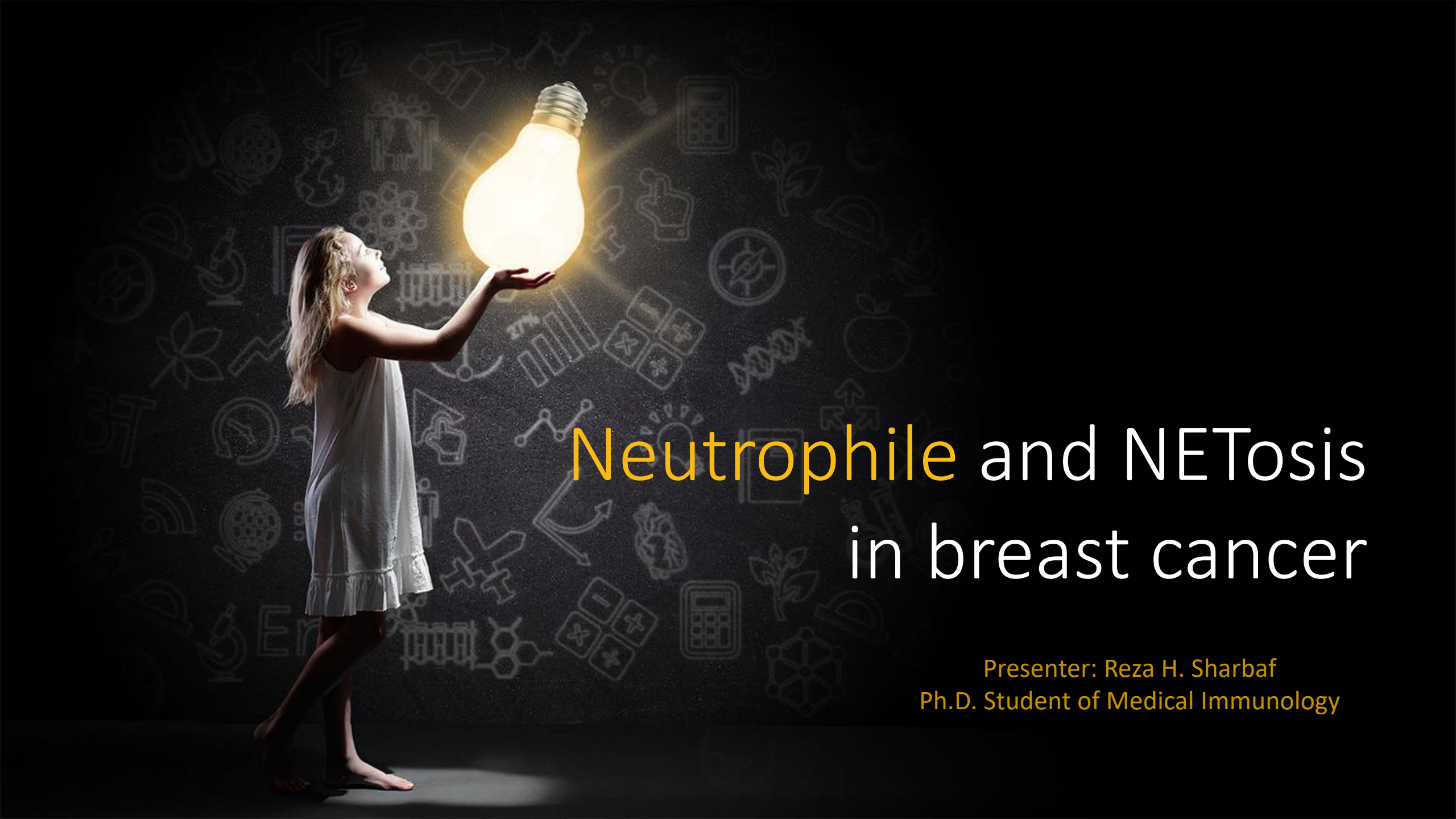


Neutrophile and NETosis in breast cancer

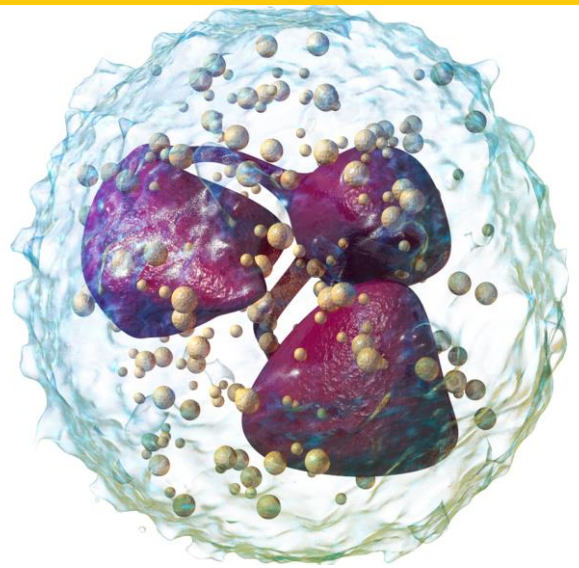
Presenter: Reza H. Sharbaf
Ph.D. Student of Medical Immunology

Agenda Infographic

Neutrophile in cancer

There is an urgent need to understand more about the impact of neutrophils on cancer progression and how they switch between anti-tumor and pro-tumor phenotypes.

Origin and distribution in vivo of neutrophils



Neutrophils, originating from the bone marrow granulocyte monocyte progenitor (GMP)

They constitute the firs significant line of defense against **bacterial** and **fungal** infections.

In healthy people, neutrophils are constantly renewed, but most are stored in the **bone marrow**, with only a tiny proportion **(1–2%)** entering the blood circulation.

They can actively adapt to tissues, acquiring organ-specific phenotypes and functions from their respective tissue microenvironments, acting as and influencing tissue homeostasis.

Subtype

Many studies have classified neutrophils according to **N1** and **N2** neutrophil density gradients, morphology, surface markers, cytokines, or chemokines.

Of neutrophiles

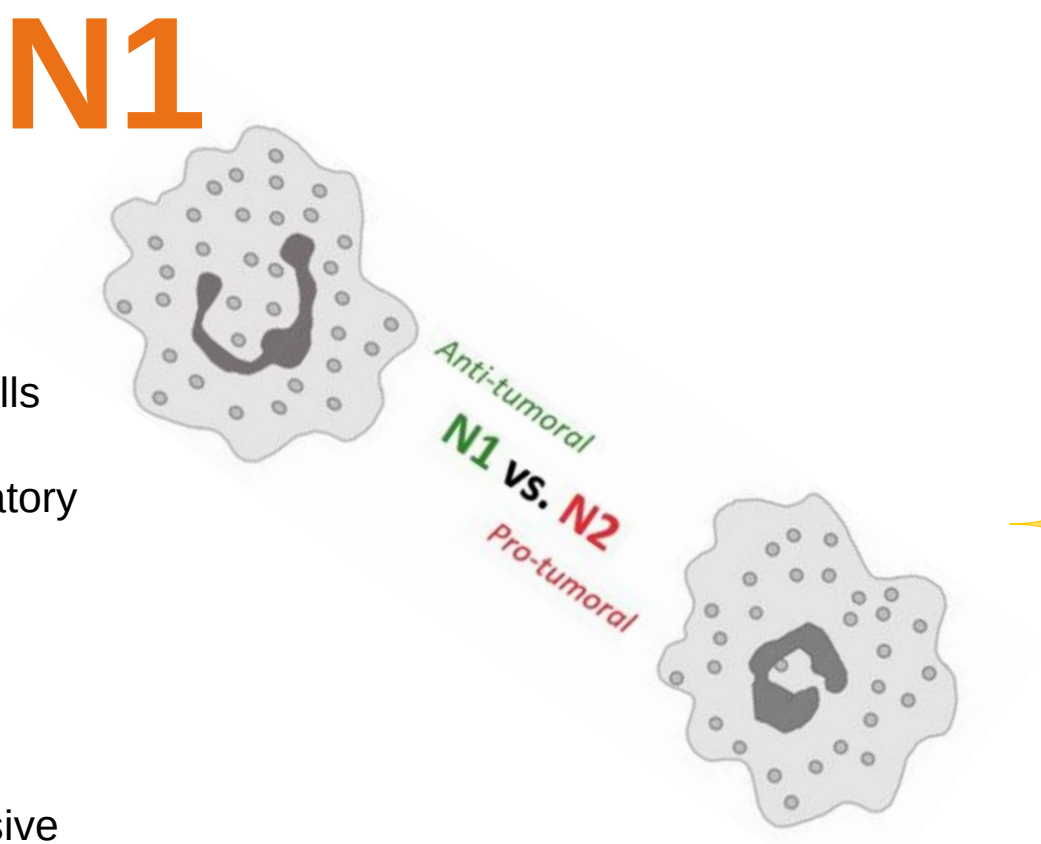
N1

- ✓ Oxidative burst
- ✓ Enhanced phagocytosis and migration ability
- ✓ No inhibition on T cells
- ✓ Potent cytotoxicity on tumor cells
- ✓ Releasing more pro-inflammatory factor such as :

Tumor necrosis factor (TNF-a),
intercellular

Adhesion molecule-1 (ICAM-1),

thus exerting a tumor-suppressive effect.



N2

- ✓ Inhibited T cell activity
- ✓ exhibit reduced cytotoxicity against tumor cells
- ✓ exhibiting higher levels of:

CD184(CXCR4),

arginase-1,

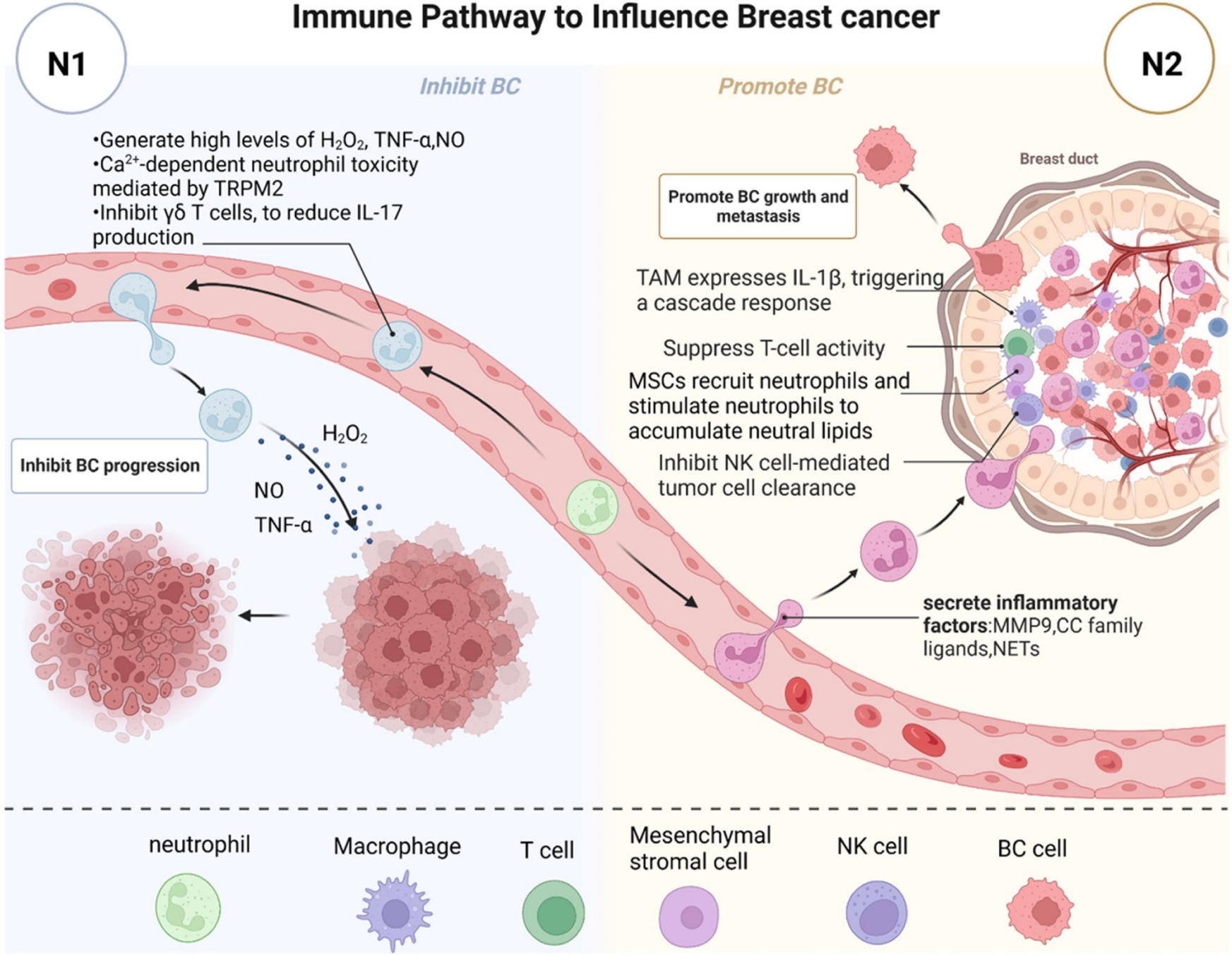
CCL2,

vascular endothelial growth factor (VEGF),

interleukin 8 (IL-8),

matrix metalloproteinase 9 (MMP-9),

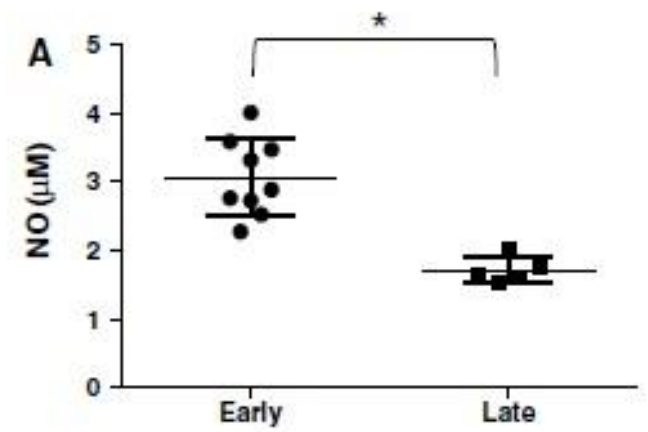
which can promote tumor growth, invasion, and metastasis

