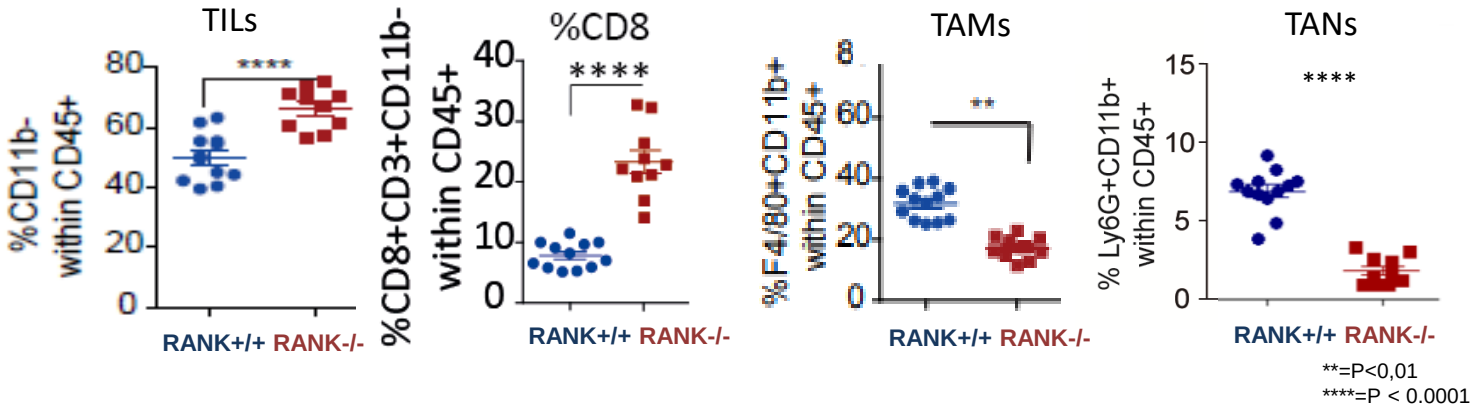
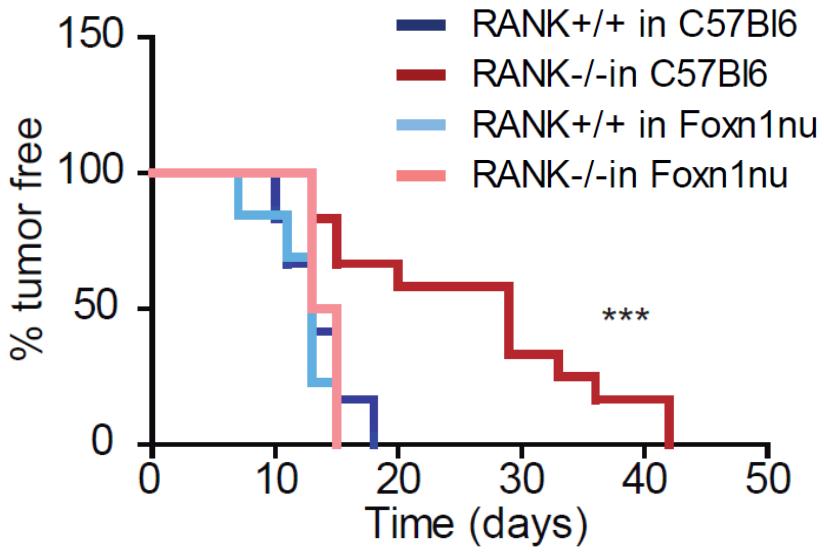


TILs: Tumour-infiltrating lymphocytes
TANs: Tumour-associated neutrophils
TAMs: Tumour-associated macrophages

Yoldi et al., Can Res 2016; Gomez-Aleza et al., Nat Comm. 2020

RANK-/- tumor cells show delayed latency and reduced tumor initiating ability when transplanted in syngeneic hosts



Tumor Initiating Ability: limiting dilution assays

TICS

RANK+/+: 1/ 285

RANK -/-: 1/ 1078*

p-value = 0.05 χ2=3.58

TICS

RANK+/+: 1/ 47

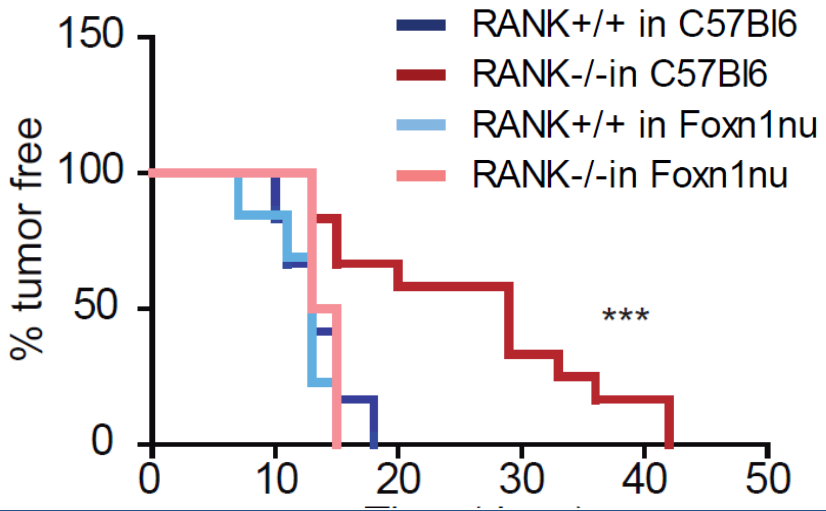
RANK -/-: 1/ 16

p-value = 0.196 χ2=1.67

TICs: Tumor initiating cells

Gomez-Aleza et al., Nat Comm. 2020

RANK-/- tumor cells show delayed latency and reduced tumor initiating ability when transplanted in syngeneic hosts



