20.GEM GEM4 Summer School: Cell and Molecular Biomechanics in Medicine: Cancer
Summer 2007

From Cell Physiology and Biology to Cellular Biomechanics:

Role of Hydrodynamics, Chemokines and Adhesion in Leukocyte-mediated Melanoma Extravasation

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Scan of USA Today article.

USA Today (Nov. 10, 2004) interviewed leading medical experts about the relationship between inflammation and cancer:

- Doctors believe that inflammation is involved in a wide variety of cancers.
- Scientists say they can't be sure whether inflammation produces cancer; or cancer leads to inflammation; or the two processes interact.
- Doctors suspect that long-term inflammation or infection is involved in up to 20% of cancers, including those of the esophagus, colon, skin, stomach, liver, bladder, breast and some kinds of lymphoma.

Cancer Metastasis Cascade

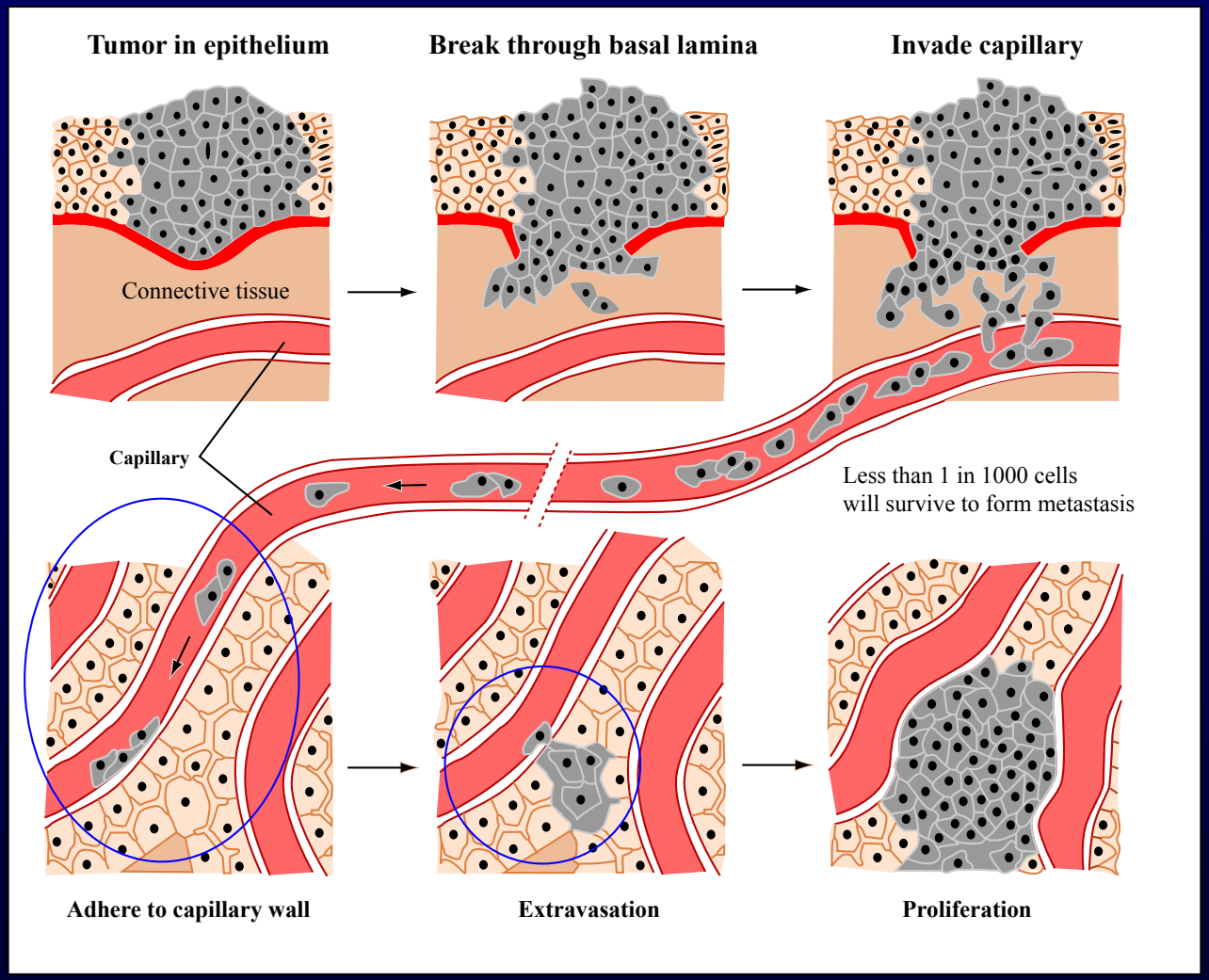

Illustration by MIT OpenCourseWare. After Alberts, et al., 1994

Alberts *et al*., 1994

Inhibiting ^{V600E}B-Raf reduced melanoma lung metastasis *in vivo*

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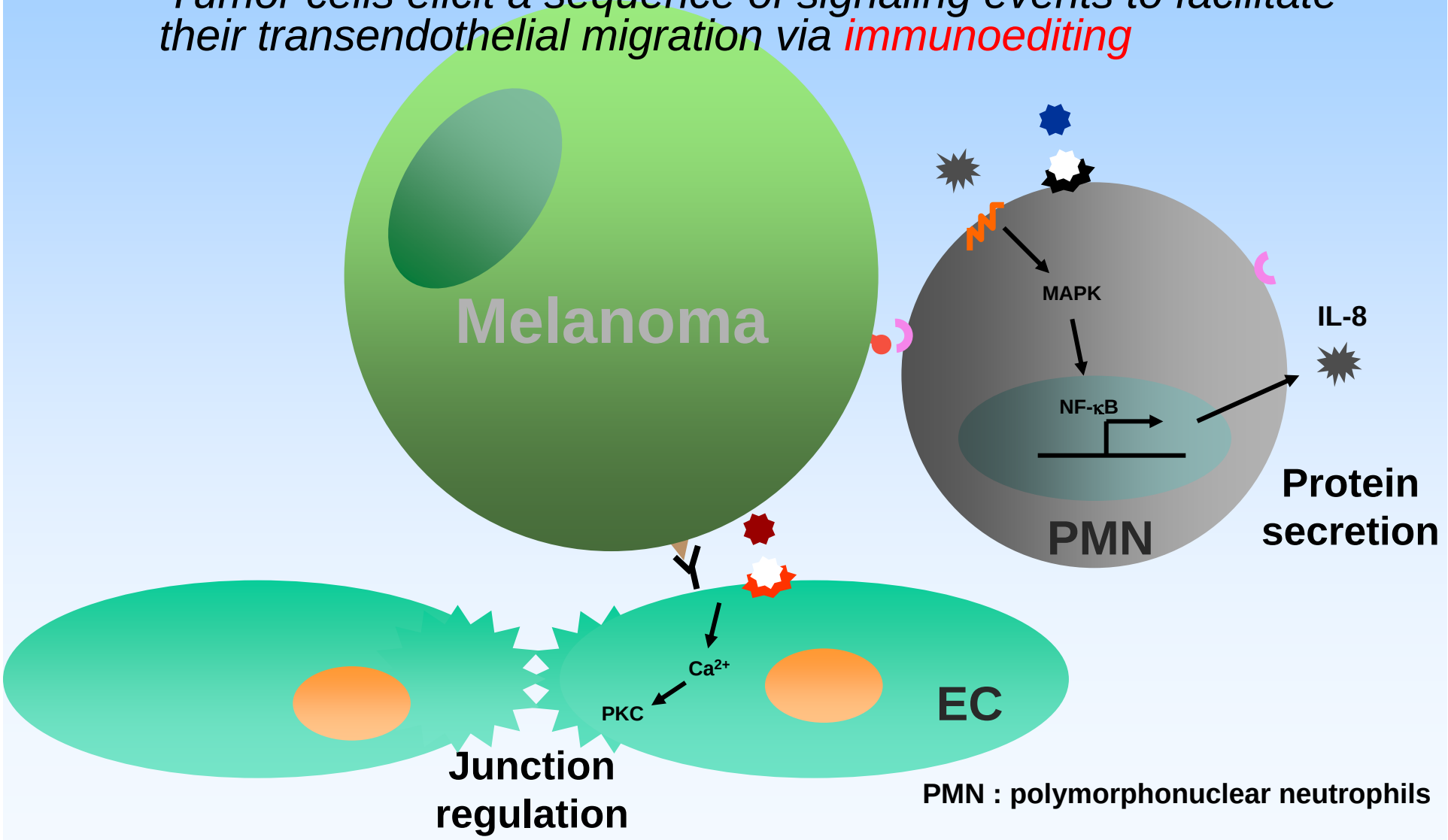
A, siRNA-mediated inhibition of V600EB-Raf inhibits melanoma lung metastasis. Left panel, 1205 Lu cell; right panel, UACC 903M cell. Number of tumors within particular size ranges (<1500 or >1500 pixels) were quantified in a minimum of 6 fields per lung from 5 to 10 animals. Values are means ± SEM. ***B***, co-localization of human melanoma cells and mouse PMNs *in vivo*.