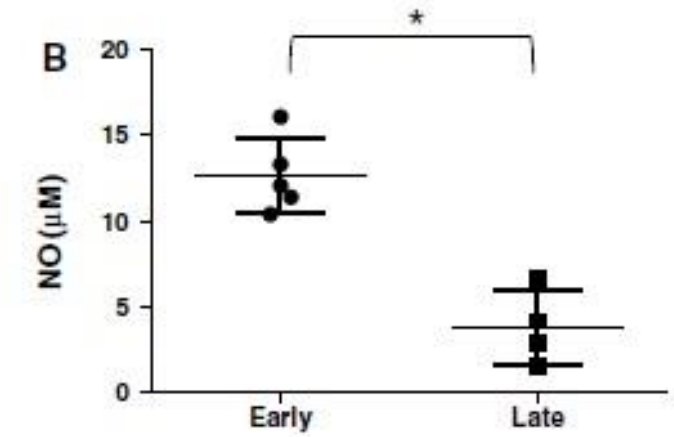


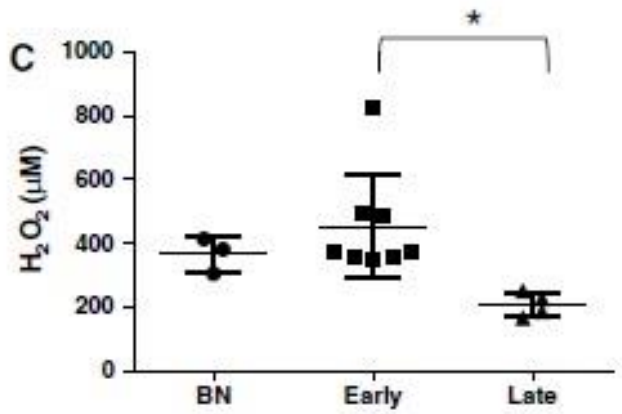
https://doi.org/10.1016/j.phrs.2023.106996

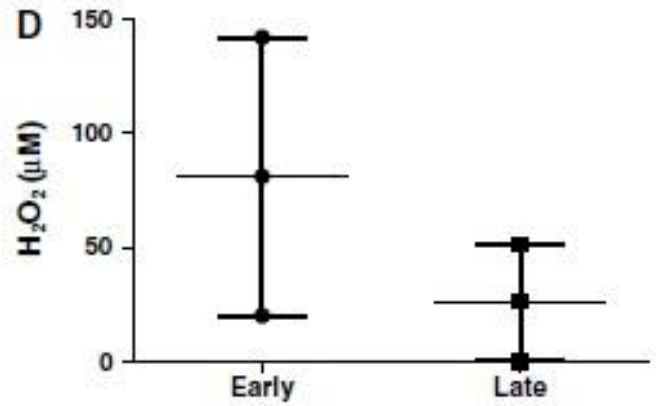
Tumor-associated neutrophils (TAN) develop pro-tumorigenic properties during tumor progression

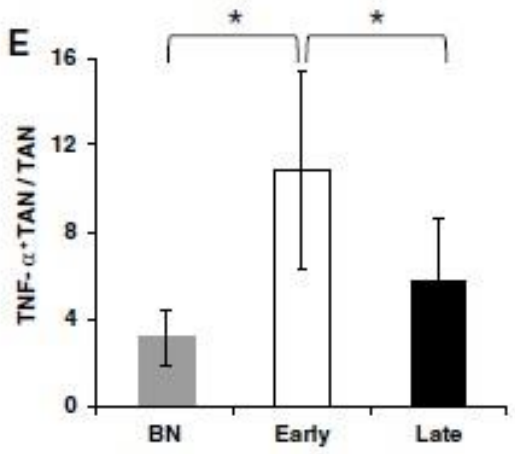
Original Article | Published: 04 October 2013 | 62, 1745–1756 (2013)

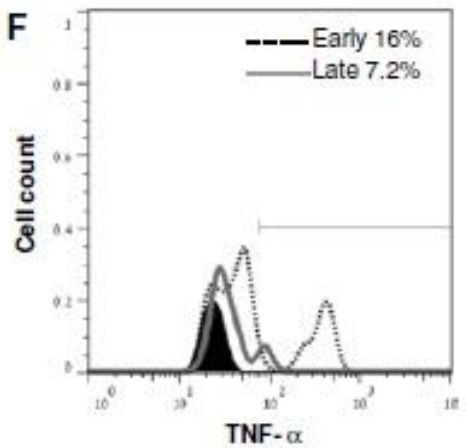




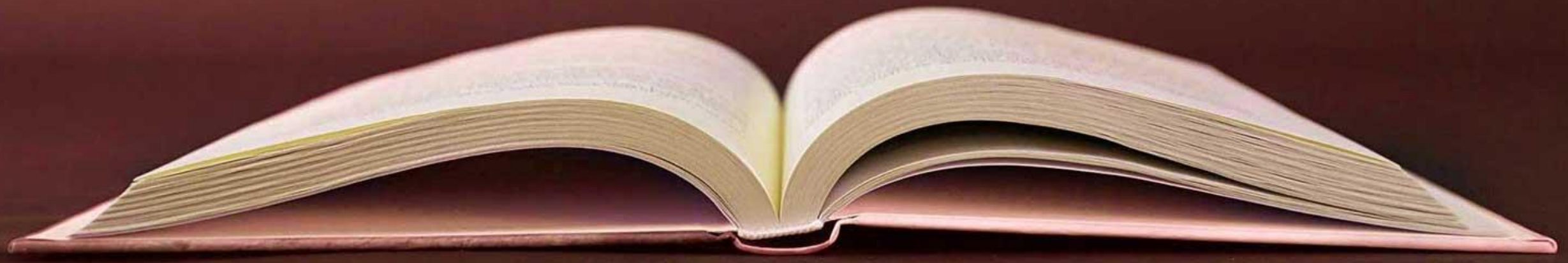








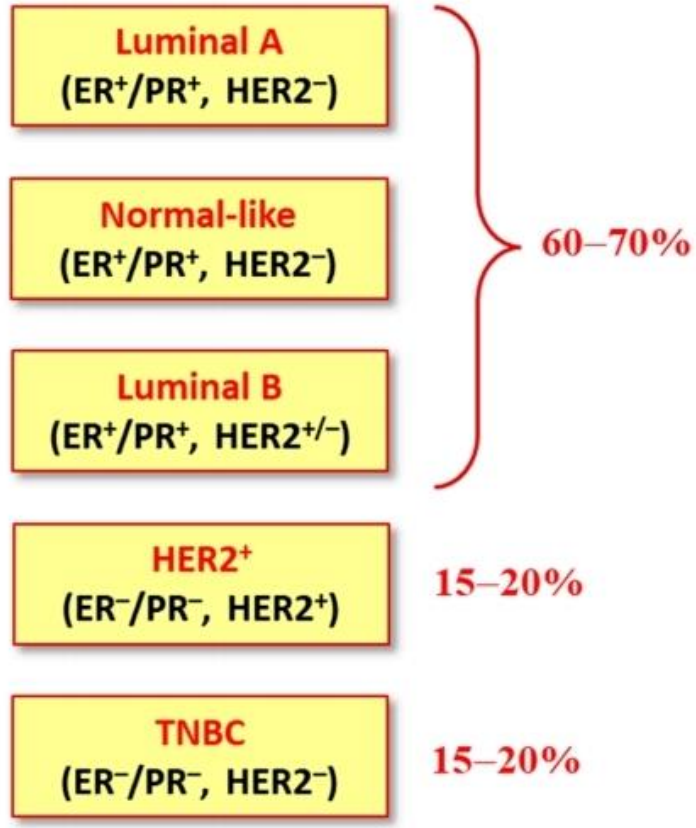
Recent investigations have progressively clarified the correlation between neutrophil profiles and BC outcomes



The close relationship between neutrophils and BC

Classification of BCs

- It is generally categorized into five major subtypes based on the presence or absence of receptors expressed by tumor cells.



The luminal A, B, and HER2+ subtypes are positive for hormone receptors (HRs), i.e., estrogen receptor (ER) and/or progesterone receptor (PR).

TNBC was so named ("triple negative") because it lacks the expression of the three molecular markers (ER, PR, and HER2), and is the most aggressive subtype, poorly prognosed, often observed in young women, representing the **15-20%** of all BCs.

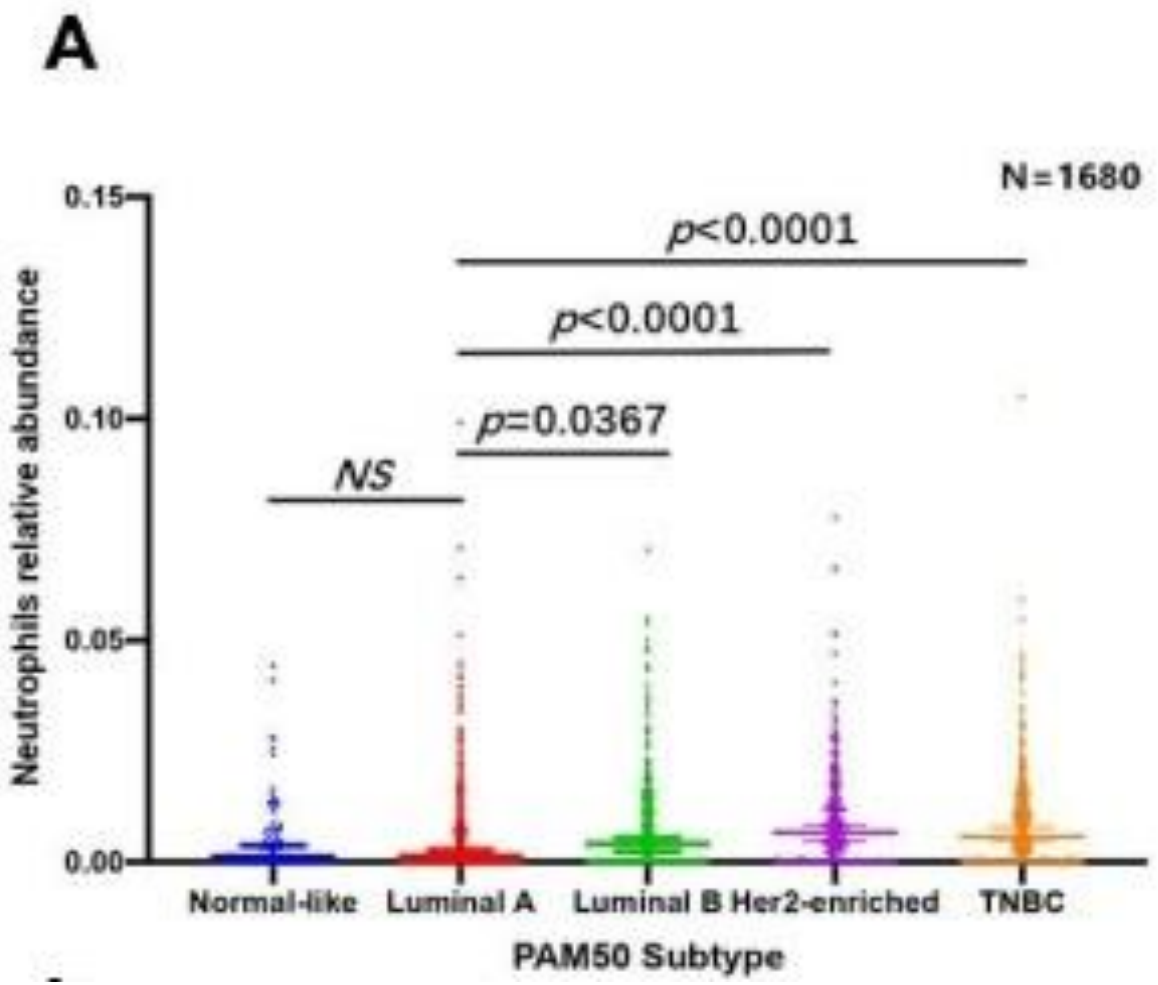
Clinical evidence of neutrophils in BC

- ✓ In patients with BC, increased **absolute neutrophil count** (ANC) and **neutrophil lymphocyte ratio** (NLR) in peripheral blood are commonly accepted symbols of advanced disease, poor prognosis, and poor response to treatment .

- ✓ In a large cohort of BC patients **(2374 patients),** the NLR were found to be significant predictors of poor overall survival (OS) in BC patients.

- ✓ High neutrophil infiltration is associated with aggressiveness and **treatment resistance** in BC .