The immune system mediates the differences in tumor latency and tumor initiation in RANK-/- tumors

Tumor Initiating Ability: limiting dilution assays

TICS ↓

RANK+/+: 1/ 285

RANK -/-: 1/ 1078*

p-value = 0.05 χ2=3.58

TICS =

RANK+/+: 1/ 47

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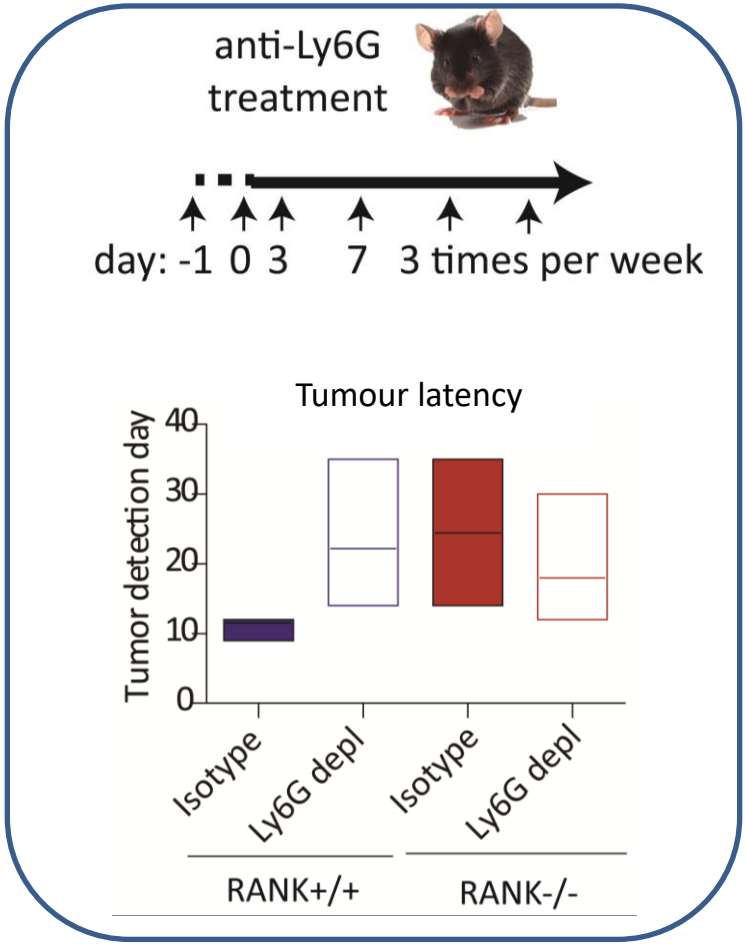
p-value = 0.196 χ2=1.67

TICs: Tumor initiating cells

Gomez-Aleza et al., Nat Comm. 2020

RANK-/- tumour delayed latency is mediated by neutrophils and CD8+ T cells

LOF approach



Gomez-Aleza et al., Nat Comm. 2020

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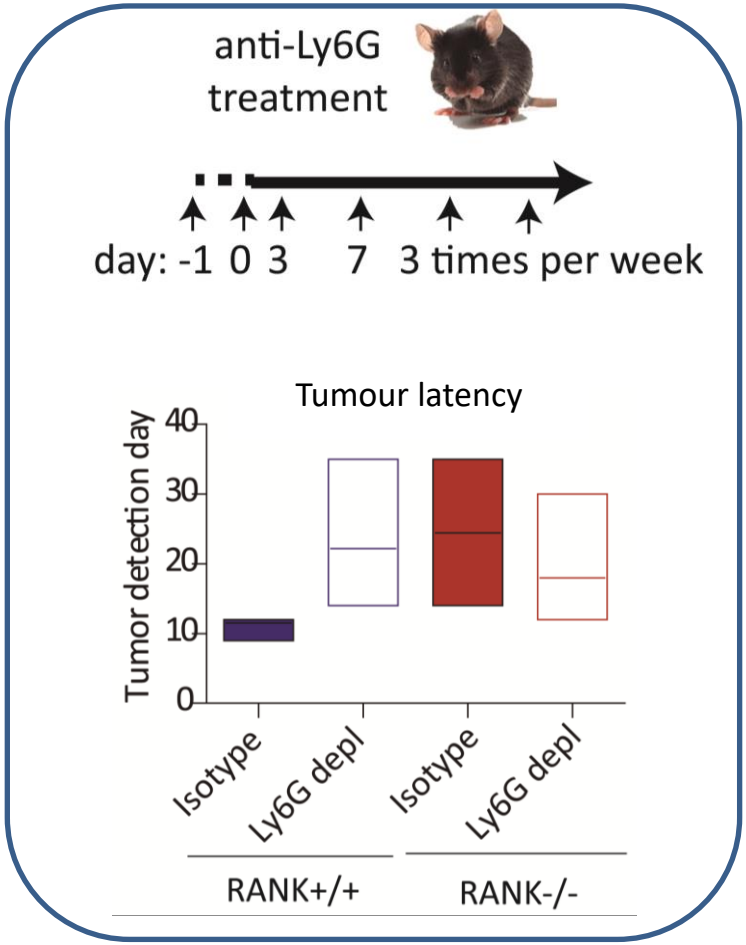
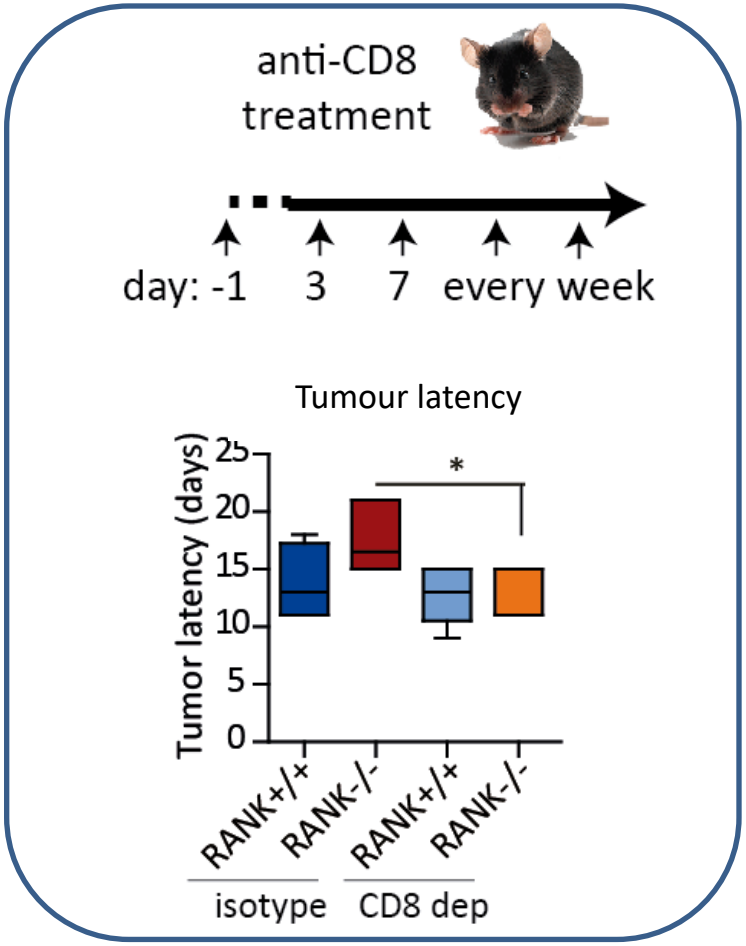


