



Tumor Immune Microenvironment: A Holistic Approach Workshop

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#SITCworkshop



Society for Immunotherapy of Cancer

Breast Tumor Cell Heme Metabolism Alters Macrophage Polarization and Function to Support Lung Metastasis

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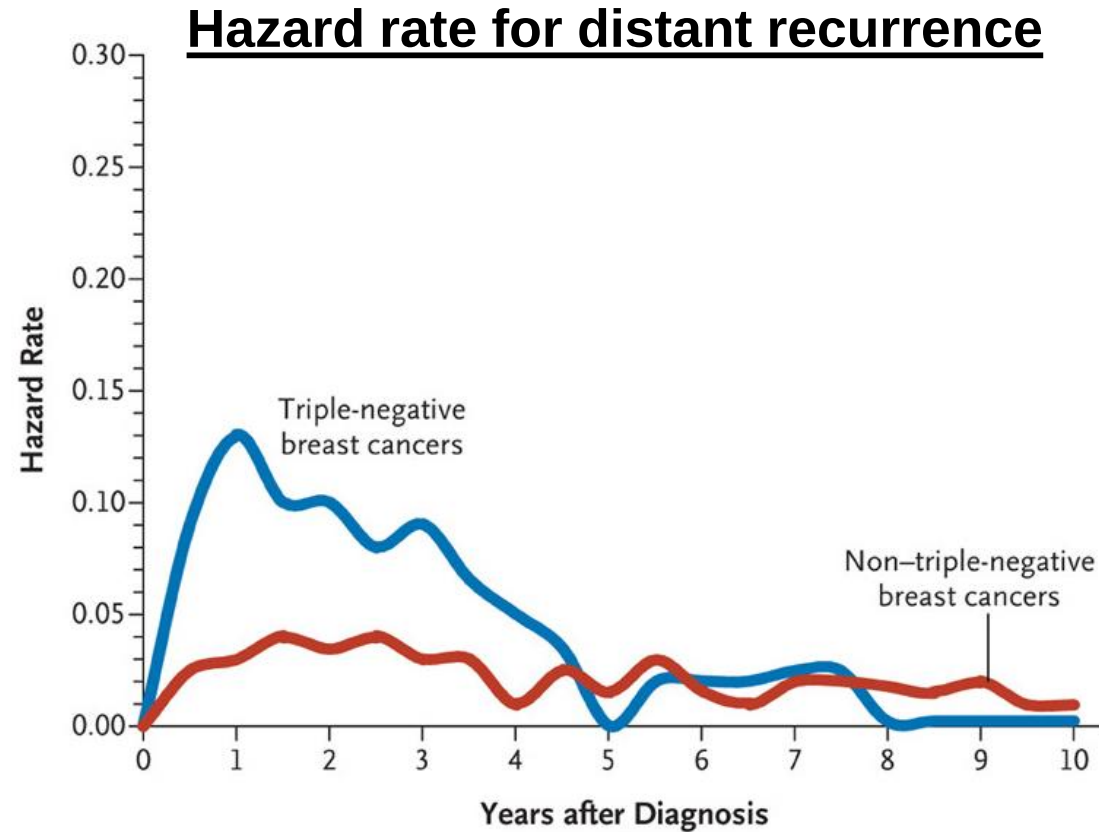
Organizers/Abstract Selection Committee