On-going Studies

Overall Hypothesis :

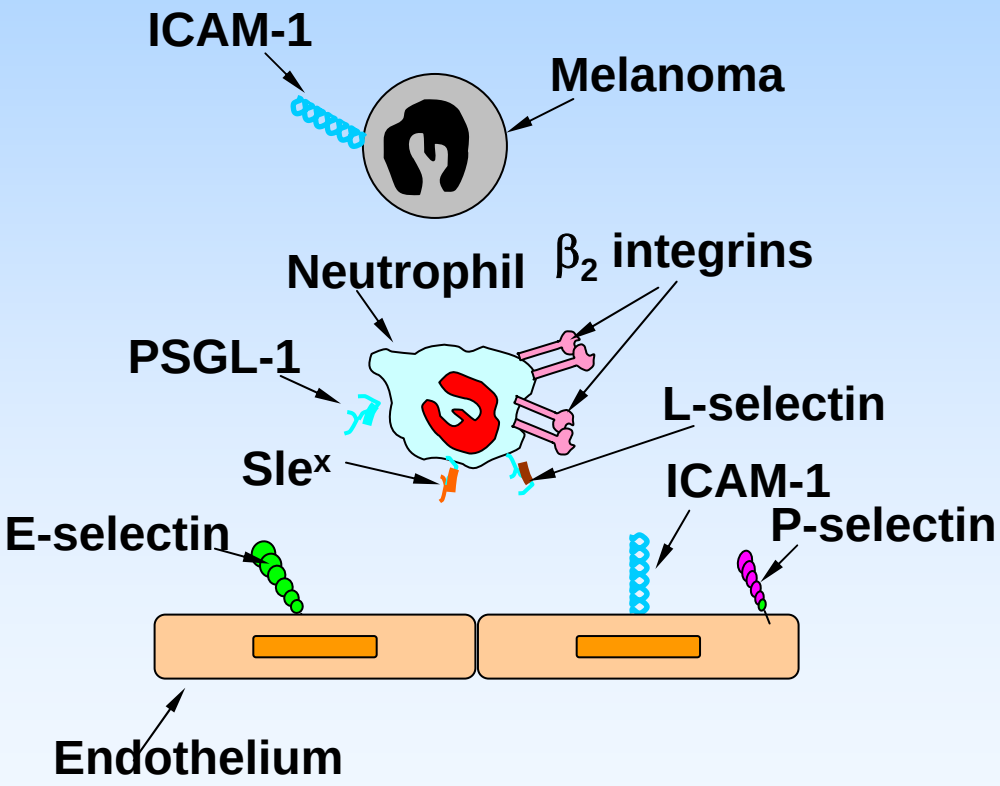
Tumor cells elicit a sequence of signaling events to facilitate their transendothelial migration via immunoediting



PMN : polymorphonuclear neutrophils

Working Hypothesis:

Neutrophils (PMN) facilitate melanoma adhesion leading to increased migration under flow conditions – an important step in tumor cell extravasation during metastasis.