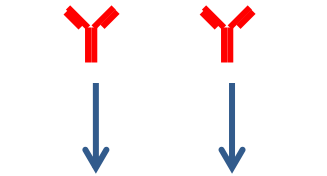


Gomez-Aleza et al., Nat Comm. 2020

D-BIOMARK: neoadjuvant denosumab in early BC. (60 patients, pre/postmenopausal, lum/TNBC)

D-BEYOND: neoadjuvant denosumab in luminal, premenopausal early BC (24 patients, pre, lum)

Biopsy (pre-denosumab)



Two Denosumab doses

Surgery

A Vethencourt, EM Trinidad
C Falo, S Pernas
A Urruticoechea
T Soler, A Petit
M Garcia, V Navarro

ICO Institut Català d'Oncologia

IDIBELL Institut d'Investigació Biomèdica de Bellvitge

INSTITUT JULES BORDET INSTITUUT

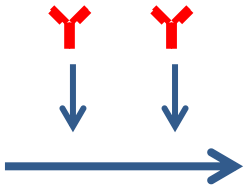
Collaboration:
C Sotiriou
B Nguyen
M Piccard

D-BEYOND: neoadjuvant denosumab in premenopausal early BC (luminal) does not change tumor cell proliferation

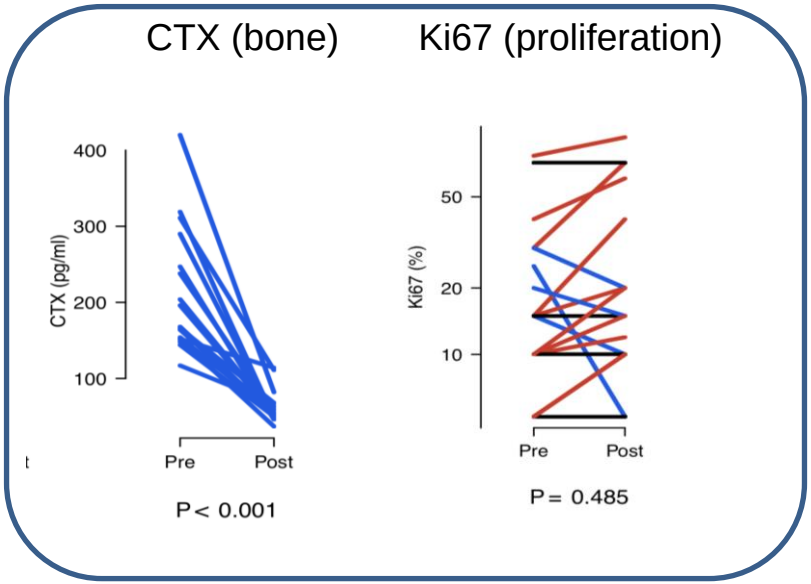
Patients

- LumA 10
- LumB 9
- HER2 4
- Basal 1

Biopsy (pre-denosumab)



Surgery



Collaboration:
C Sotiriou
B Nguyen
M Piccard

Gomez-Aleza et al., Nat Comm. 2020

D-BEYOND: neoadjuvant denosumab increases tumor immune infiltration in premenopausal early BC (luminal)

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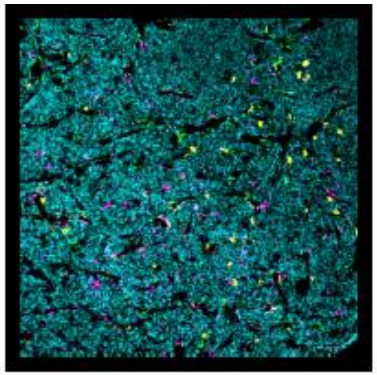
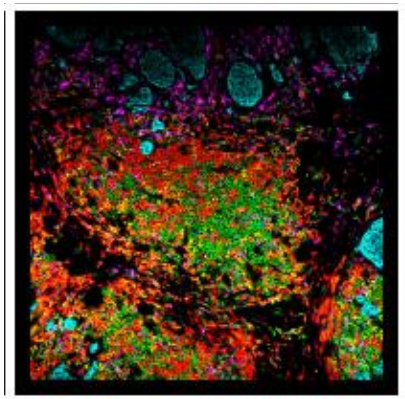
LumA	10
LumB	9
HER2	4
Basal	1