 @mwilli323

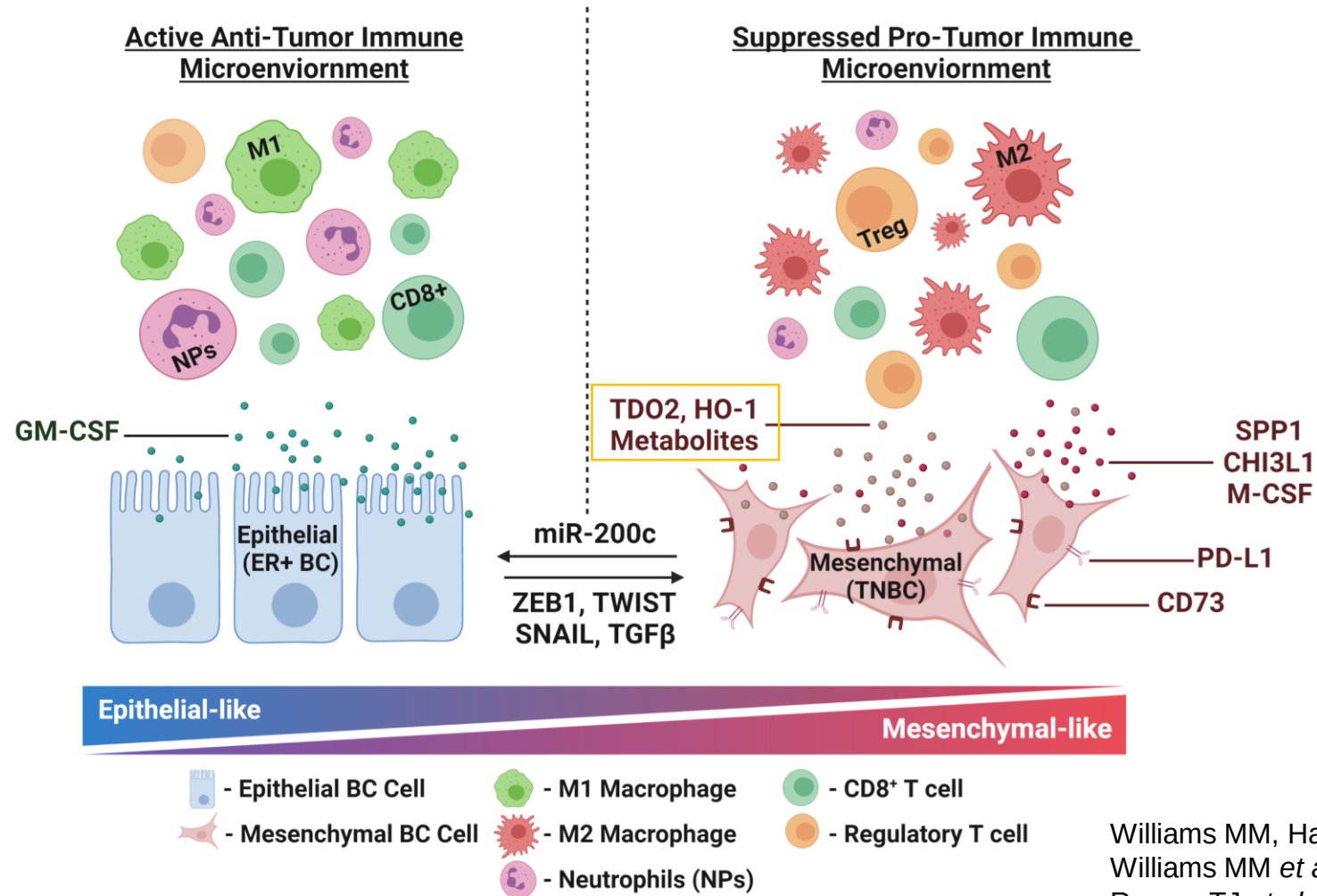
Triple-negative breast cancers (TNBC) metastasize more rapidly than other BC subtypes

>20% of TNBC patients will recur within the first 5 years



Foulkes WD *et al.* NEJM. 2010.

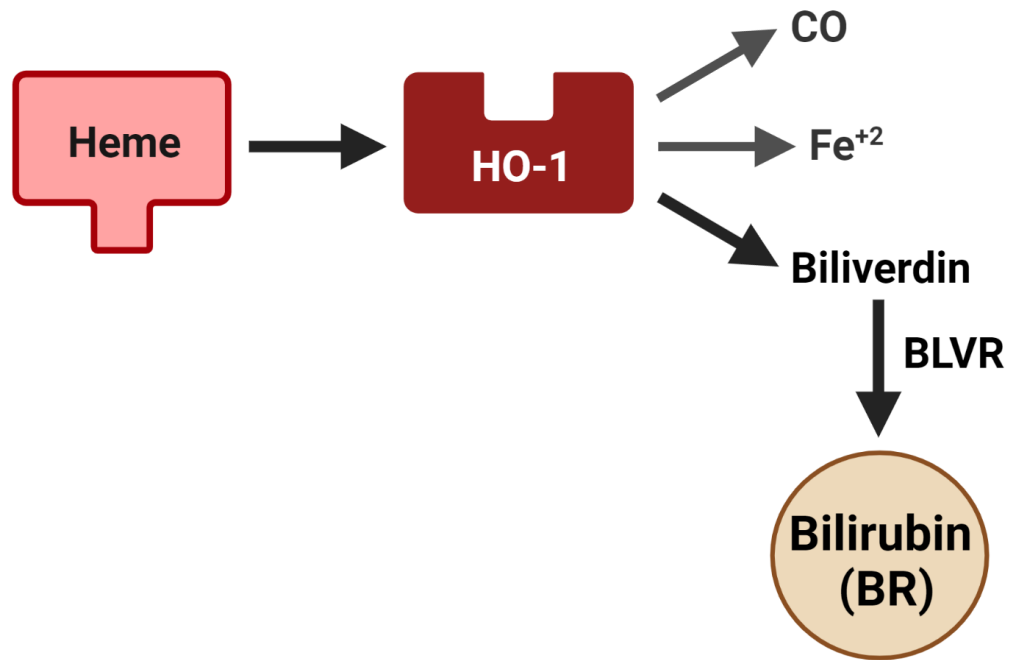
Manipulation of EMT is a tool to reveal pro-metastatic, immune suppressive pathways in TNBC