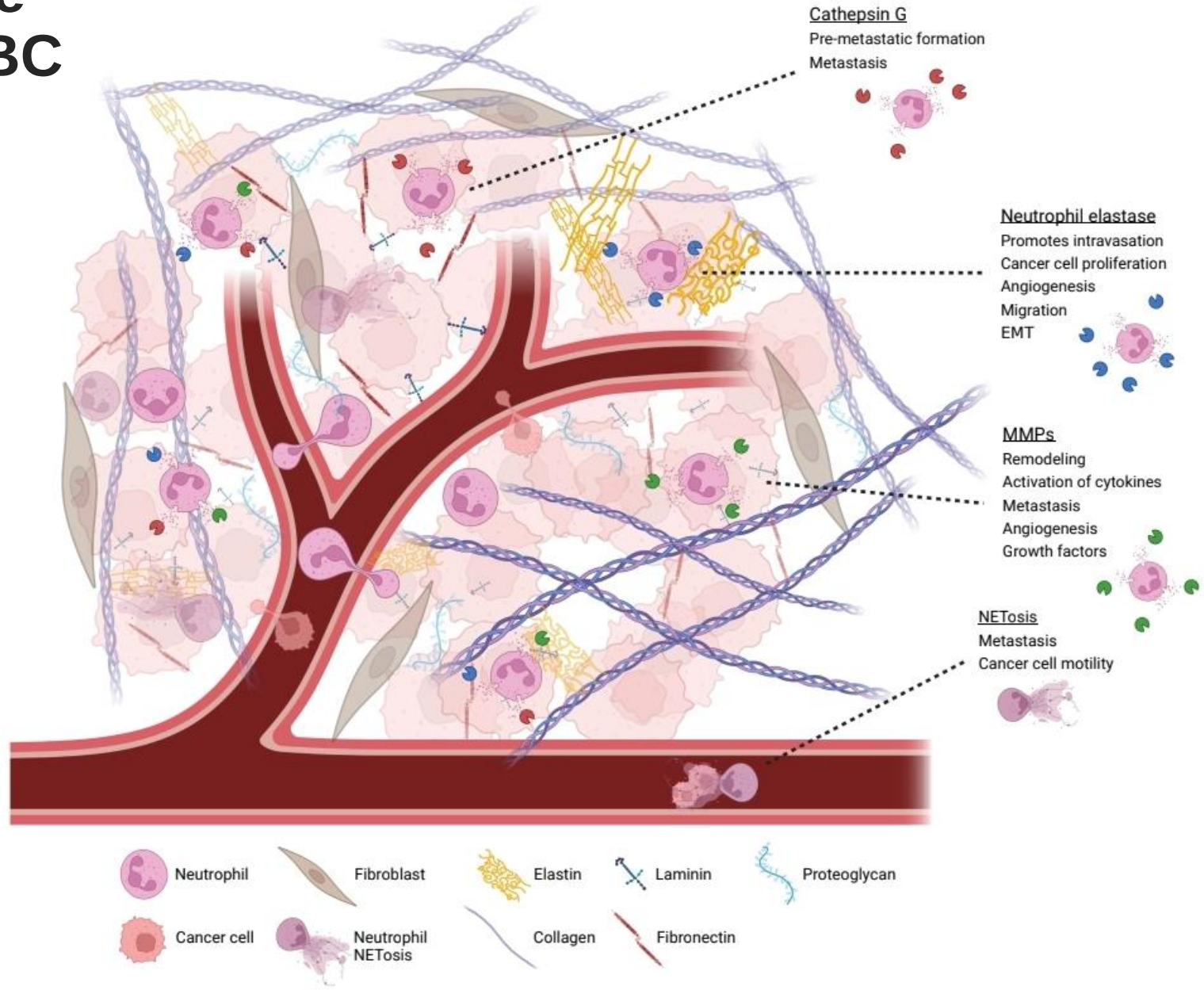
Pre-Clinical evidence of neutrophils in BC



DOI: 10.3389/fimmu.2020.01779

Neutrophils accelerate the growth and metastasis of BC

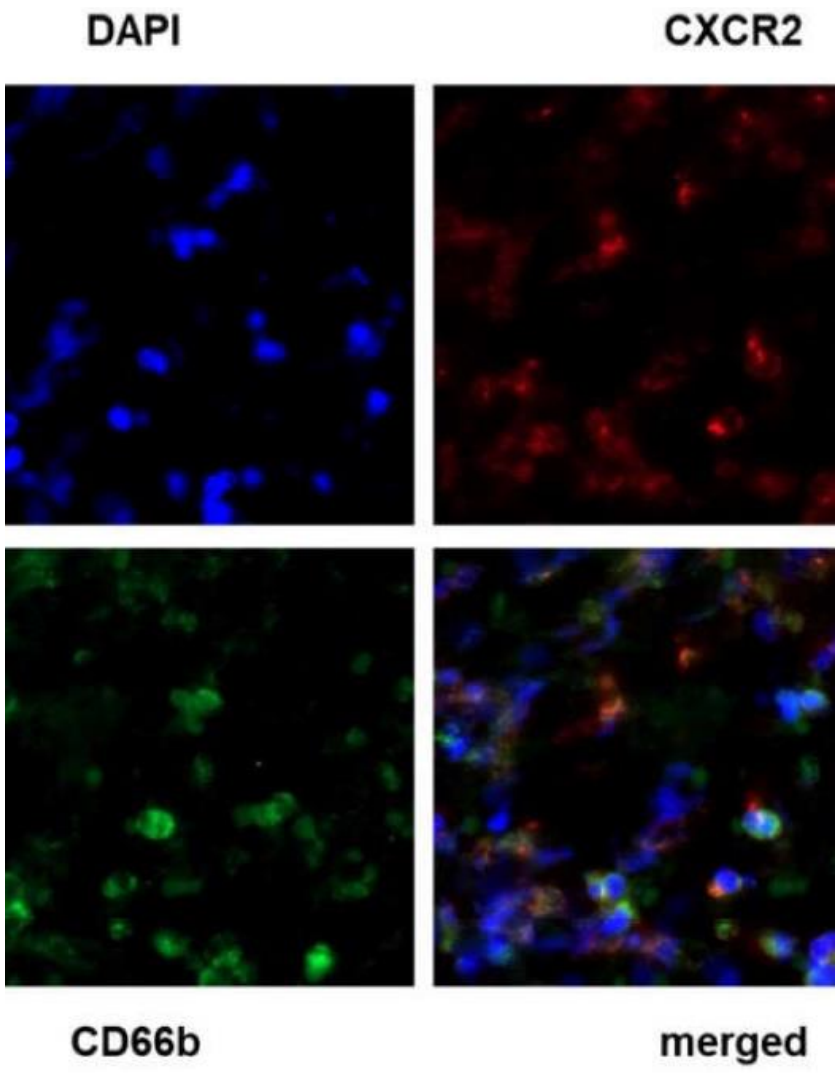
Neutrophils promote tumor growth and metastasis in the TME through the secretion of proteases such as matrix metalloproteinase **(MMP) 9, MMP2**, the formation of inflammatory factors including **IL-1ß, CC family ligands**, **NETs,** and interaction with other cells in the TME.



Cancers (Basel). 2020 Aug; 12(8): 2076.
Published online 2020 Jul 27. doi: 10.3390/cancers12082076

Prognostic Value of CXCR2 in Breast Cancer

Florence Boissière-Michot,[1] William Jacot,[1,2] Julien Fraisse,[1]
Gwendal Lazennec[3,4,*]



Chemokines

The release of neutrophils from the bone marrow is mainly dependent on the interaction between C-X-C motif chemokine receptor 4 (**CXCR4) and CXCR2** and their ligands.

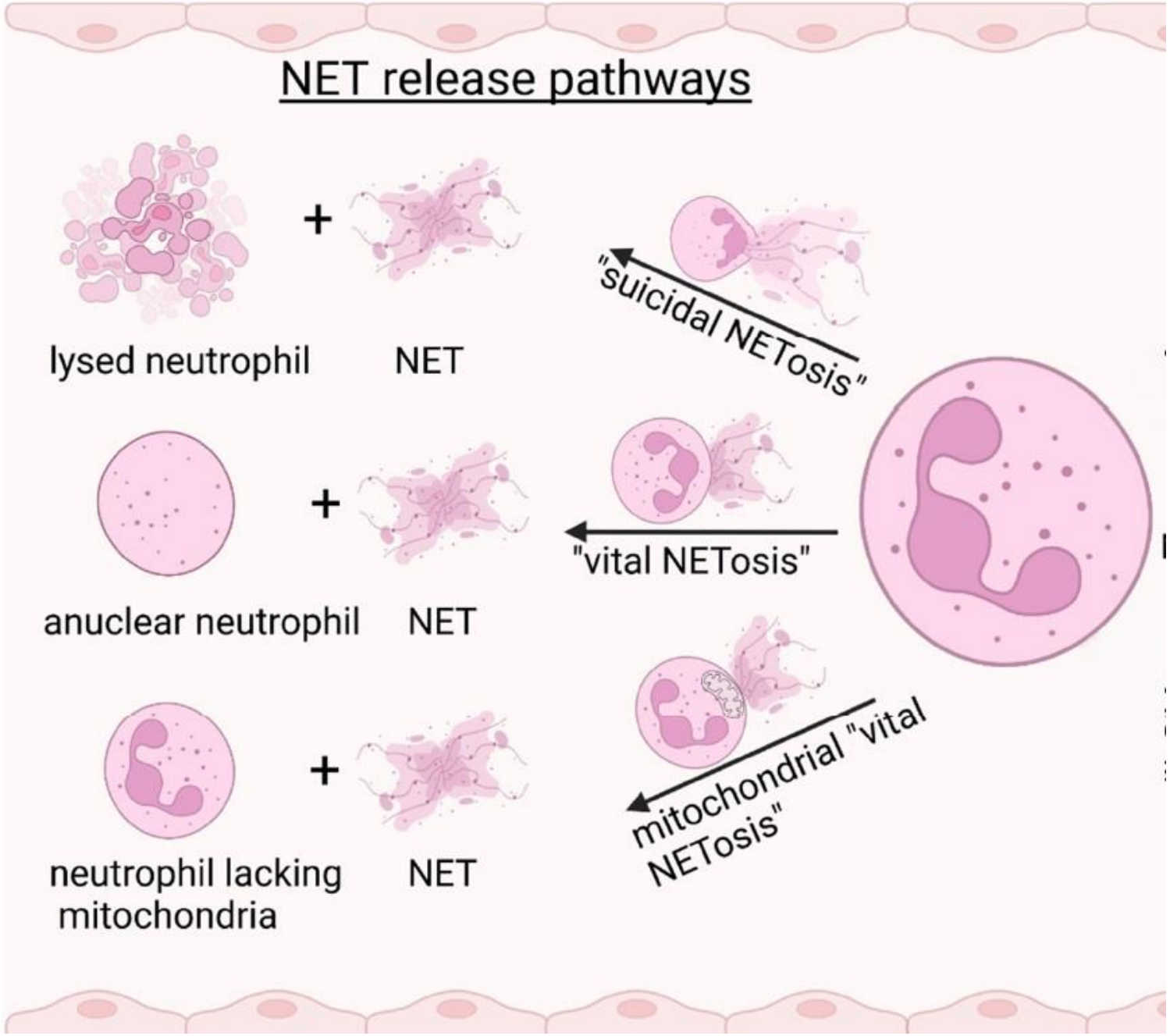
CXCR4 acts as a **homing agent** for neutrophils in the bone marrow , and CXCR2 are mainly responsible for the **release of neutrophils** into the circulation.

✓ CXCR2 receptors in neutrophils respond to the upregulation of CXCR2 ligands at tumor sites, resulting in increased recruitment of neutrophils to tumor-associated sites.

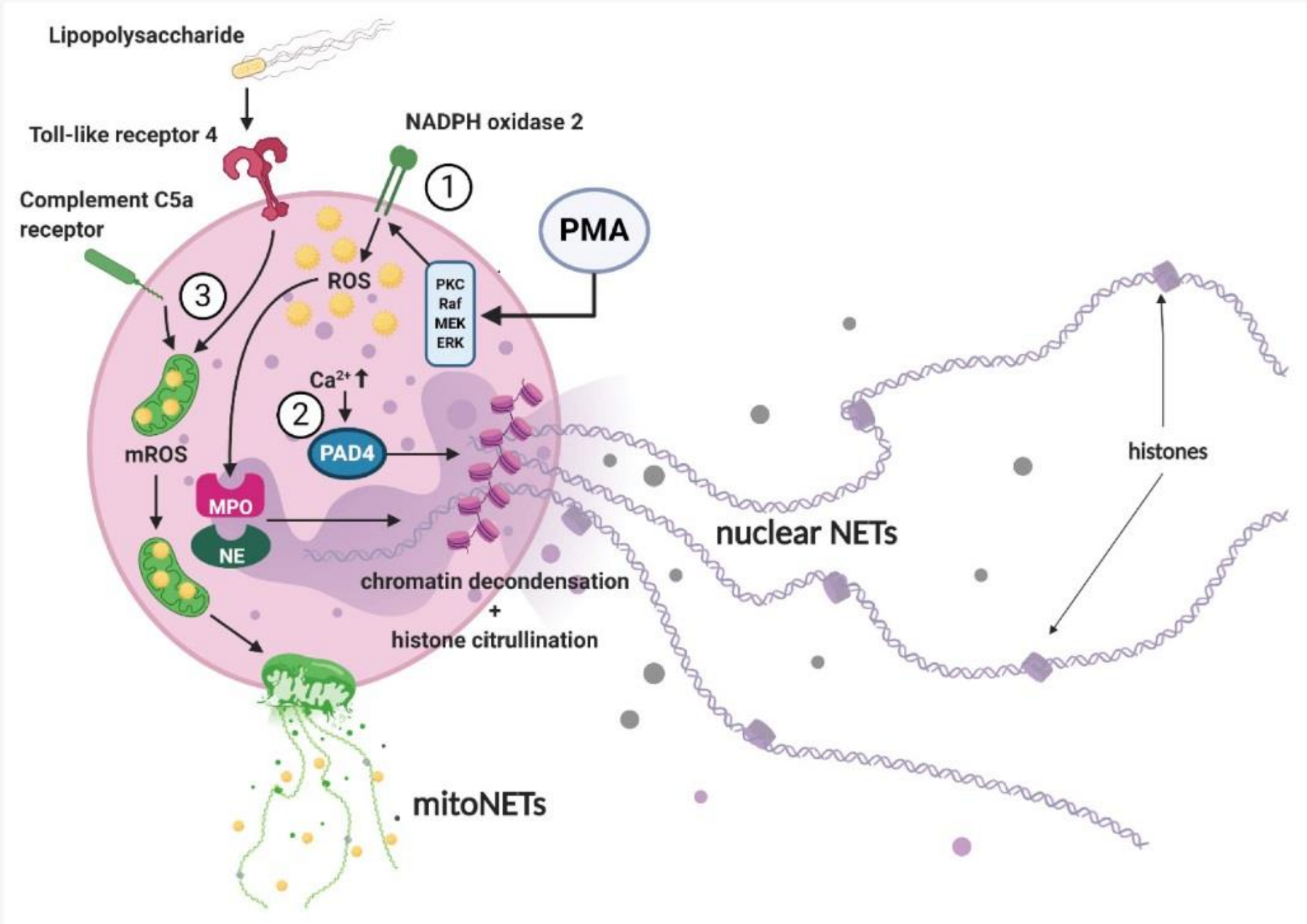
THE ROLE OF NETs in breast cancer

NETs have also been associated with tumor cell **proliferation** and **metastasis**, **cancer-related thrombosis** and primary tumor growth.