Biopsy (pre-denosumab)



Two Denosumab doses

Surgery



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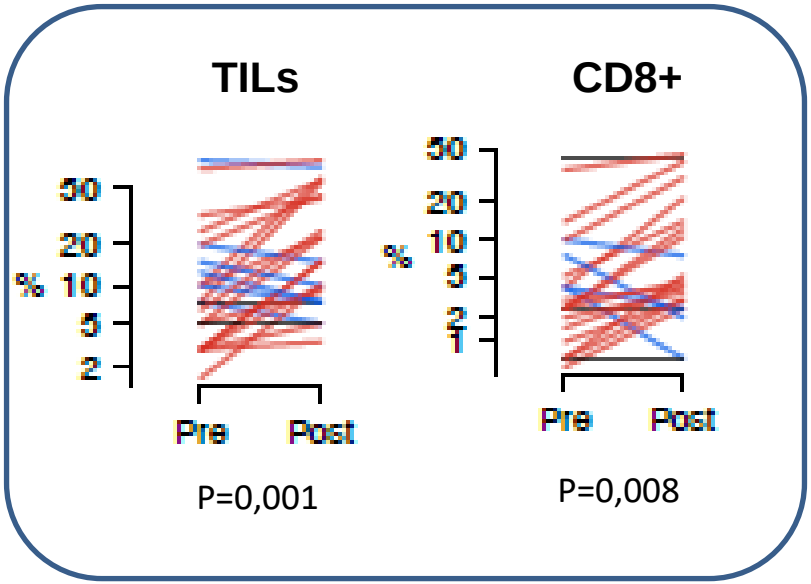

Gomez-Aleza et al., Nat Comm. 2020

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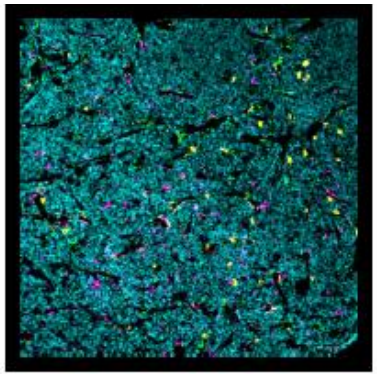
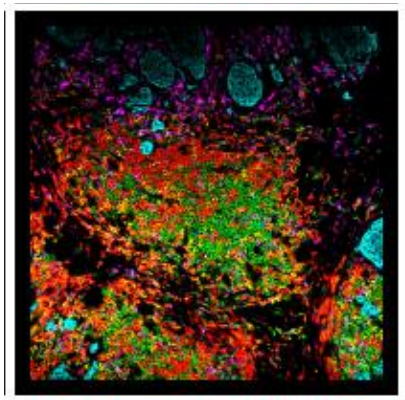


Two
Denosumab doses



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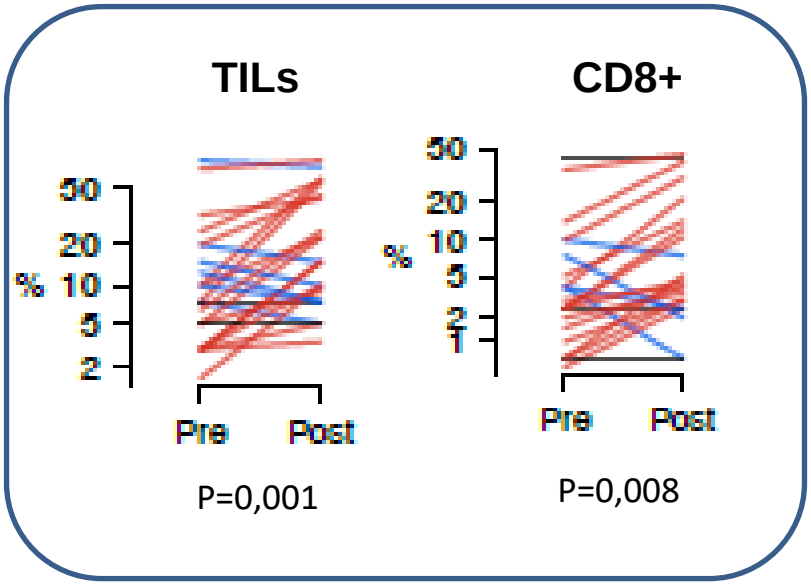
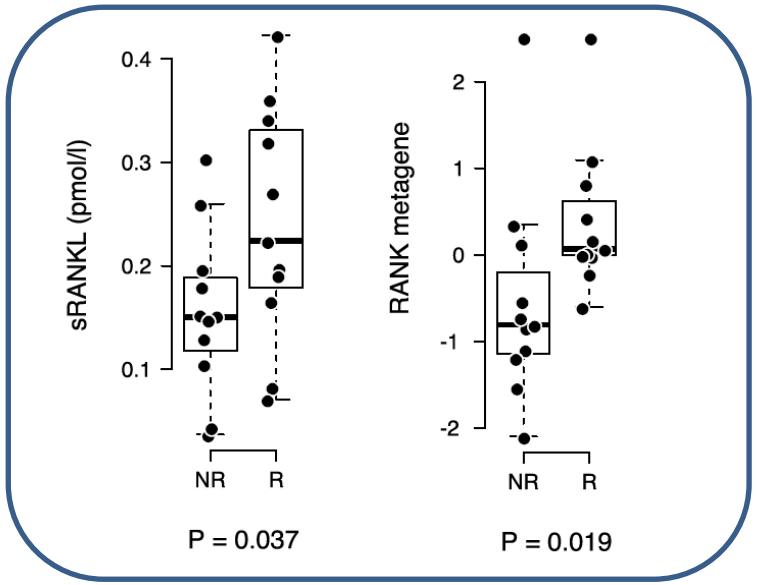
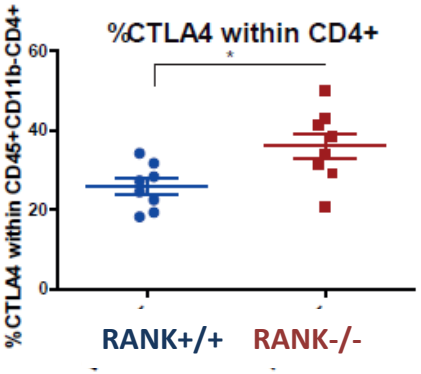