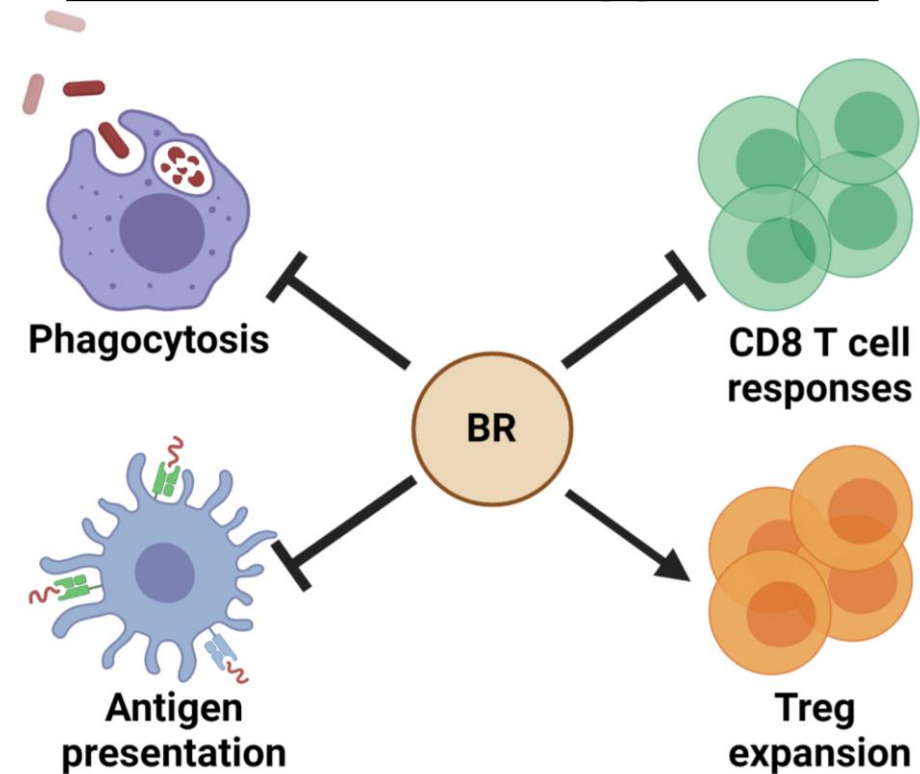
Williams MM, Hafeez SA *et al.* Pharmaceuticals. 2021.
 Williams MM *et al.* NPJ Breast Cancer. 2021.
 Rogers TJ *et al.* MCR. 2019.
 Dongre A *et al.* Can Res. 2017, and Can Disc. 2021.

Heme oxygenase-1 (HO-1) produces immune suppressive metabolites

Heme metabolism via HO-1



BR and immune suppression

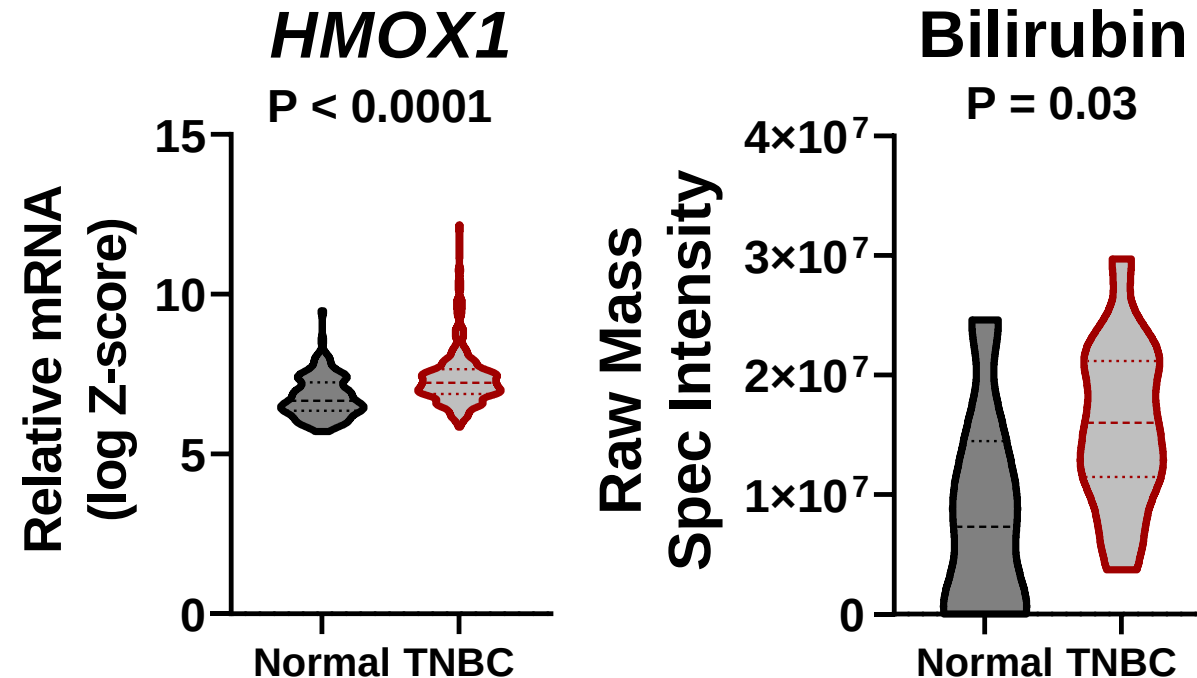


BR has never been studied as an immune modulator in cancer

Images created with Biorender.com
Jangi S *et al.* Int J Biochem & Cell Bio, 2013.