TYPE OF NETOSIS



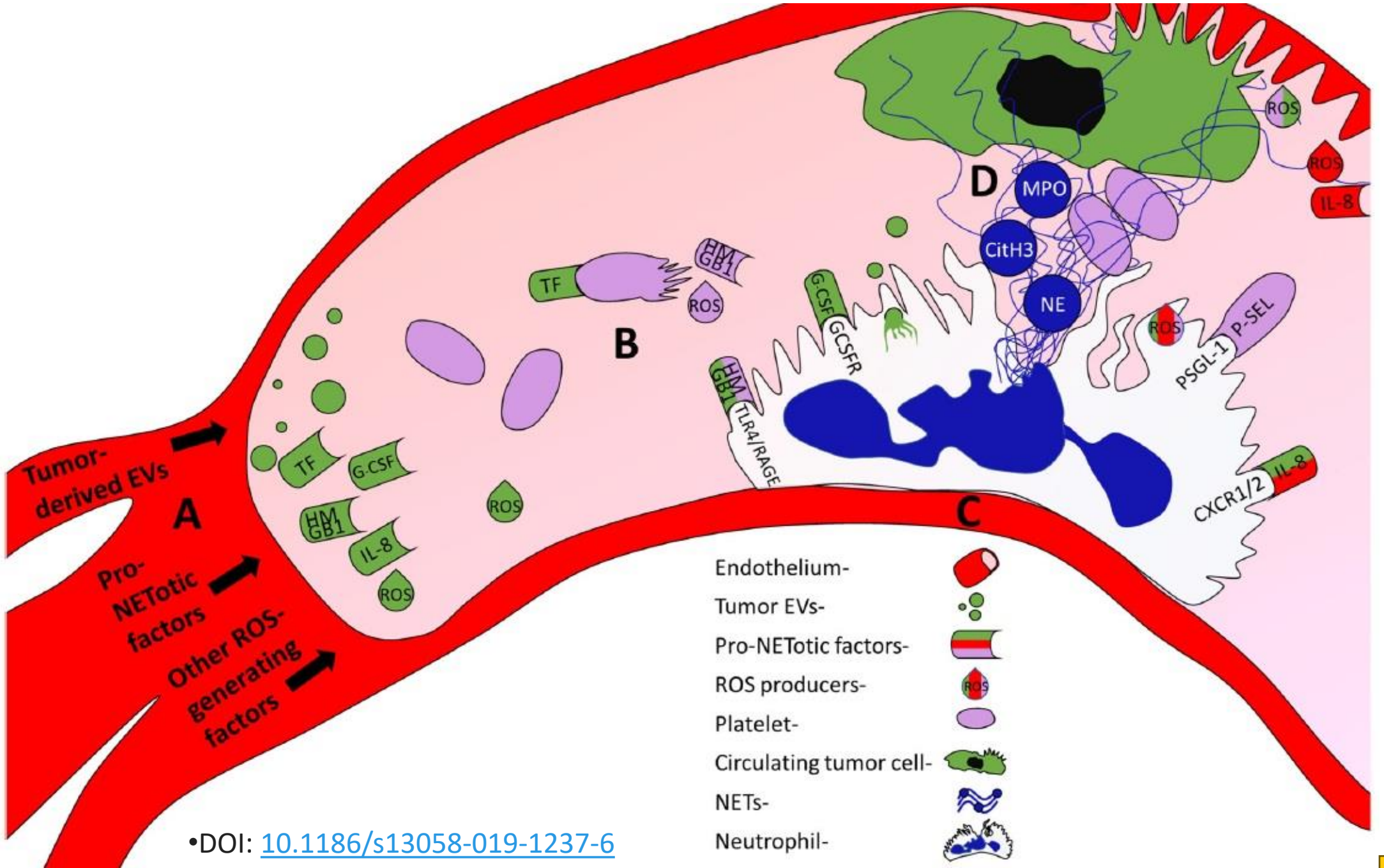
doi: 10.3390/diagnostics12071715



https://doi.org/10.3390/ijms22020559

Cellular and molecular stimulants of NETosis

Stimulus	Relevance to cancer progression	Origin
Platelet-activating factor [19][M]	Promotes tumor cell proliferation, neovascularization, and immunosuppressive phenotype	Leukocyte, platelet, and endothelial secretion in inflammation
HMGB1 [14, 25, 33][HM]	Associates with existing NETs; role in platelet and neutrophil activation; synergizes with LPS and thus may exacerbate response to infection	Leukocyte and platelet secretion in inflammation; expressed in some tumors; released during cell death
IL-8 [5, 34, 35][H]	Drives neutrophilia; positive correlation with poor outcome in women with breast cancer	Expressed in some tumors; released from activated endothelial cells
G-CSF [19, 36, 37][M]	Drives neutrophilia; positive correlation with metastasis; potentiates extracellular vesicle driven NETosis	Expressed in some tumors
PAD4 [38–40][HM]	Catalyzes histone citrullination; inhibition prevents NETosis in most circumstances	Neutrophils; expressed in some tumors
P-selectin [41][M]	Facilitates neutrophil motility; drives platelet-neutrophil aggregation	Endothelial cells; platelets
TF [42–44][H]	Activates platelets which activate neutrophils and causes NETosis, potentially through multiple pathways	Secreted during NETosis; expressed in some tumors; contained in tumor EVs

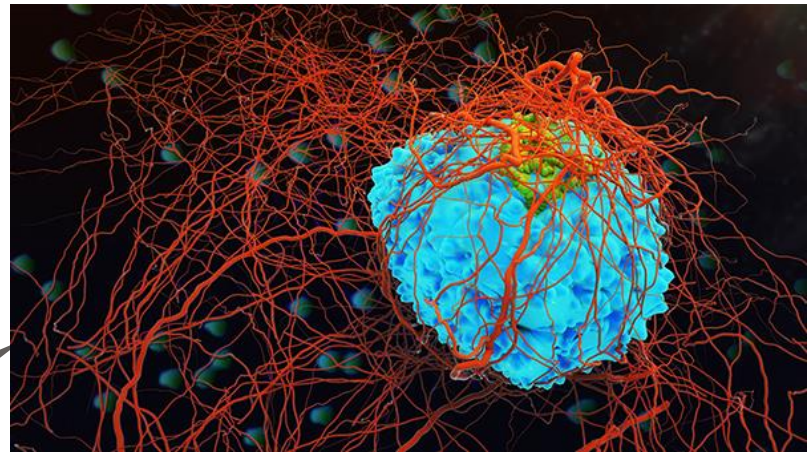


•DOI: [10.1186/s13058-019-1237-6](https://doi.org/10.1186/s13058-019-1237-6)

Breast Tumor cells factors

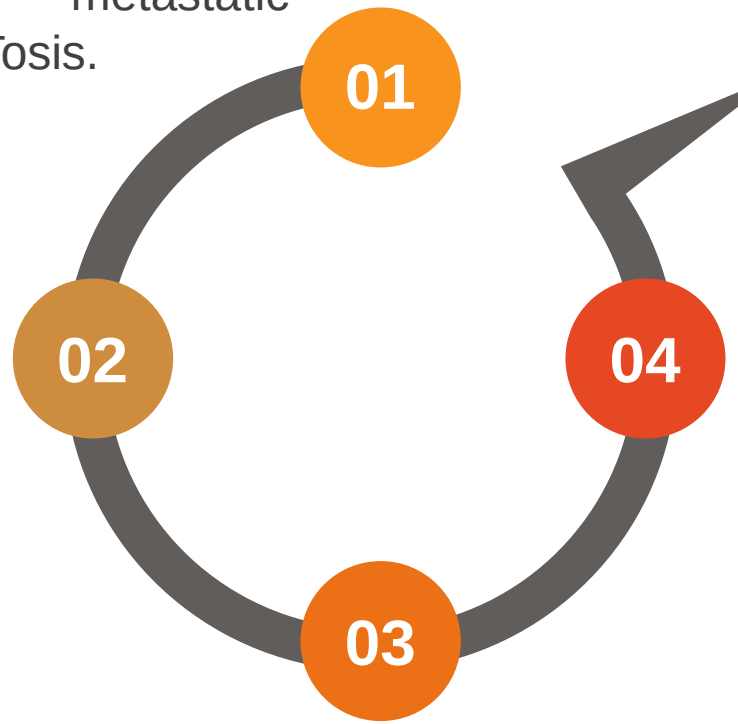
GCSF

Overexpression of G-CSF in breast cancer. can result in an overabundance of neutrophils in the blood, **ROS generation** in neutrophils, enhanced metastatic potential and subsequent NETosis.



IL-8

IL-8 plays an important role in recruiting neutrophils to sites of inflammation, IL-8 production has also been associated with increased **metastatic potential** .



Endothelial cells

Activated Ecs release inflammatory cytokines and growth factors and also express several adhesion molecules on their surface such as **P-selectin, E-selectin, and ICAM-1** to facilitate neutrophil rolling, adhesion, and transmigration to the inflamed sit.

Cathepsin C

Cathepsin C released from breast cancer cells can induce NETosis by activating **NE** on the neutrophil cell membrane.