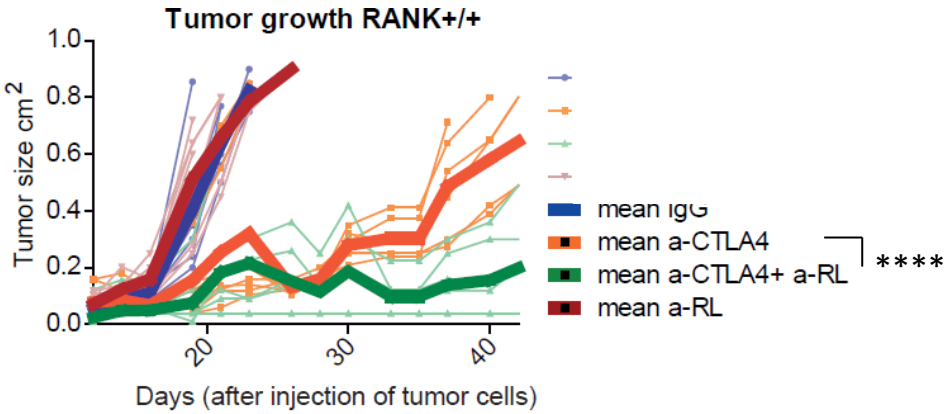


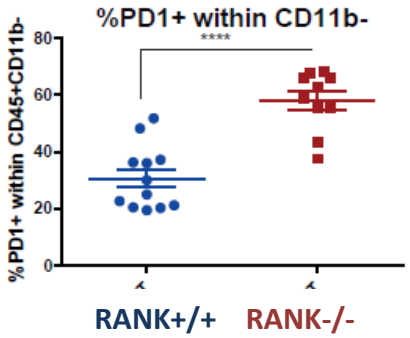
Gomez-Aleza et al., Nat Comm. 2020

Rankl inhibition improves response to immunotherapy in breast cancer

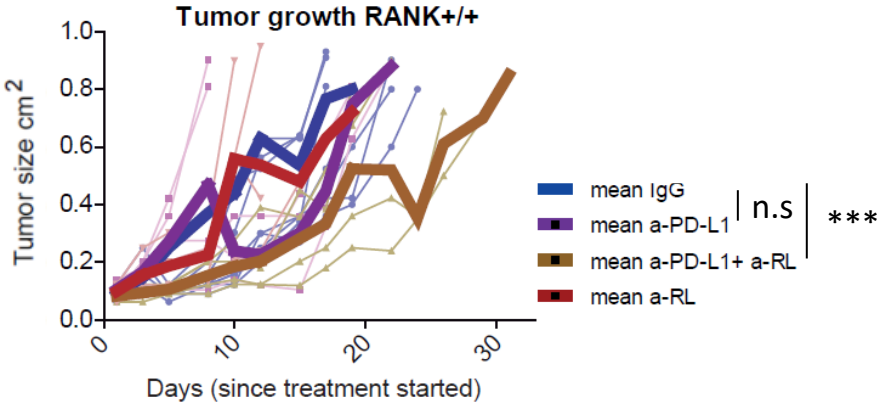


Early setting:





Late setting:



Gomez-Aleza et al., Nat Comm. 2020

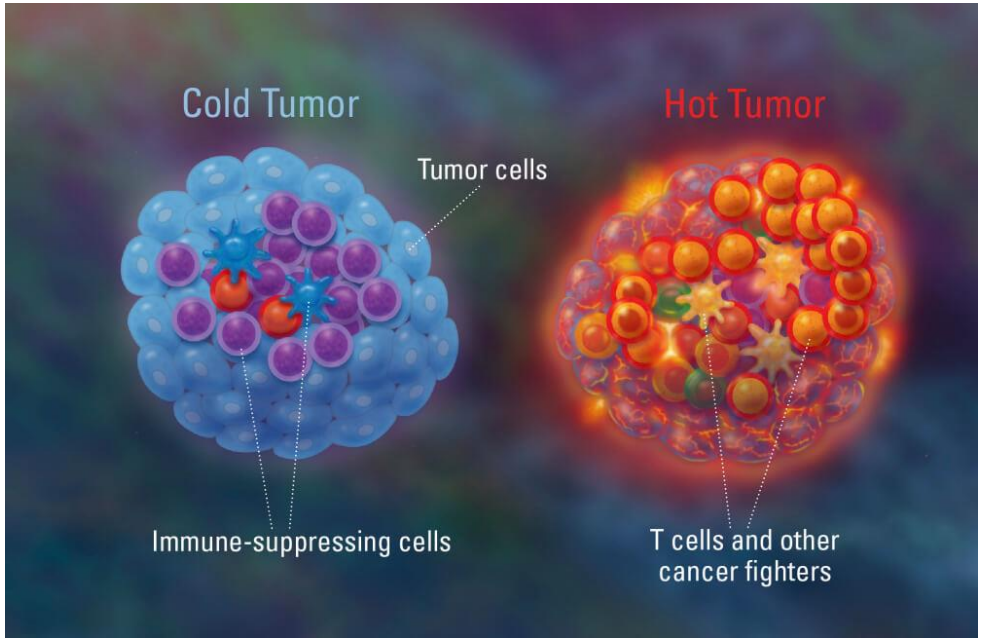
RANK PATHWAY INHIBITORS FOR

BREAST CANCER PREVENTION

AND TREATMENT

not only for BRCA1!