Heme metabolism is increased with TNBC tumorigenesis

Normal breast and primary TNBC specimens



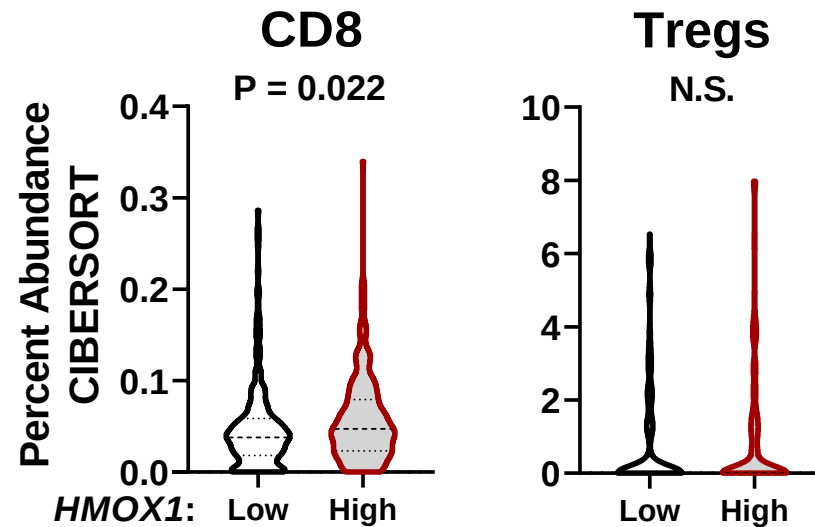
***HMOX1* = gene encodes HO-1**

The Cancer Genome Atlas, CBioPortal.com, Nature 2012, Nature 2019, Nature Comm. 2016.

High expression of *HMOX1* predicts a more suppressive macrophage microenvironment