Impact of NETosis on VTE and metastasis

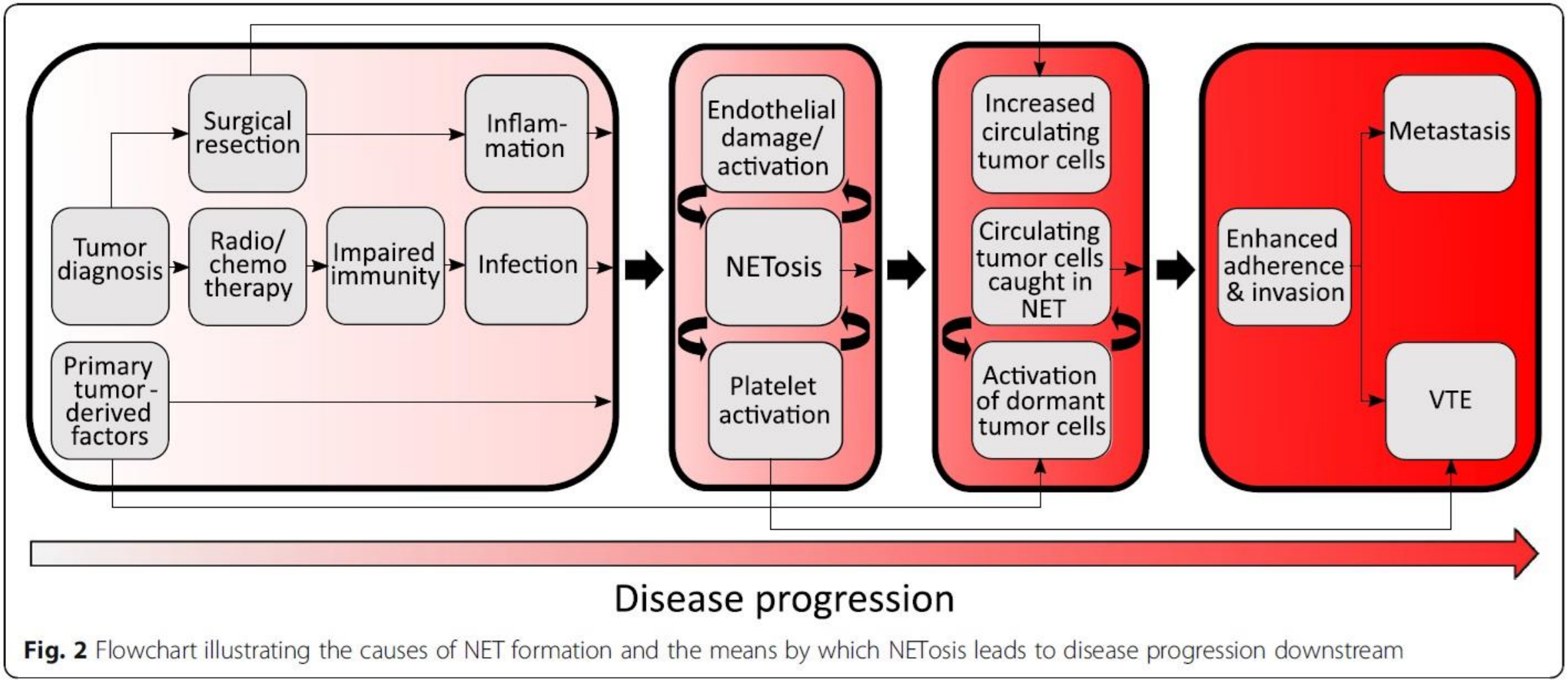
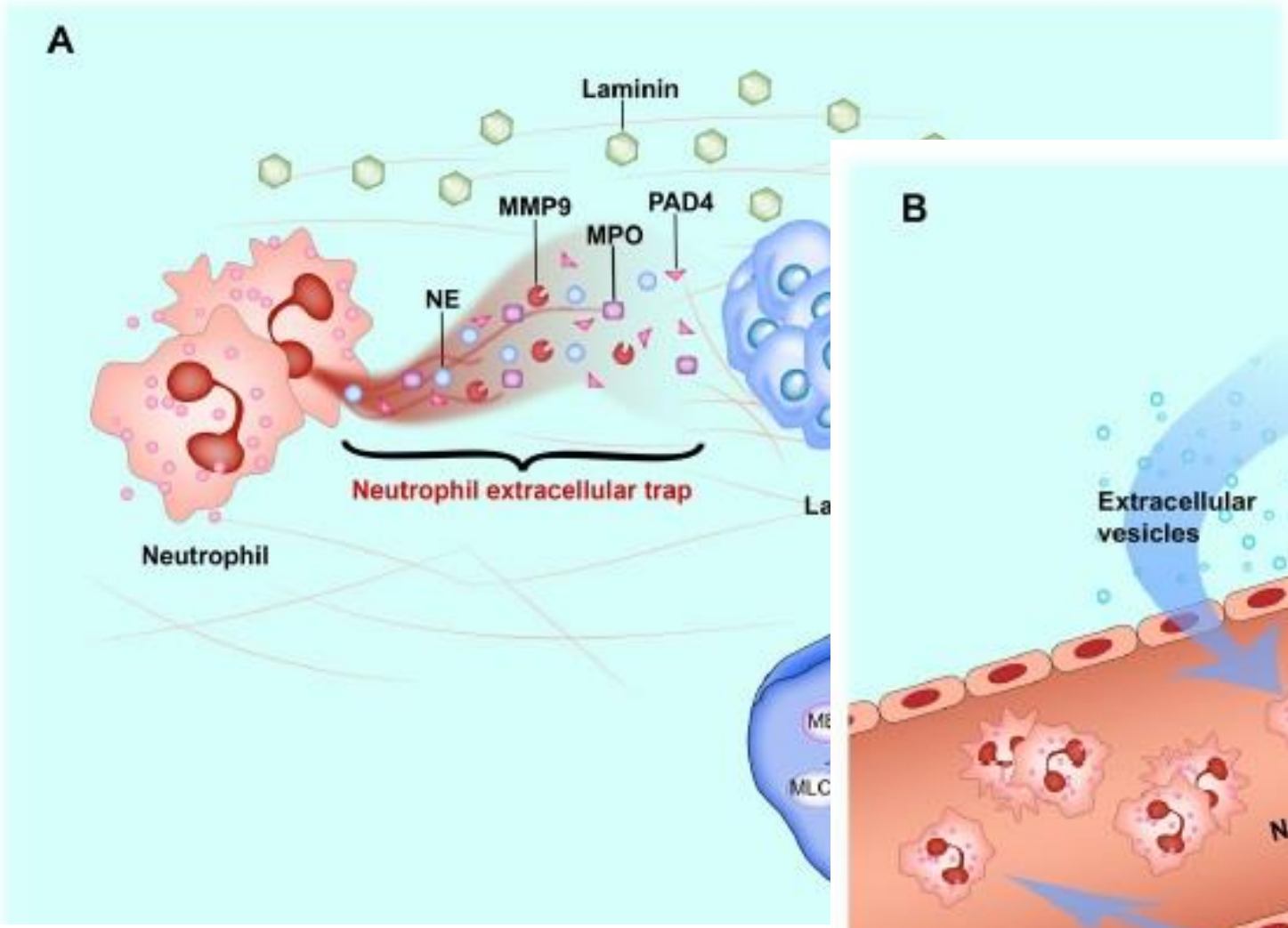
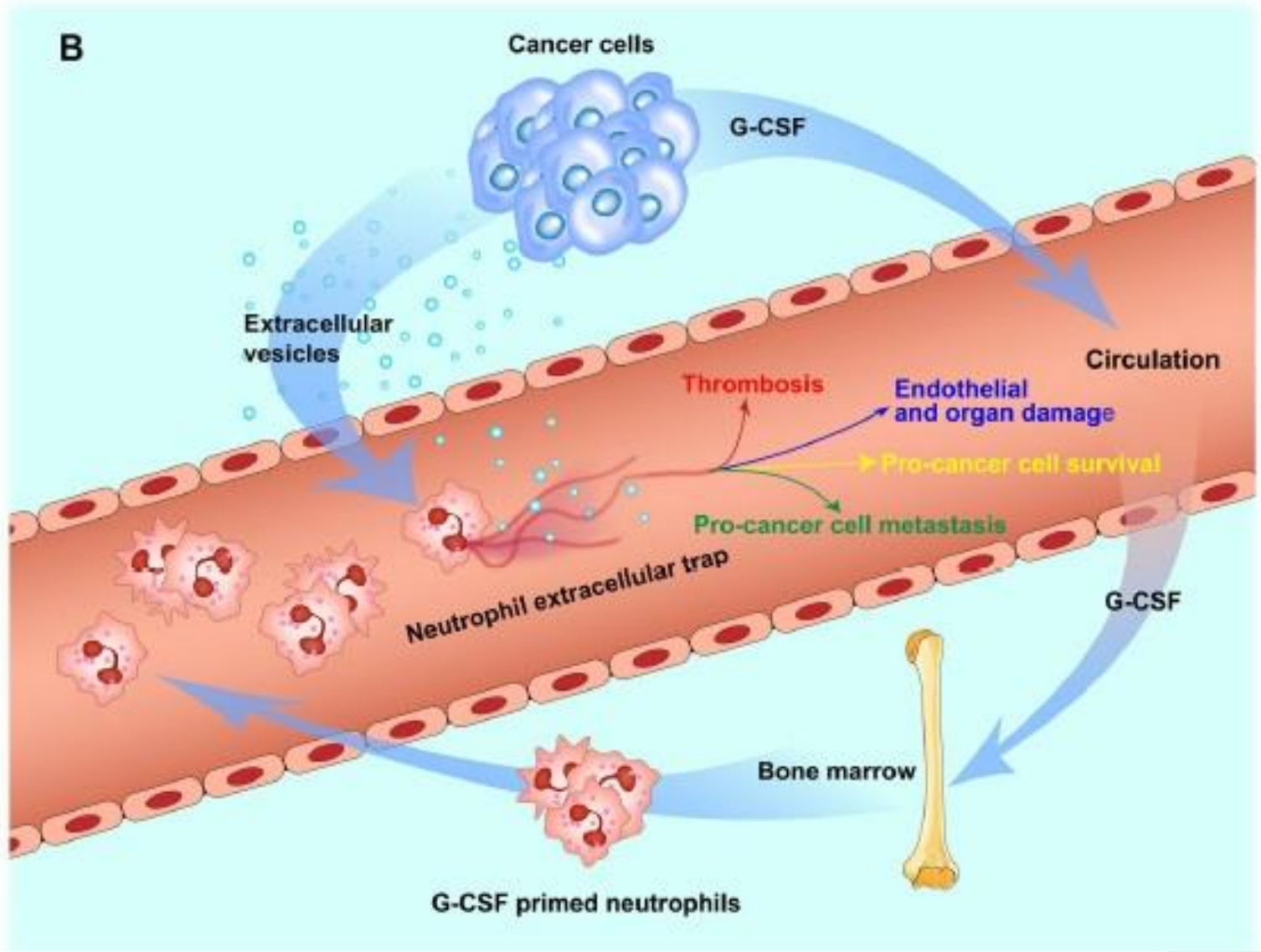


Fig. 2 Flowchart illustrating the causes of NET formation and the means by which NETosis leads to disease progression downstream

•DOI: 10.1186/s13058-019-1237-6

Roles of NETs in and awakening dormant cancer cells





Neutrophils in triple-negative breast cancer: an underestimated player with increasingly recognized importance

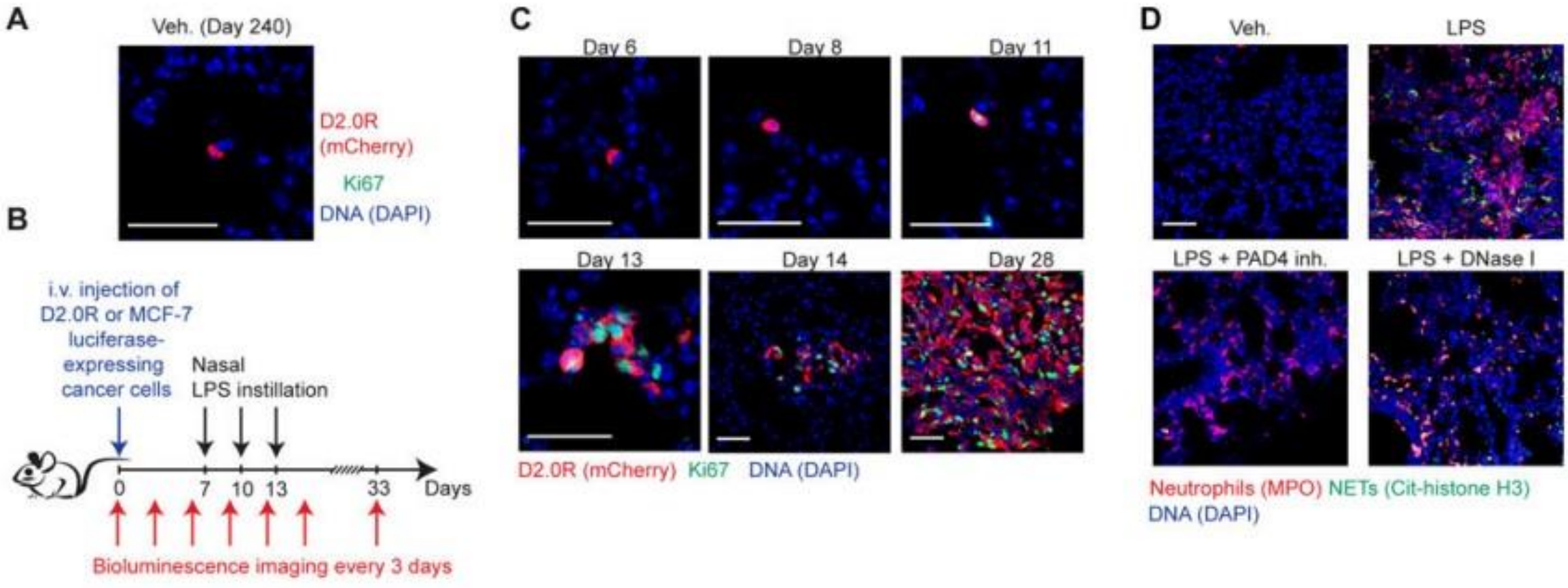
Chanjuan Zheng, Xi Xu, Muyao Wu, Lian Xue, Jianyu Zhu, Hongzhuo Xia, Siyu Ding, Shujun Fu, Xinyu Wang, Yian Wang, Guangchun He, Xia Liu ✉ & Xiyun Deng ✉

Breast Cancer Research **25**, Article number: 88 (2023) | Cite this article

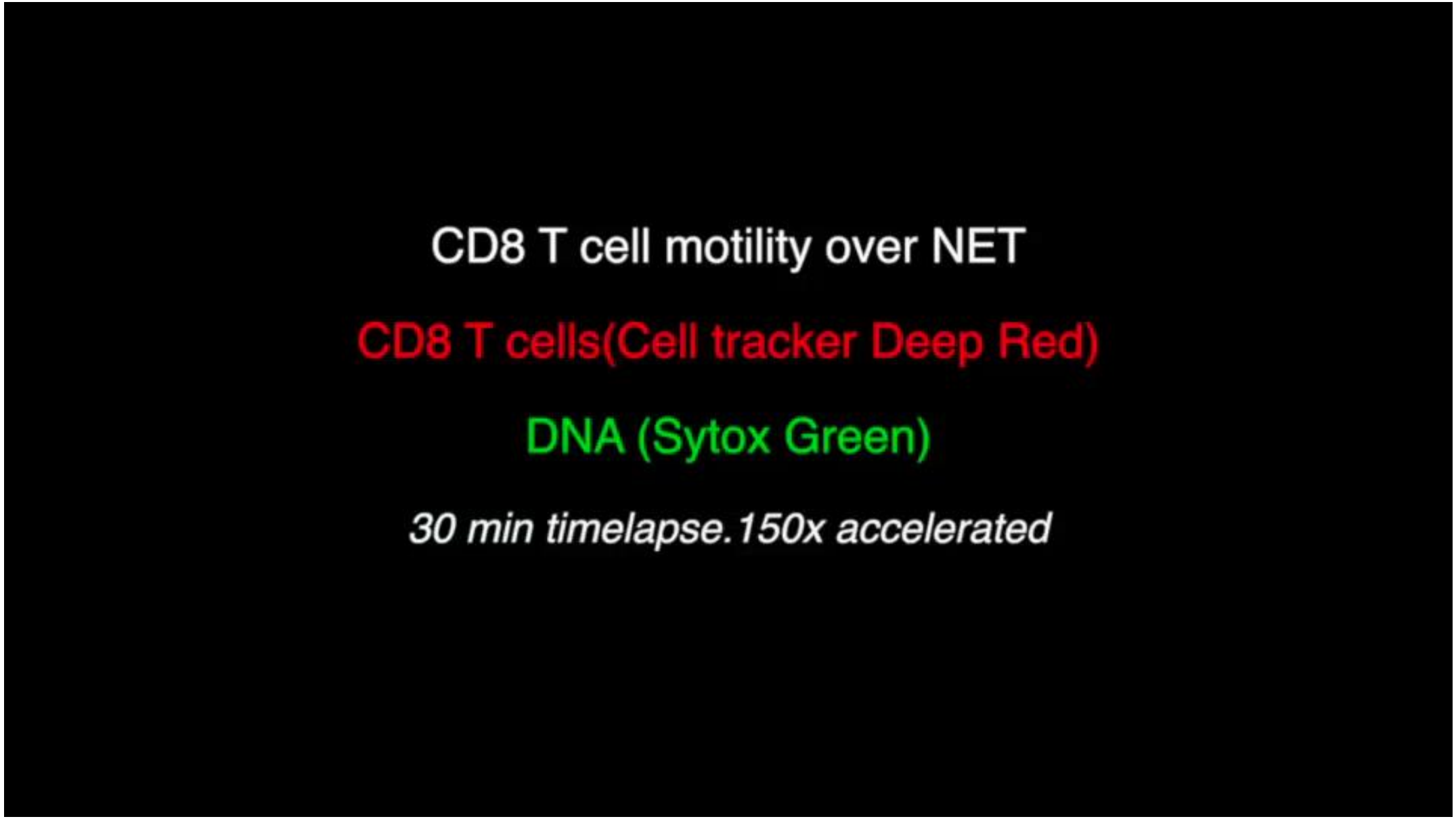
Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice

JEAN ALBRENGUES, MARIO A. SHIELDS, DAVID NG, CHUN GWON PARK, ALEXANDRA AMBRICO, MORGAN E. POINDEXTER, PRIYA UPADHYAY, DALE L. UYEMINAMI, ARNAUD POMMIER, [...], AND MIKALA EGEBLAD +13 authors Authors Info & Affiliations