Denosumab → immunomodulator



Post-menoupausal?

TNBC?

Control arm?

NCT03691311

D-BIOMARK

Enrollment : 60 patients

- 24 post /pre-menopausal
- 12 TNBC

ICO Institut Català d'Oncologia

IDIBELL Institut d'Investigació Biomèdica de Bellvitge

AMGEN

Acknowledgements

IDIBELL Institut d'Investigació Biomèdica de Bellvitge

cnio stop cancer

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Andrea Vethencourt

Maria Jimenez
Patricia G Santamaria
Gema Perez Chacón
Marina Ciscar
Alex Collado
Alexandra Barranco
Jaime Redondo

Clara Gómez
Jorge G Miragaya
Guillermo Yoldi
Héctor P Montoyo
Adrian Sanz
Maria Zafeiroglou
Kim Pedersen
Sandra Benitez
Enrique Hernández
Pasquale Pellegrini
Alex Cordero
Marta Palafox
Manuel Gris
Ilaria DiBenedictis

Bastien Nguyen & Christos Sotiriou
Thierry Walzer; Lourdes Planelles
M Collado, I Palmero, M Serrano

Clinical team
Catalina Falo &
Teresa Soler &
Anna Petit (APA)
Sonia Pernas
Ander Urruticoechea

ICO Institut Català d'Oncologia

erc

AMGEN

aecc Catalunya Contra el Càncer

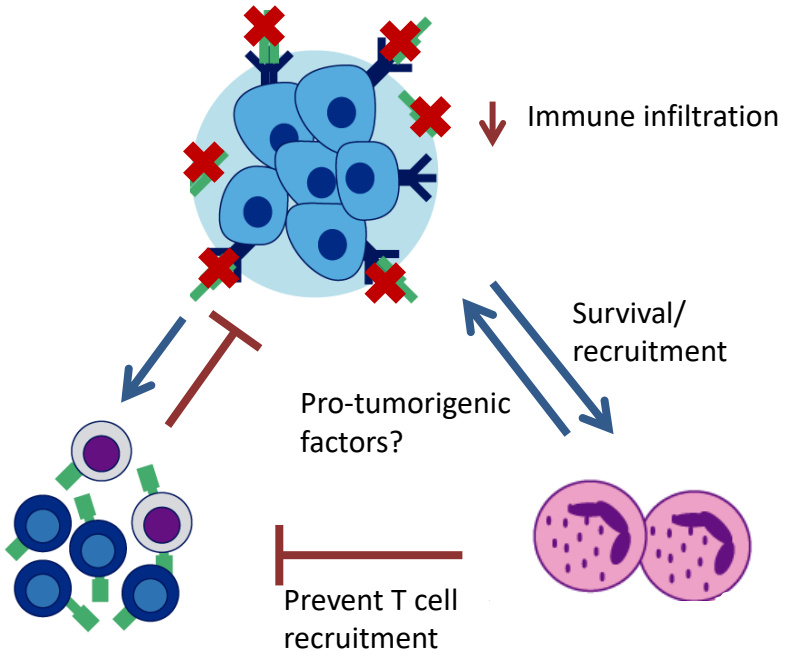
Fundació La Marató de TV3

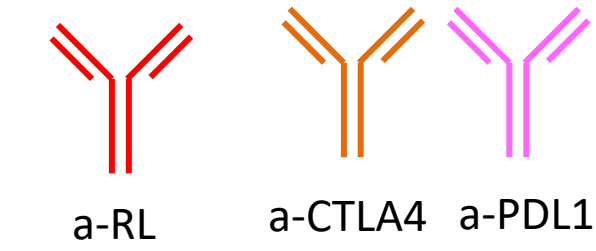
AGAUR

Instituto de Salud Carlos III

Conclusions

RANK PATHWAY INHIBITORS FOR IMMUNOTHERAPY IN BREAST CANCER



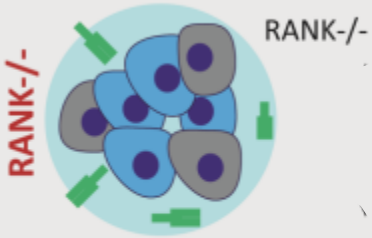


Denosumab + immunotherapy

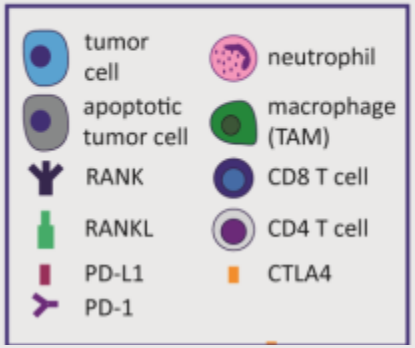
Summary

Conclusions

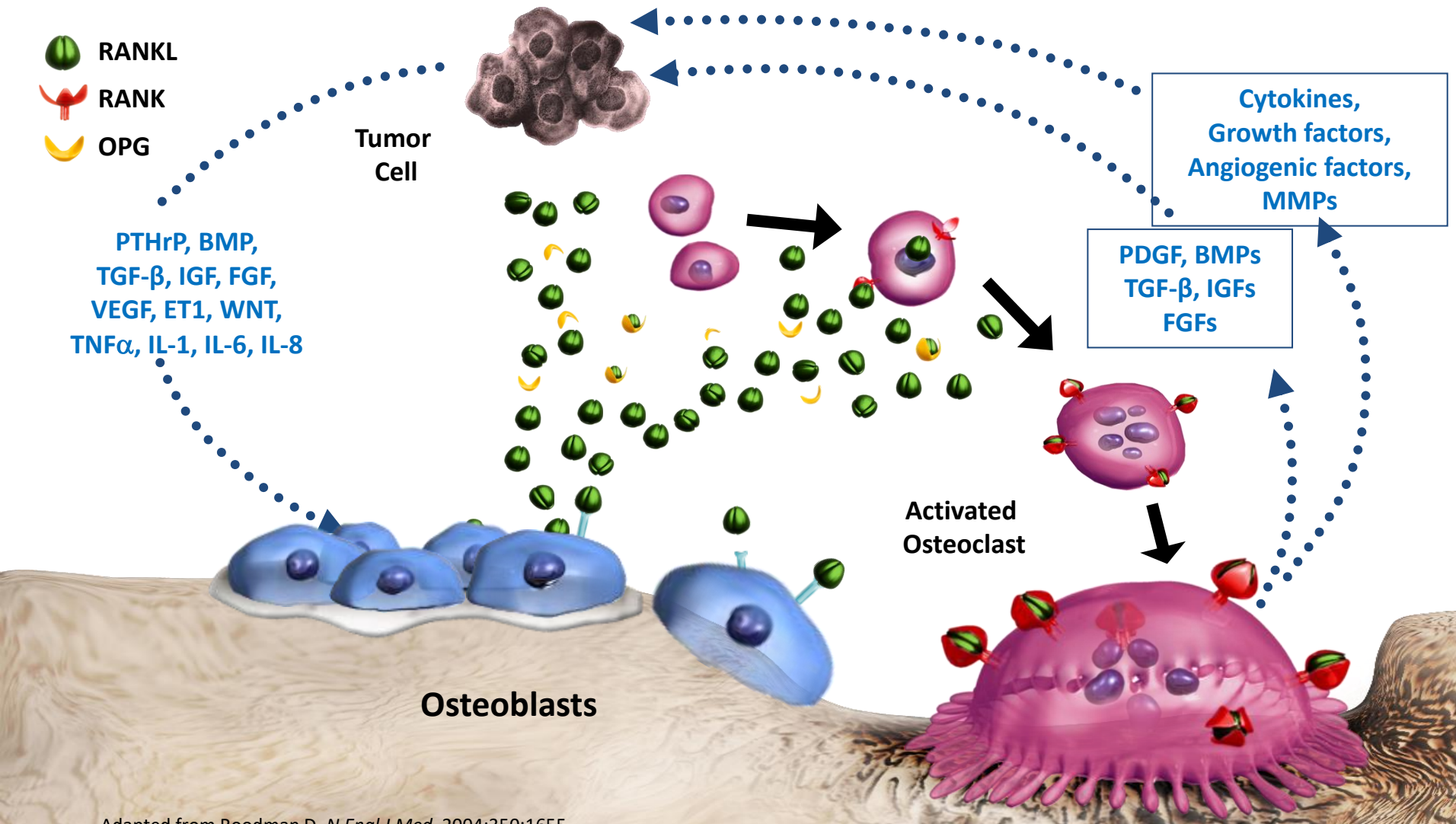
↓immune infiltration
"cold tumor"



↑immune infiltration
"hot tumor"