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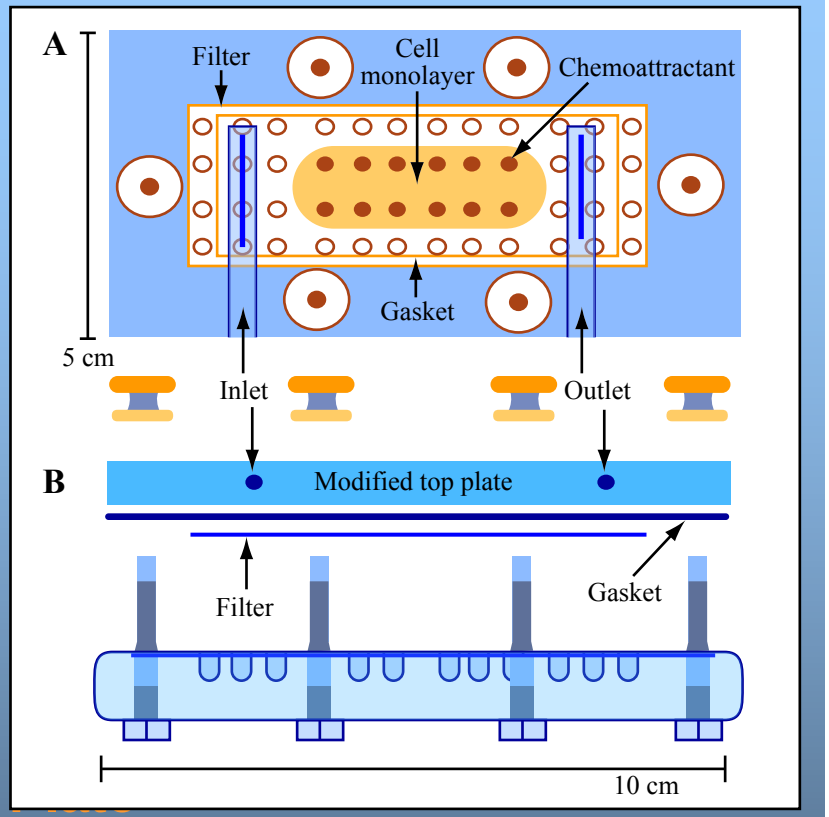
Flow-Migration Assay

Figure by MIT OpenCourseWare.