SCIENCE · 28 Sep 2018 · Vol 361, Issue 6409 · DOI: 10.1126/science.aao4227



NETosis Limits **Cytotoxic Immunity** against 4T1 Tumor Cells in Subcutaneous Tumors and at Metastatic Niches



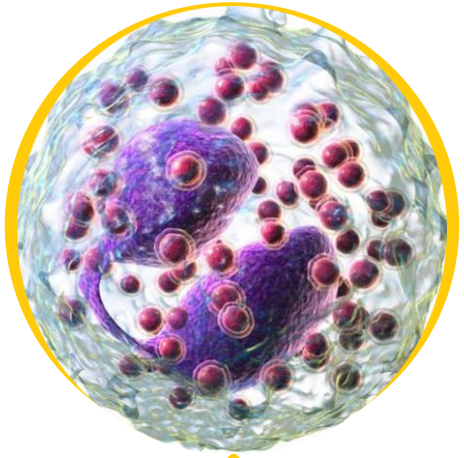
https://doi.org/10.1016/j.immuni.2020.03.001

The crosstalk between neutrophils and other cells in TME

Neutrophils can interact with other cells in the TME to participate in tumor **progression.**

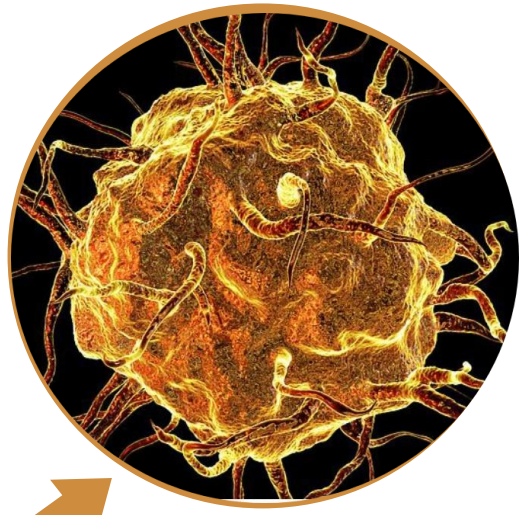
NK cells

In Spiegel's in vivo experimental systems study of mouse breast cancer 4T1 cells, CD11b+ / Ly6G+ neutrophils were found to **inhibit NK** cell-mediated clearance of tumor cells to increase the survival time of luminal cancer cells and play a tumor-promoting role.



Macrophages

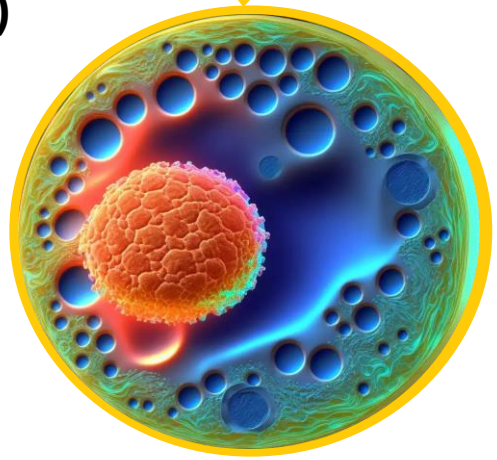
CCL2 production by BC cells promotes IL1ß expression in tumor-associated macrophages (TAM), which triggers a series of cascade responses involving γδT-cell IL17 expression, leading to G-CSF-induced pro-metastatic neutrophil expansion and enhanced pro-metastatic systemic inflammation.



Mesenchymal stromal cells (MSCs)

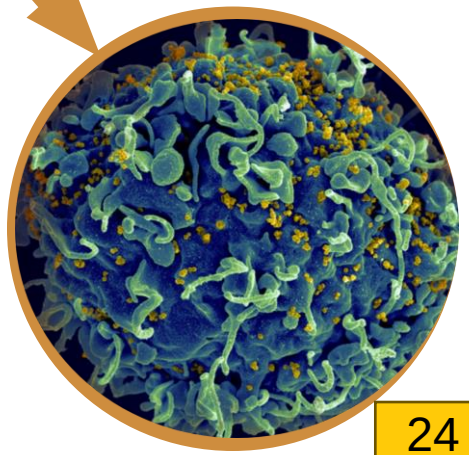
TNF-α-activated MSCs can recruit neutrophils and promote lung metastasis of BC.

Neutrophils, stimulated by lung-resident mesenchymal cells, could accumulate **neutrophilic lipids**, leading to lung metastasis of BC cells .



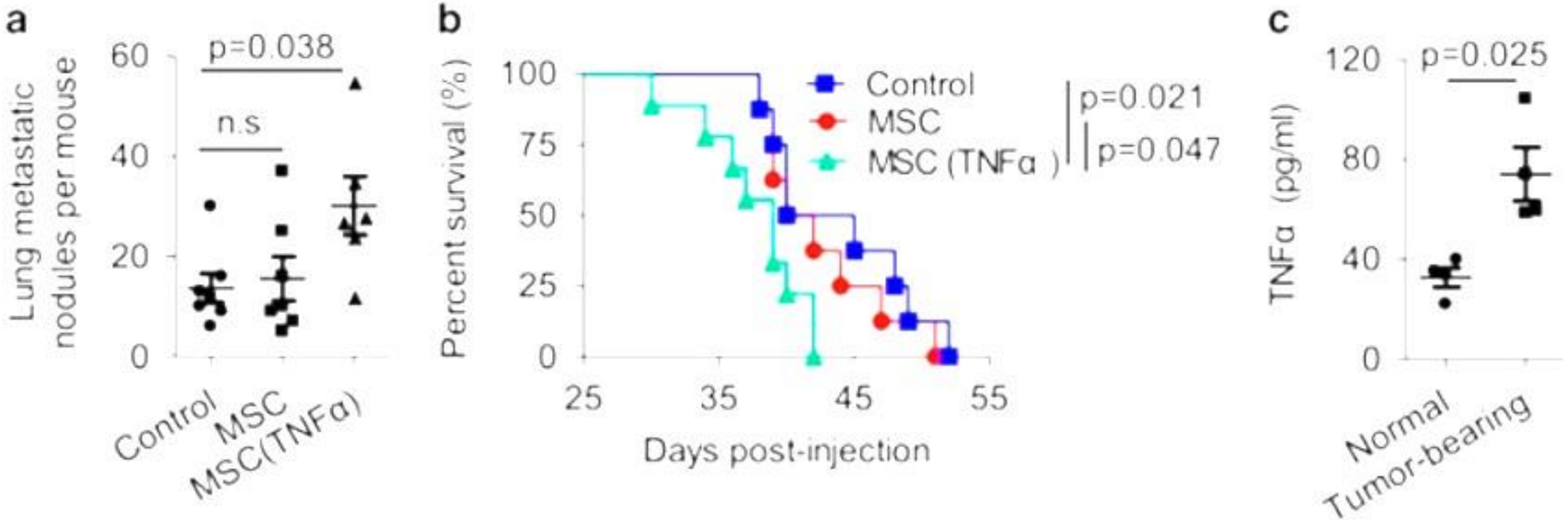
T cells

C-C motif chemokine ligand 20 **(CCL20)** secreted by BC cells activates neutrophils infiltrating into TME and induces the expression of **PD-L1** in neutrophils, thus promoting the involvement of T-cell immunosuppression in tumor progression in BC.



TNFα-activated mesenchymal stromal cells promote breast cancer metastasis by recruiting CXCR2$^+$ neutrophils

P F Yu,[1] Y Huang,[1] Y Y Han,[1] L Y Lin,[1] W H Sun,[1] A B Rabson,[2] Y Wang,[1,4,*] and Y F Shi[1,2,3,4,*]