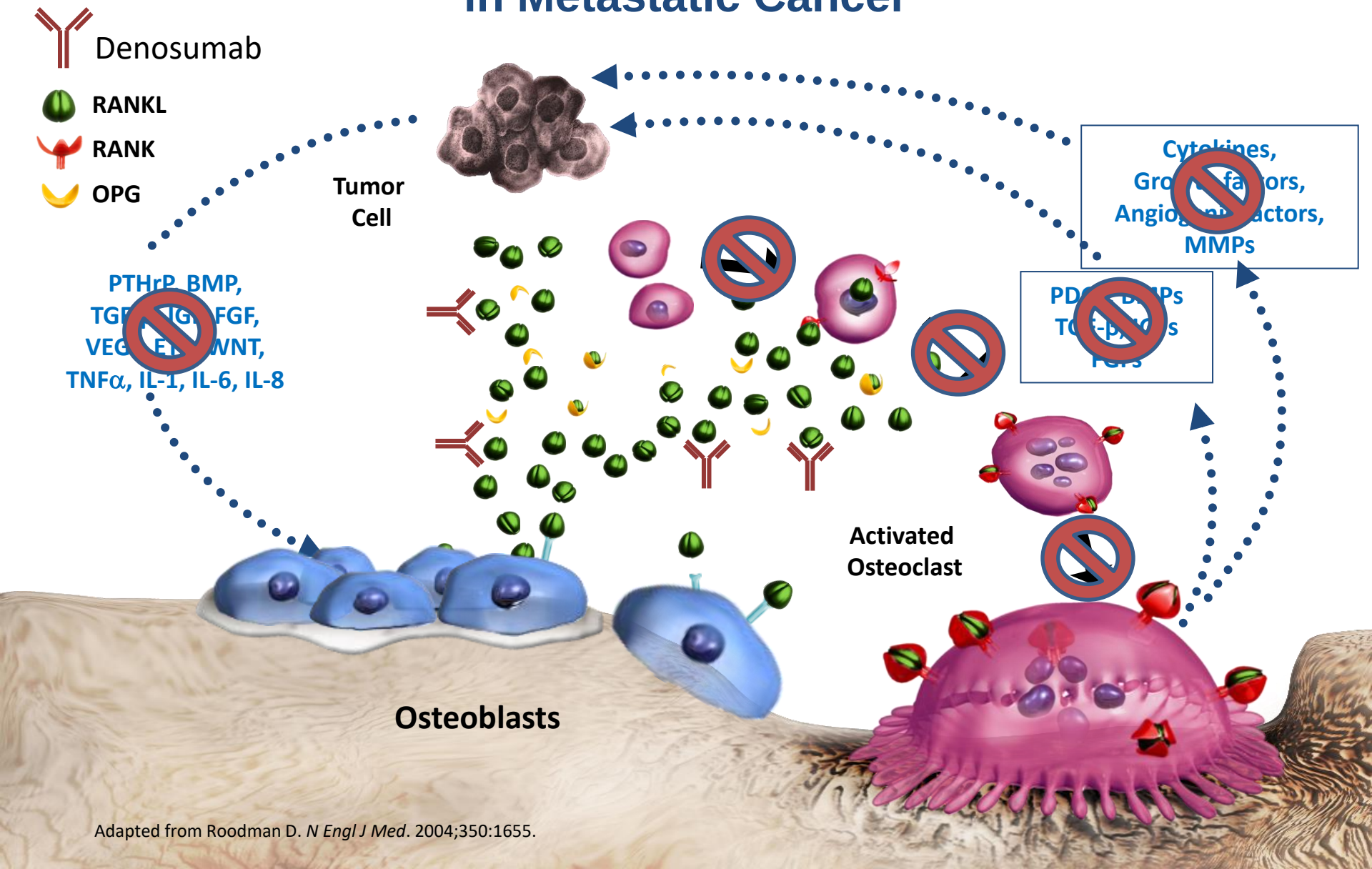


The "Vicious Cycle" Hypothesis of Bone Destruction in Metastatic Cancer



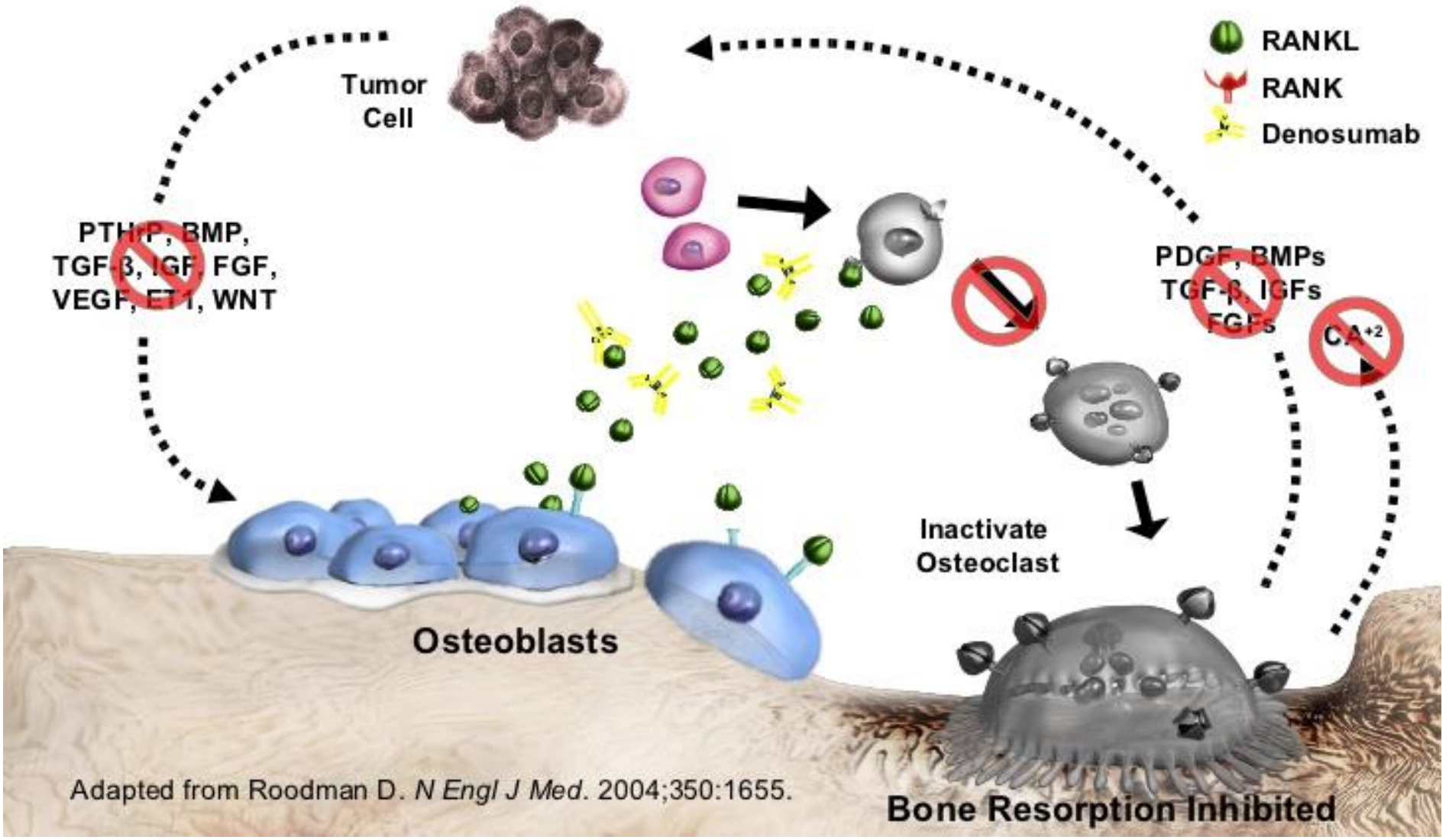
Adapted from Roodman D. *N Engl J Med*. 2004;350:1655.

The "Vicious Cycle" Hypothesis of Bone Destruction in Metastatic Cancer



Adapted from Roodman D. *N Engl J Med*. 2004;350:1655.

Denosumab Binds RANK Ligand and Inhibits Osteoclast Formation, Function and Survival



Adapted from Roodman D. *N Engl J Med*. 2004;350:1655.