CIBERSORT of TNBC specimens

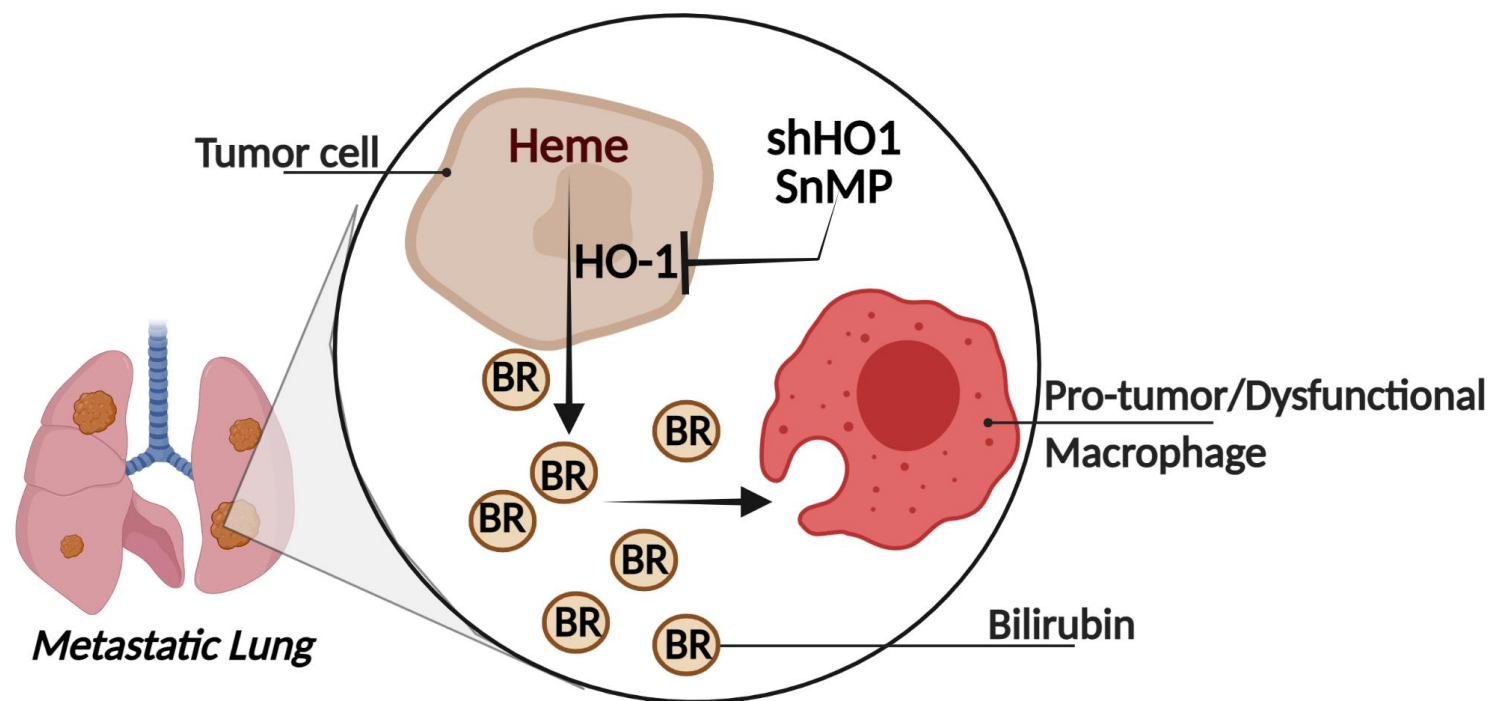
T Cells



***HMOX1* = gene encodes HO-1**

The Cancer Genome Atlas, CBioPortal.com, Nature 2012, Nature 2019, Nature Comm. 2016.

Hypothesis: Triple-negative breast cancer heme metabolism supports metastasis by promoting a pro-tumor immune microenvironment.

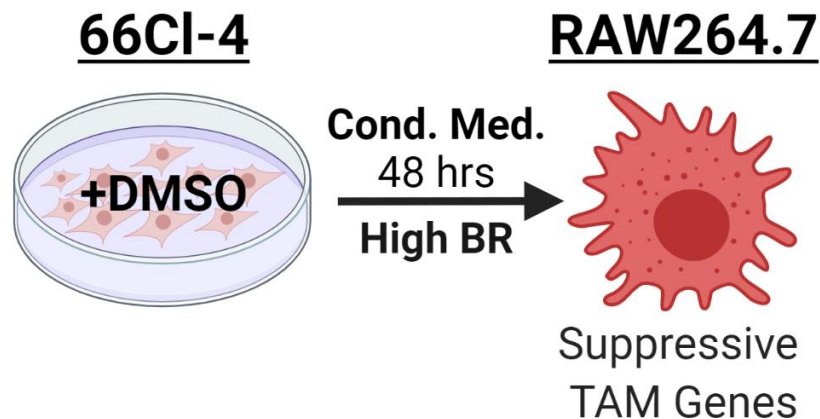


SnMP = HO-1 competitive inhibitor Tin Mesoporphyrin

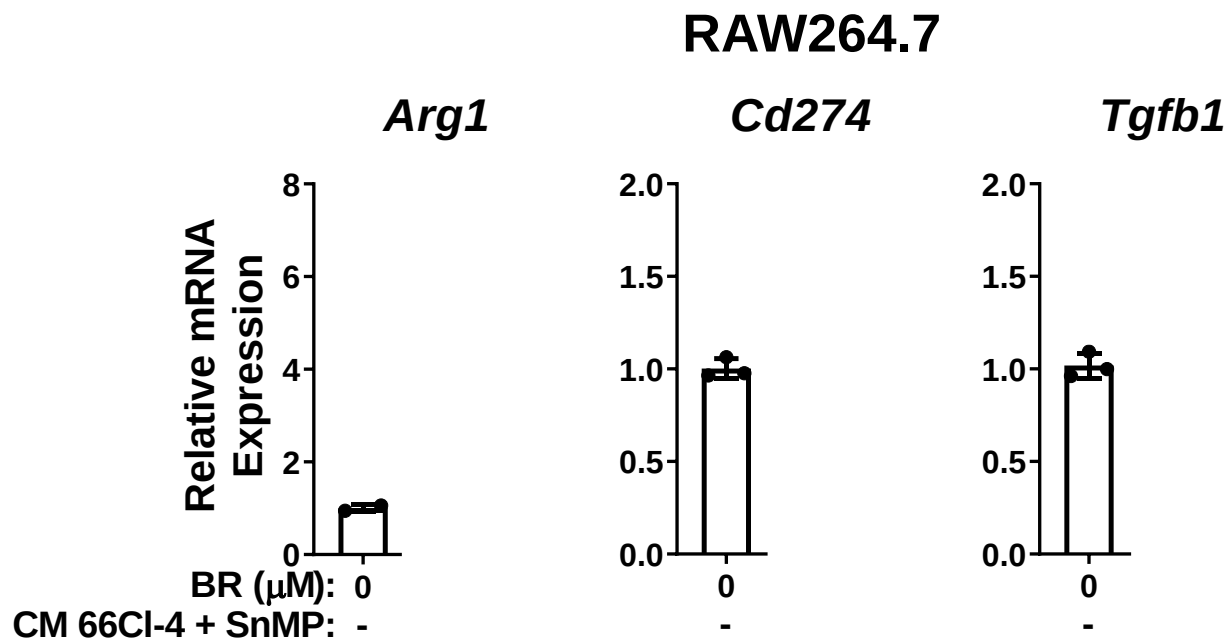
Image created with Biorender.com

Tumor cell-HO-1 supports immune suppressive macrophage markers via secreted bilirubin