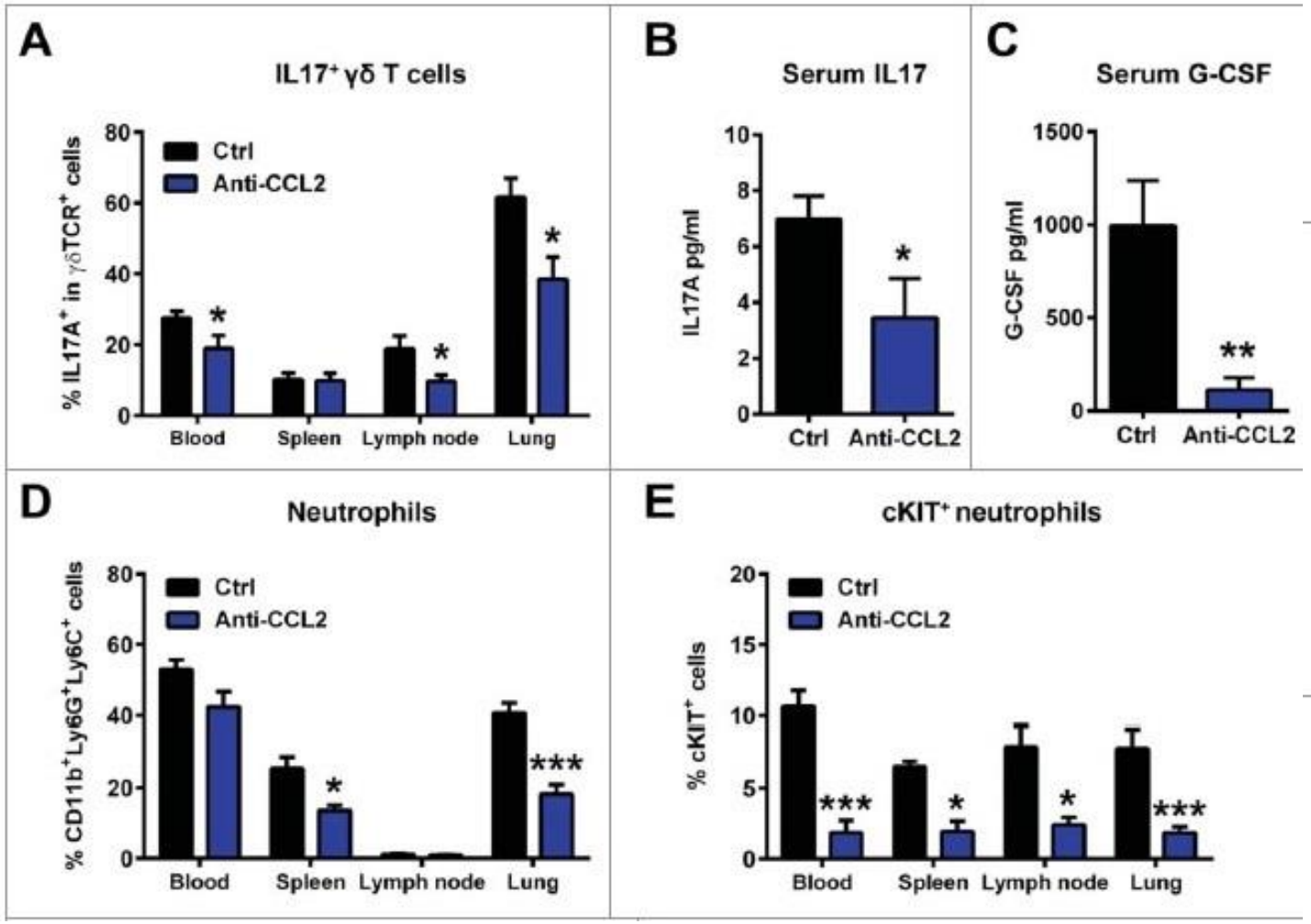


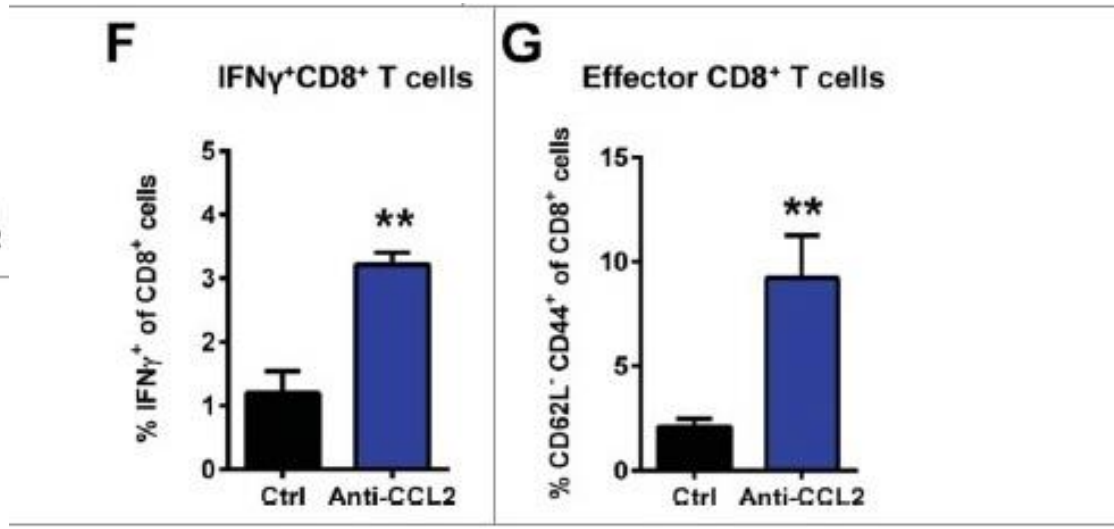
ORIGINAL RESEARCH

OPEN ACCESS Check for updates

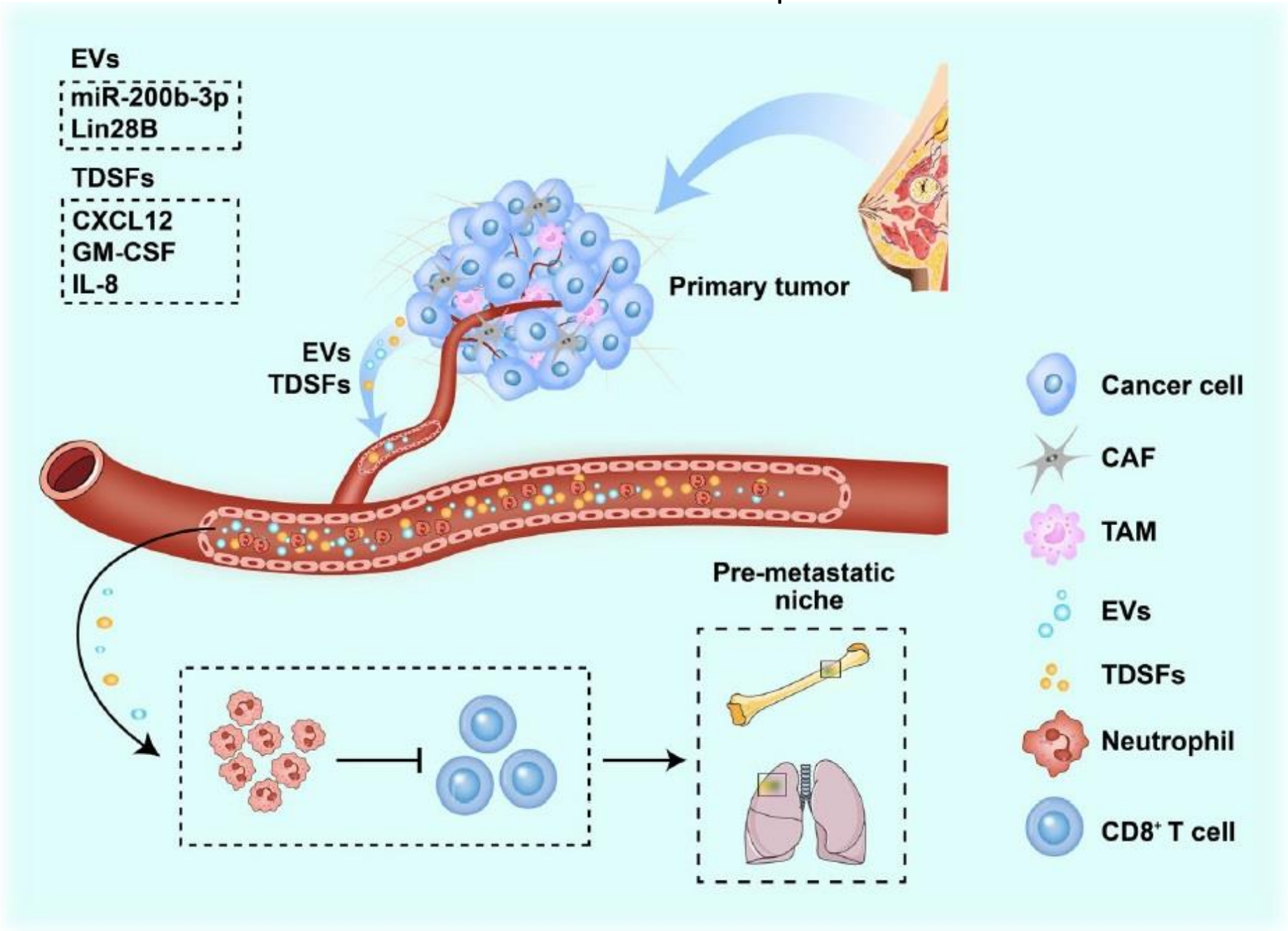
Mammary tumor-derived CCL2 enhances pro-metastatic systemic inflammation through upregulation of IL1β in tumor-associated macrophages

Kelly Kersten[a], Seth B. Coffelt[a,*], Marlous Hoogstraat[b], Niels J.M. Verstegen[a], Kim Vrijland[a], Metamia Ciampricotti[a], Chris W. Doornebal[a,c], Cheei-Sing Hau[a], Max D. Wellenstein[a], Camilla Salvagno[a], Parul Doshi[d], Esther H. Lips[e], Lodewyk F.A. Wessels[b,f], and Karin E. de Visser[a]





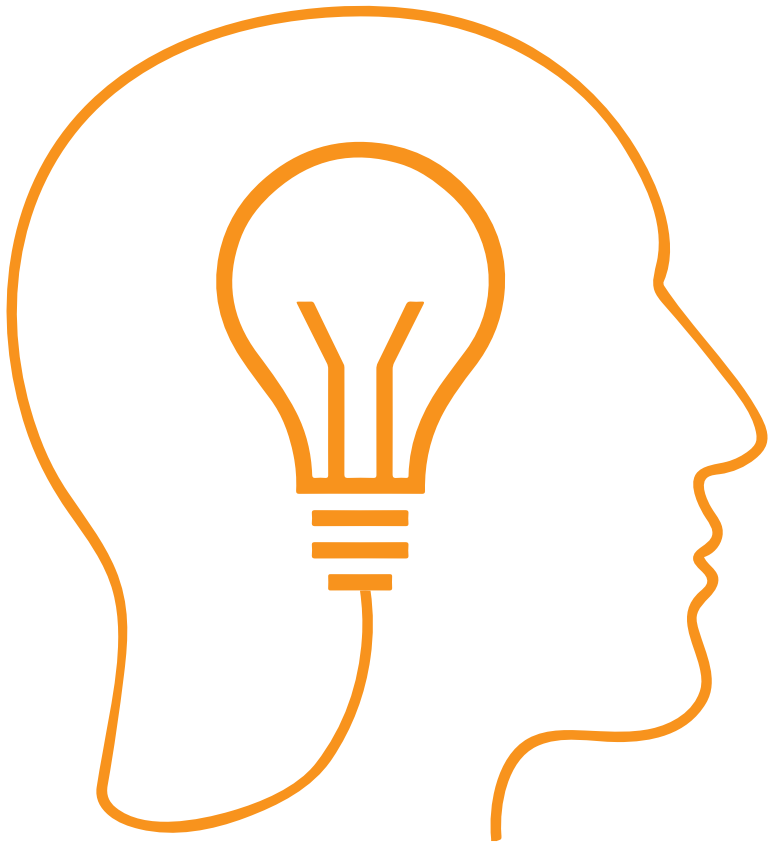
Tumor cell-induced formation of the pre-metastatic niche



Neutrophil-targeted BC treatment strategies

Multiple clinical strategies are under exploration to inhibit neutrophil recruitment, prolong survival, and encourage genetic reprogramming toward the N1 antitumor phenotype .

Strategies for inducing the N1



Cytokines

- ✓ In an **IFN-ß** enriched environment, neutrophils are converted to an N1 phenotype with anti-tumorigenic properties.
- ✓ The **TLR agonists** acting as Type I IFN inducers, such as (BCG), monophospholipid A, and imiquimod,

Compound

- ✓ **ß-glucan** induces the reprogramming of neutrophils towards an anti-tumor (N1) phenotype, polarized by type I IFN signaling in a ROS-dependent manner.
- ✓ **(BCG)** vaccination induces functional reprogramming of human neutrophils towards the N1 phenotype, characterized by increased expression of **activation markers and antimicrobial function**, enhanced neutrophil killing capacity, increased ROS production, and phagocytosis.

Strategies for inducing the N1

Hormones

- ✓ **Melatonin** is an endogenous hormone that provides a variety of biological activities.
- ✓ They observed melatonin-induced neutrophils with an N1-like antitumor phenotype and increased NETs that caused apoptosis of tumor cells through intercellular contacts.

Radiotherapy

- ✓ Radiotherapy affects **early neutrophil infiltration** by inducing mesenchymal-epithelial transition, activating neutrophil recruitment, and polarizing newly recruited neutrophils to an anti-tumor phenotype, which can be enhanced by concomitant administration of **G-CSF.**

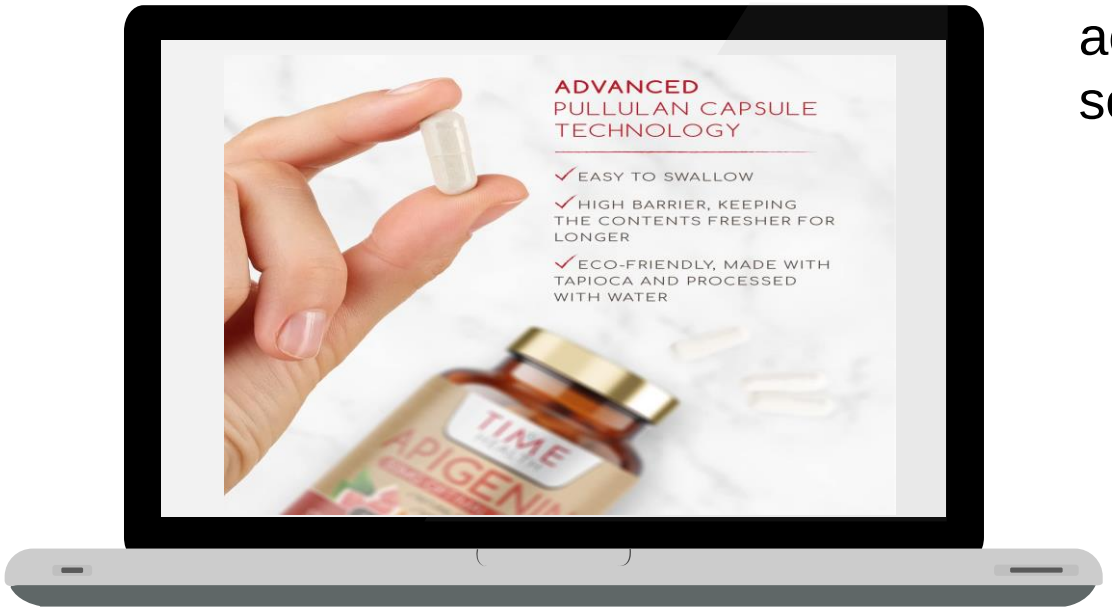
Therapeutic strategy for targeted N2 neutrophils

Chemokine receptor inhibitors

The chemokine receptors CXCR1 and CXCR2 are among the most promising targets for neutrophil targeting.

Reparixin, a CXCR1 variant inhibitor (and to a lesser extent a CXCR2 variant inhibitor) in clinical trials, interacts with CXCL8 receptors to inhibit the intracellular signal transduction events activated by the binding of CXCL8 to CXCR1 and CXCR2.

Therapeutic strategy for targeted N2 neutrophils



- ✓ **Galunisertib**, an oral small molecule inhibitor of selective **TGF-ß receptor I kinase**, has antitumor activity in animal models of BC, and it has shown some efficacy in the clinical stage of BC treatment.

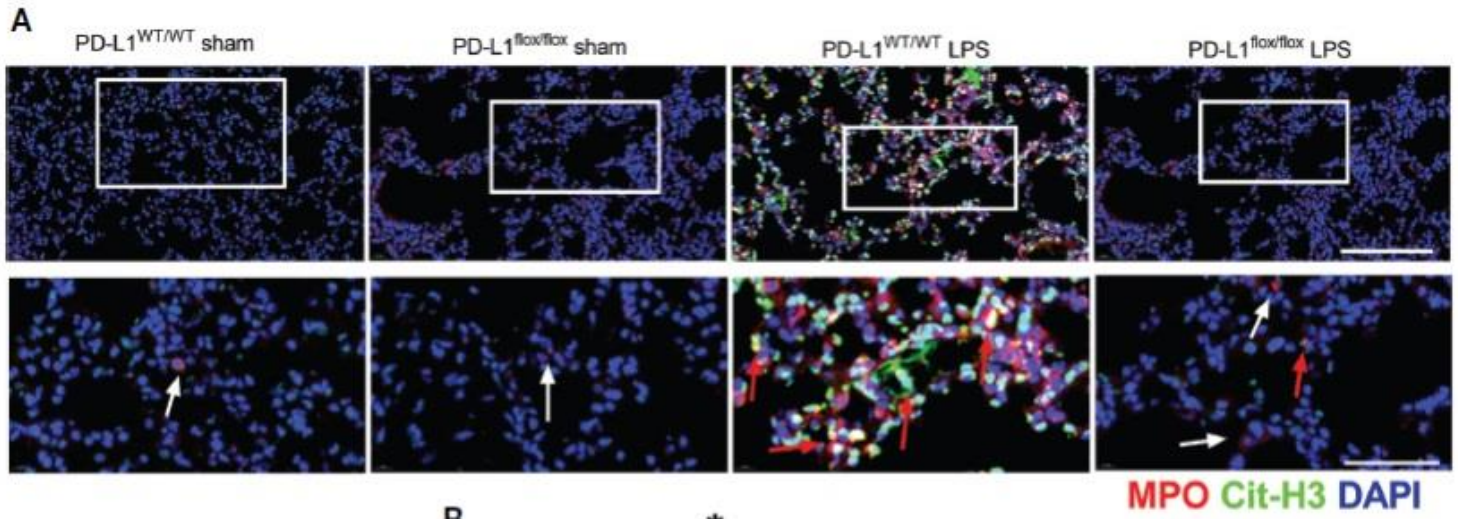
- ✓ **Apigenin** inhibits TNFa/IL-1a-induced **CCL2** release, which in turn inhibits the infiltration/ migration of **TANs**, ultimately resulting in attenuated tumor immune escape, tumor growth, angiogenesis, and metastasis.

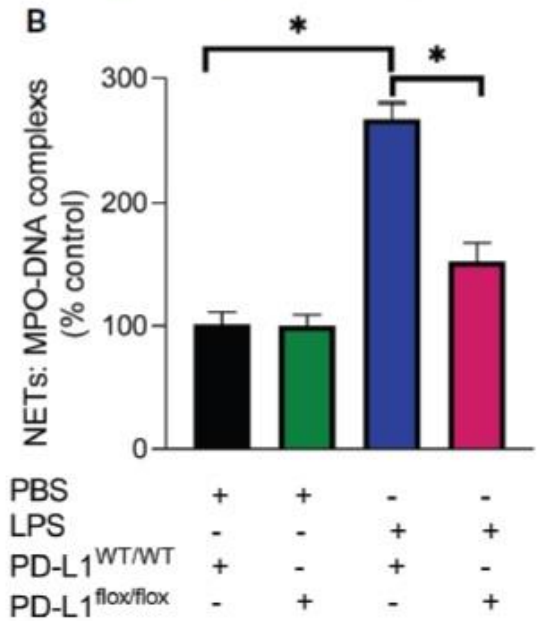
PD1/PD-L1targeting-agents

- ✓ Cancer-associated fibroblast (CAFs) in the TME can induce activation of the STAT3 pathway in neutrophils, which disrupts T cell function and accelerates tumor progression via PD1/ PD-L1 signaling pathways.