Experimental model



Suppressive TAM genes



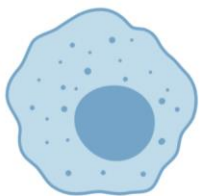
Arg1, *Cd274* (PD-L1), *Tgfb1* = immune suppressive tumor associated macrophage (TAM) markers
Secreted Tgf β 1 levels were similarly altered

Image created with Biorender.com

Bilirubin enhances macrophage PD-L1 expression

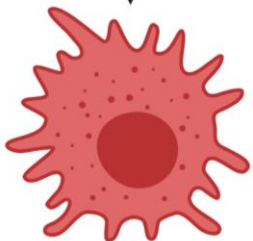
Experimental model

RAW264.7,
THP-1 or BMDM



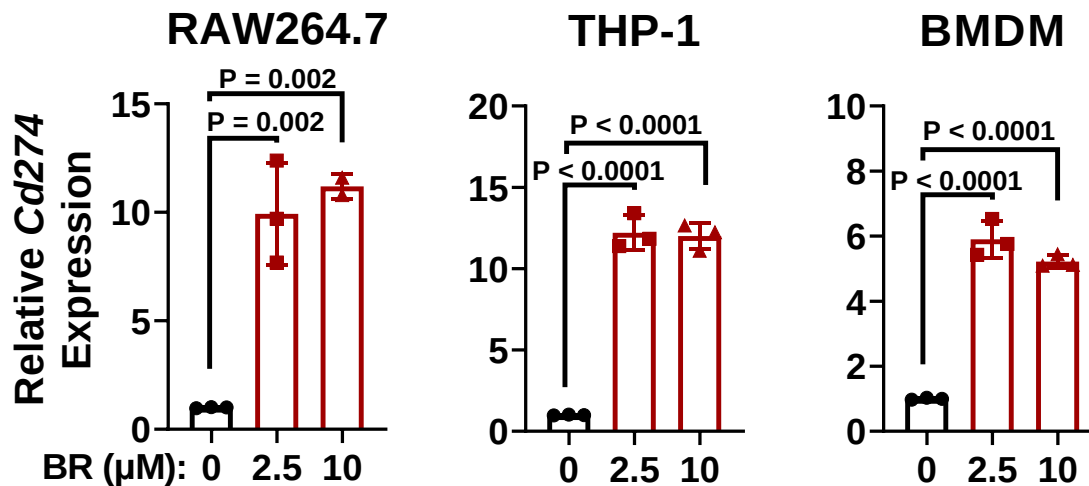
Unactivated

+ 0, 2.5,
10 μ M BR
48 hrs



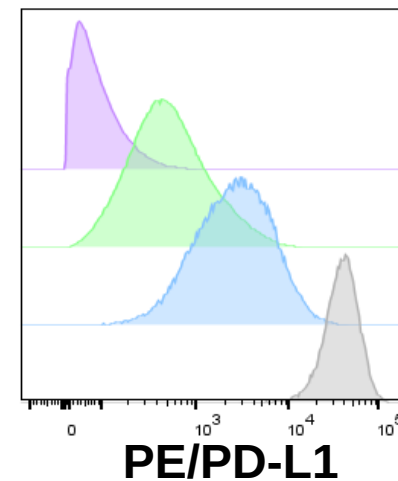
Immunosuppressive
M2/TAM-like

PD-L1 gene expression



PD-L1 protein expression

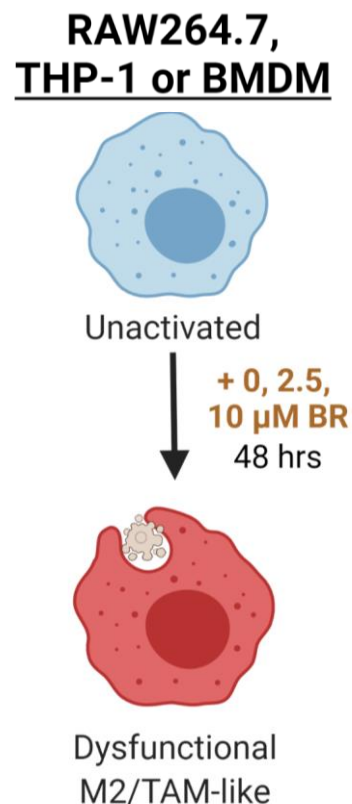
Treatment		MFI
	0 μ M BR	98.5
	2.5 μ M BR	838
	10 μ M BR	3642
	100 ng/mL IFN γ + LPS	39957