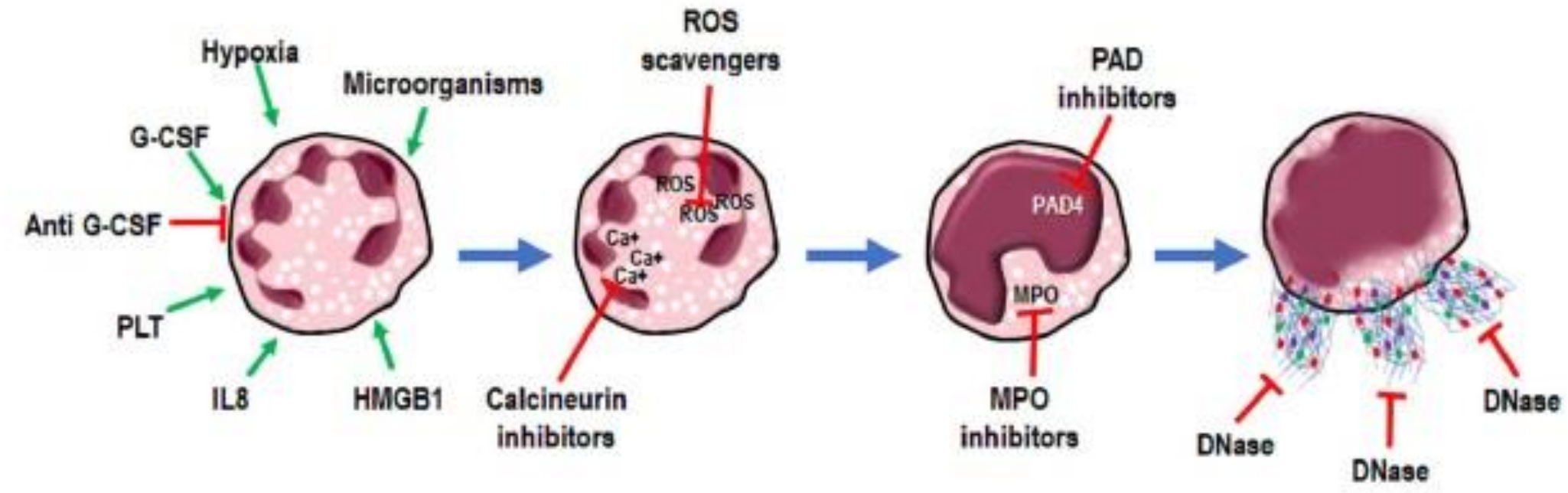
- ✓ Targeting PD1/PD-L1 crosstalk is an effective immunotherapy for a variety of cancers and can also be an effective strategy for targeting TAN.

PD-L1 maintains neutrophil extracellular traps release by inhibiting neutrophil autophagy in endotoxin-induced lung injury





NETs as targets for therapy



- ✓ The inhibitory effect of **aspirin** on NETs has yielded some promising results in animal models.
- ✓ Lapponi et al. showed that aspirin prevented NET-induced injury of the **lung endothelium** by inhibiting platelet activation and subsequent NET formation in mice. The inhibitory effect of aspirin on **NF-κB,** an inflammatory transcriptional regulator that plays a role in some pathways promoting NETosis, was also demonstrated.

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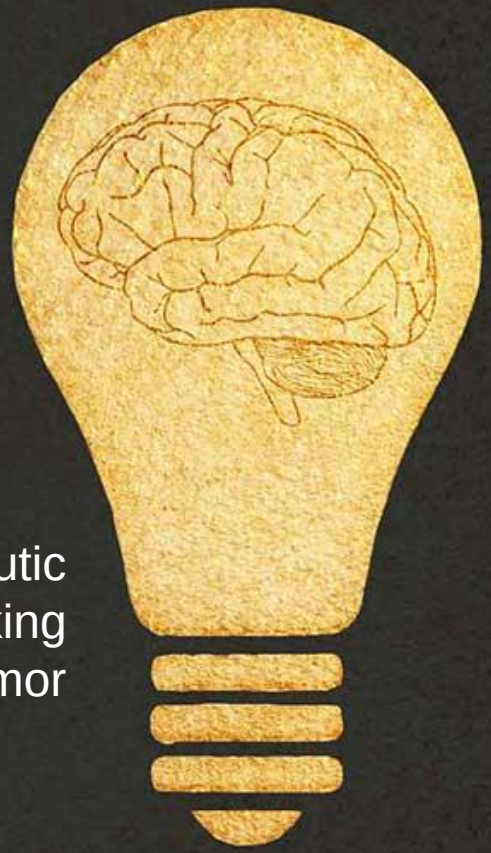
CHALLENGES

- Therapeutics targeting neutrophils are constrained by challenges such as the suboptimal classification of neutrophils, potential severe infections, and other side effects.
  - Most of the data on anti-tumor ’N1’ and pro-tumor ’N2’ are derived from mouse models, which contrasts with the gradual progression of human cancers.
    - Although neutrophils are significantly associated with treatment outcomes and prognosis of BC, as well as their informative value in predicting relevant parameters, the clinical utility of NLR or TAN is limited owing to unreliable markers for differentiating N1/N2 neutrophils and non-standardized NLR thresholds.
      - Furthermore, while antagonists such as CXCR1/2 hold therapeutic promise and have shown encouraging results in clinical trials, blocking neutrophils recruitment may amplify ongoing damage in the tumor microenvironment and promote tumor progression in the long run.



CONCLUSION

- The next vital step will be untangling the web of crosstalk between neutrophils, tumor cells, endothelial cells, platelets, and extracellular vesicles, and eventually the influence of other components of the innate and adaptive immune systems on cancer progression.

- Given the dual role of neutrophil action on tumors, the ideal goal of oncology would be to promote the enrichment of anti-tumor neutrophils while depleting tumor-promoting neutrophils without altering anti-microbial neutrophils.

Therefore, the function and phenotype of neutrophils need to be further explored, as well as better predictive markers and more effective strategies for neutrophil-targeted cancer therapy.

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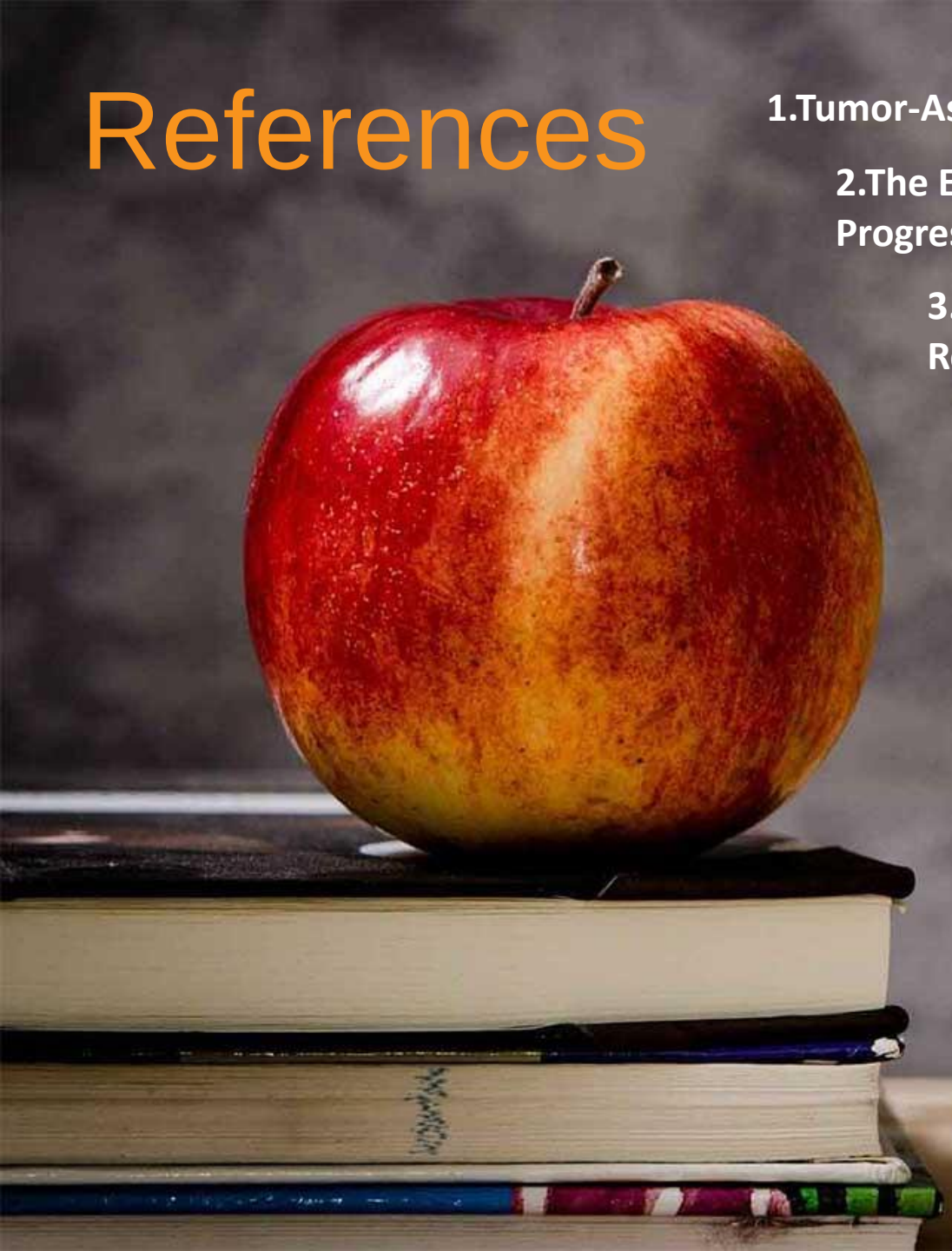

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8. Neutrophil extracellular traps in breast cancer and beyond: current perspectives on NET stimuli, thrombosis and metastasis, and clinical utility for diagnosis and treatment

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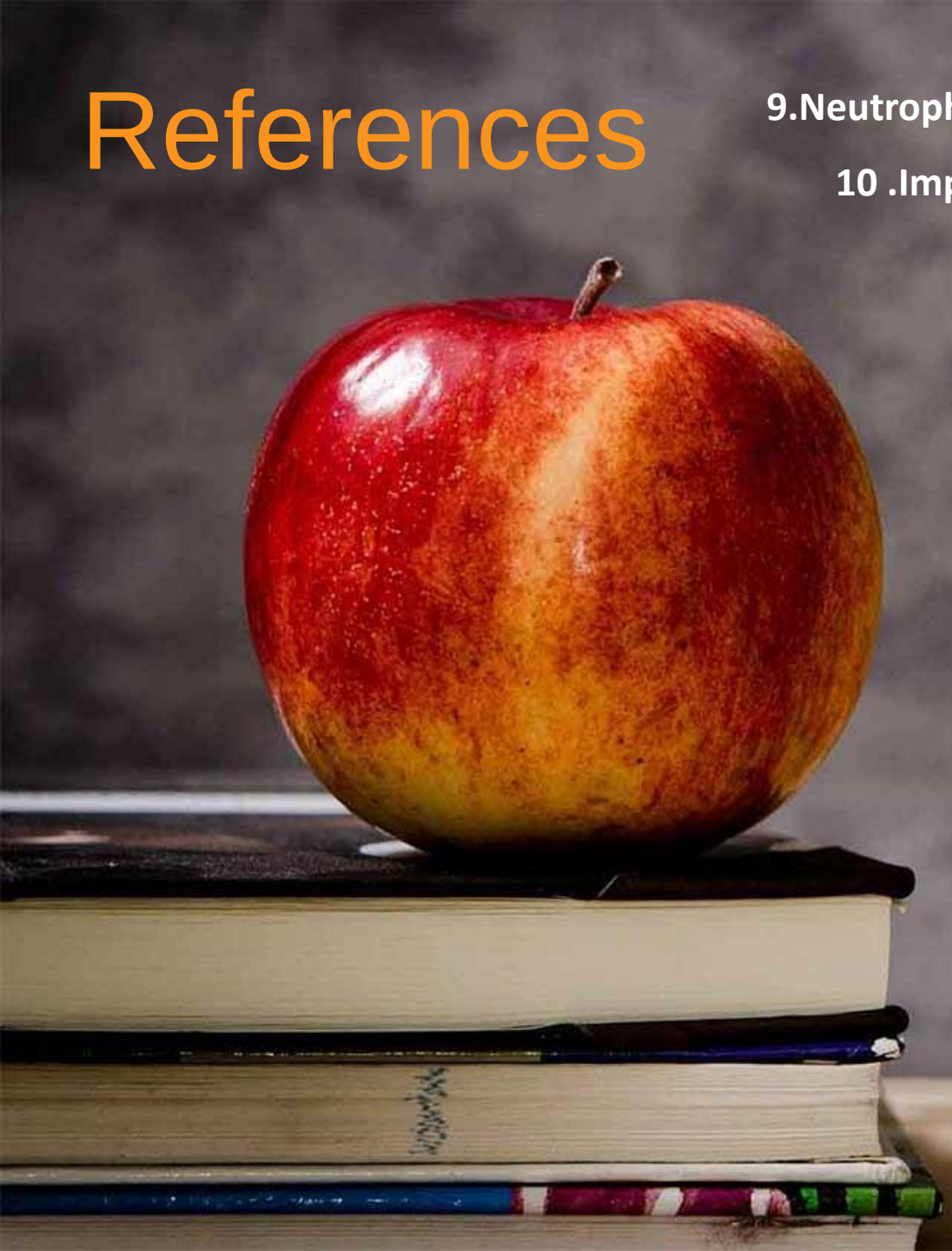

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THANK YOU