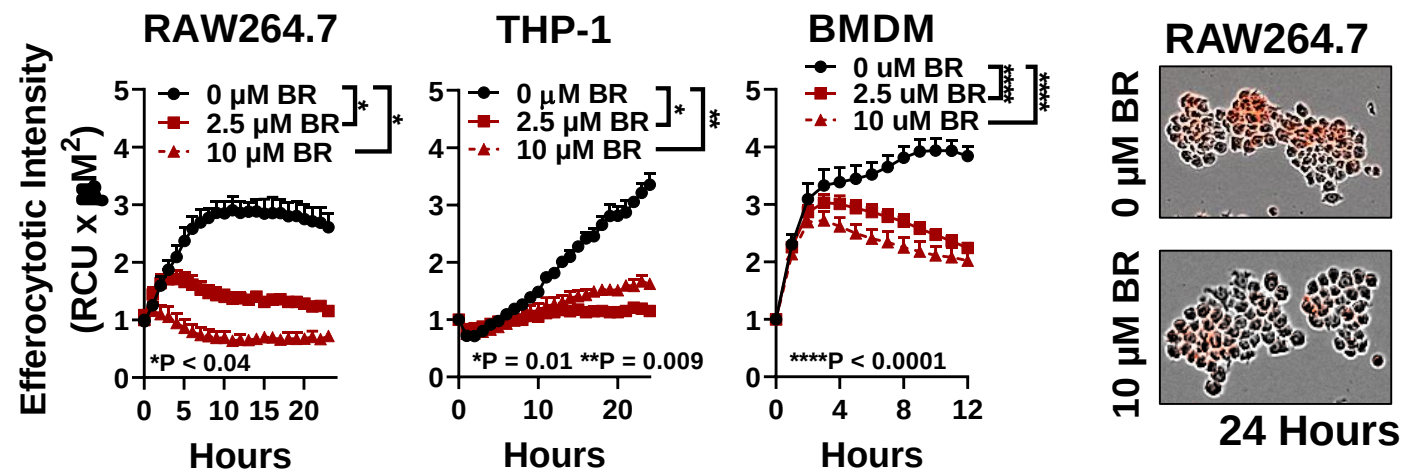
Similar results seen with 5.0 and 20 μ M BR
Image created with Biorender.com

Bilirubin decreases macrophage efferocytosis

Experimental model



Efferocytic activity



Efferocytosis receptor expression

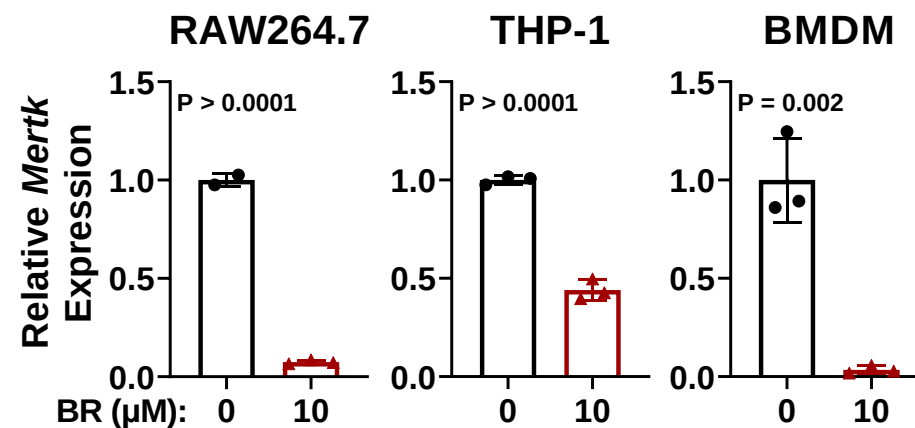
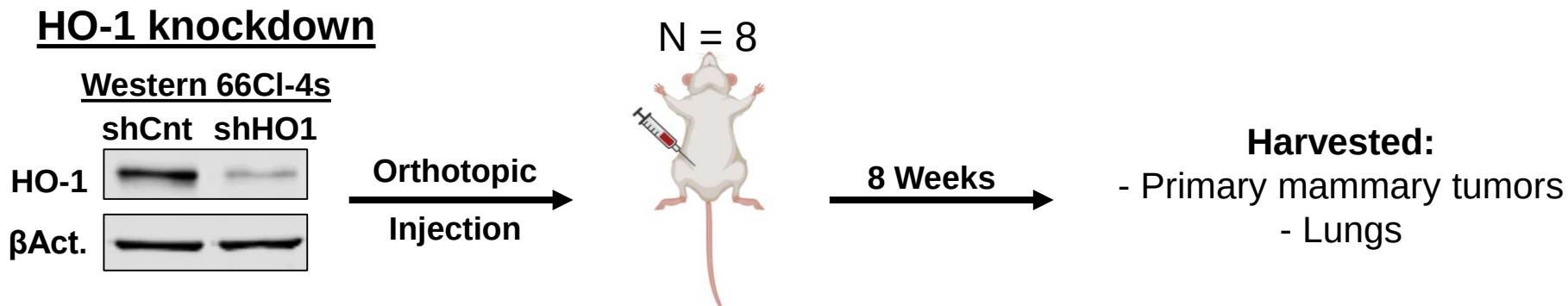
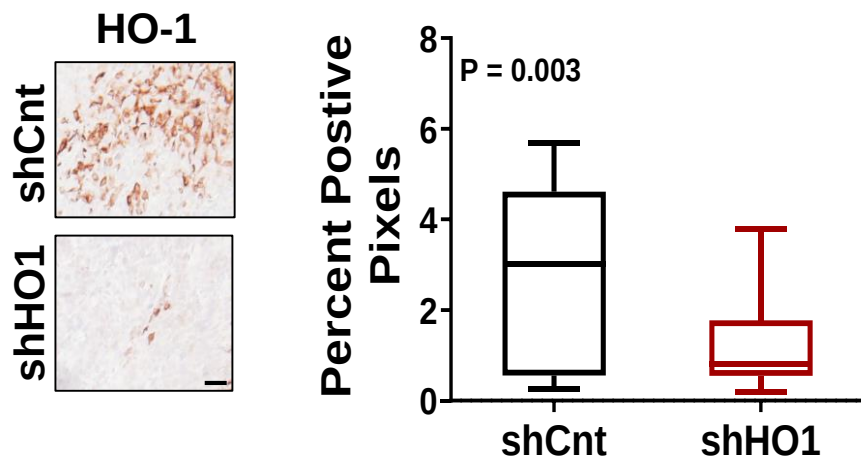


Image created with Biorender.com

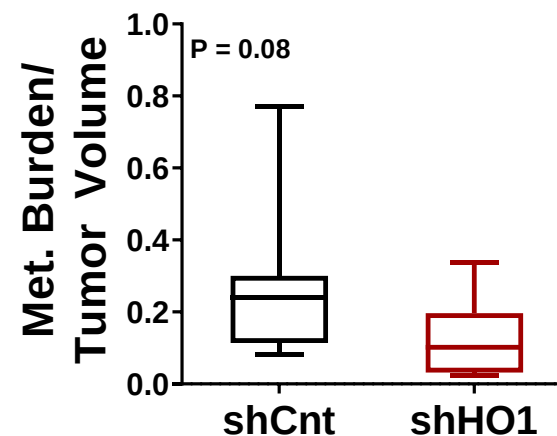
Tumor cell-HO-1 inhibition limits lung metastasis



1. HO-1 IHC primary tumors



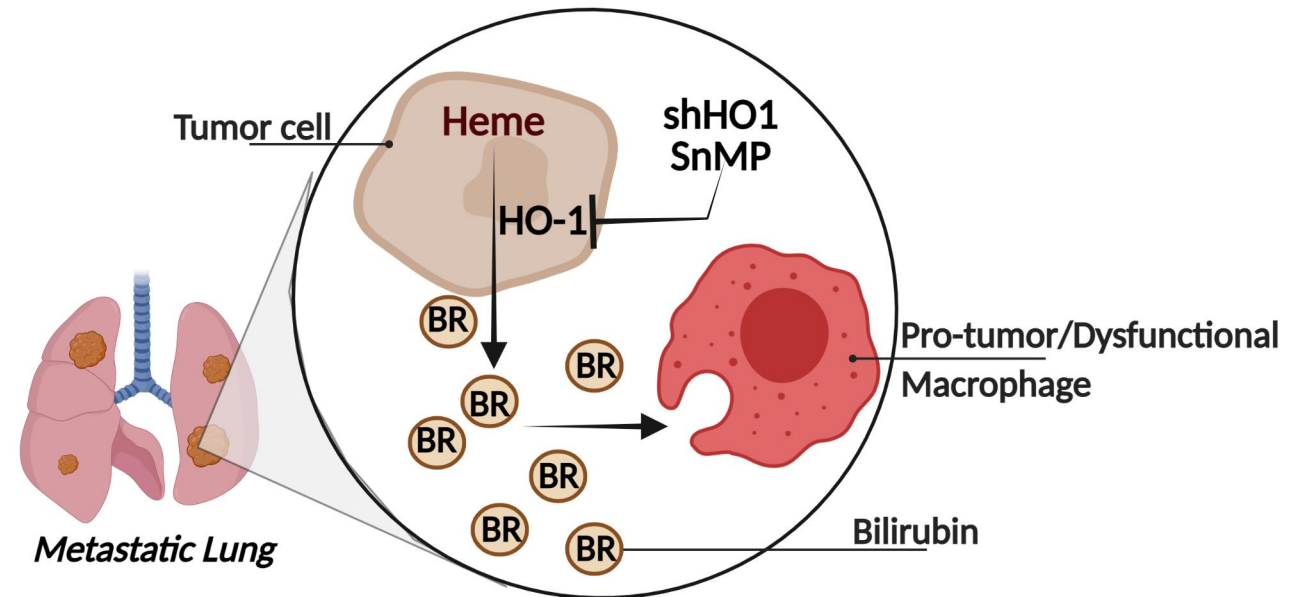
2. Lung metastatic capacity



Summary

Take home messages:

- HO-1 and its metabolite bilirubin support:
 - Macrophage immune suppression
 - Macrophage dysfunction
 - Lung metastatic capacity



Current research directions:

- Testing the impact of HO-1 inhibition on:
 - TME of primary tumors and metastatic lungs and livers (focusing on myeloid compartment)
 - sensitivity of metastases to immunotherapy