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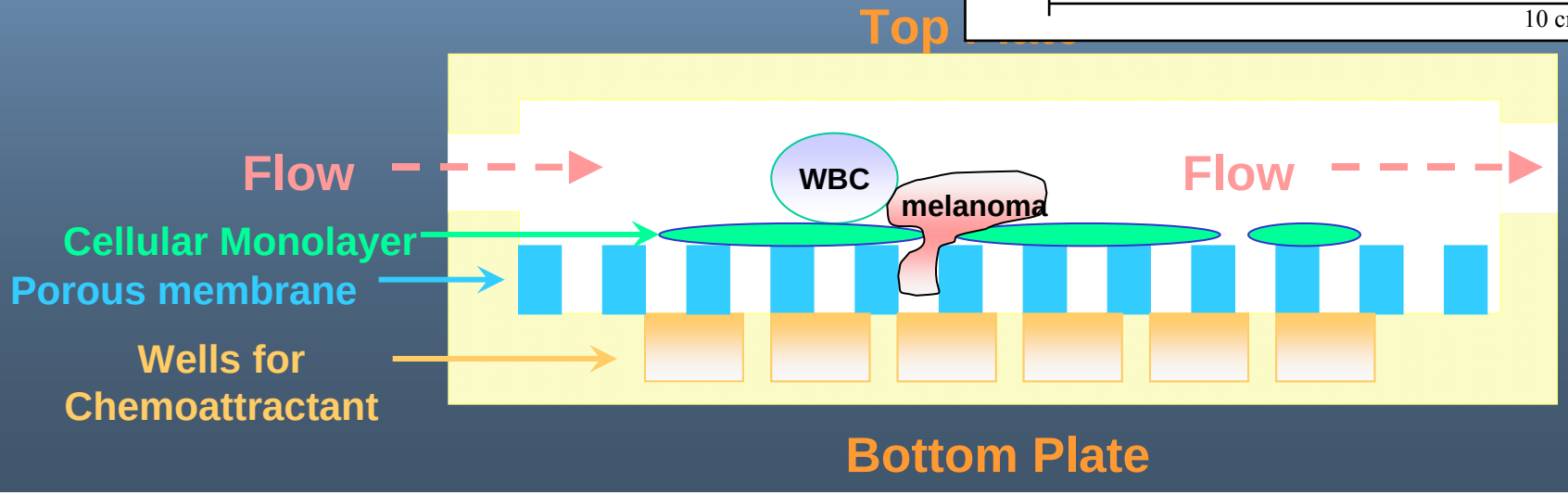
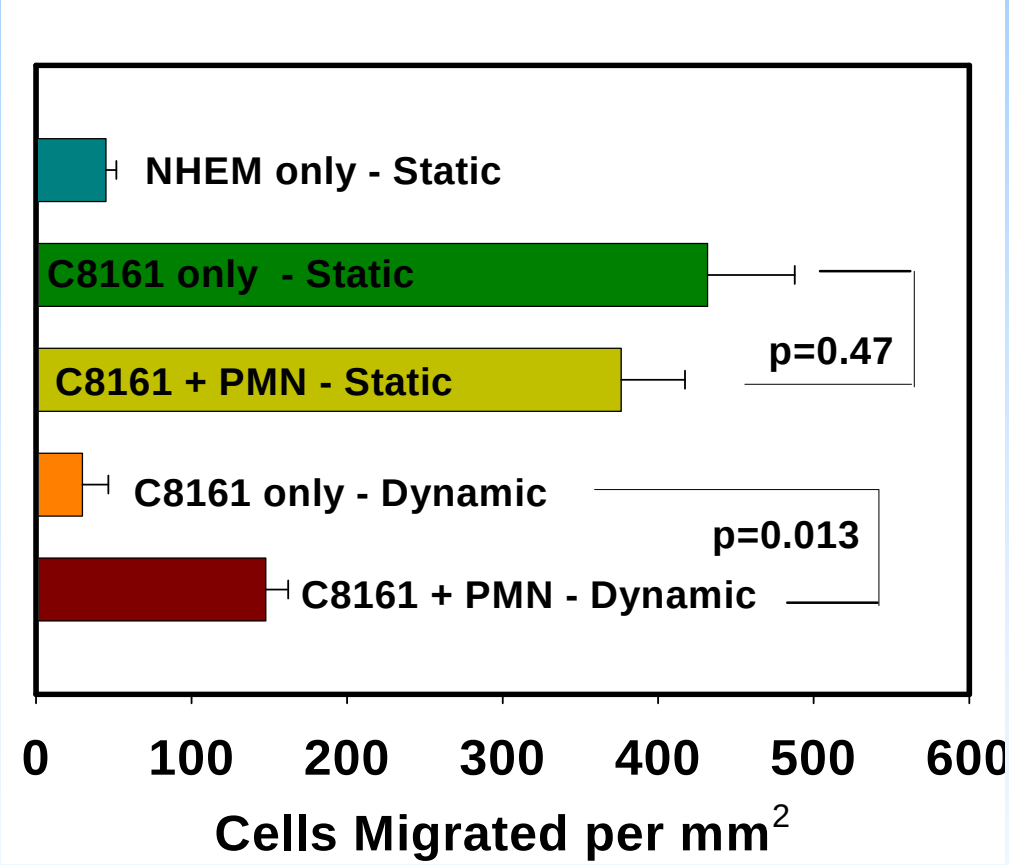
Gopalan et al. 1997 *J. Immunol.*



PMN Facilitates Melanoma Cell Migration Under Flow Conditions

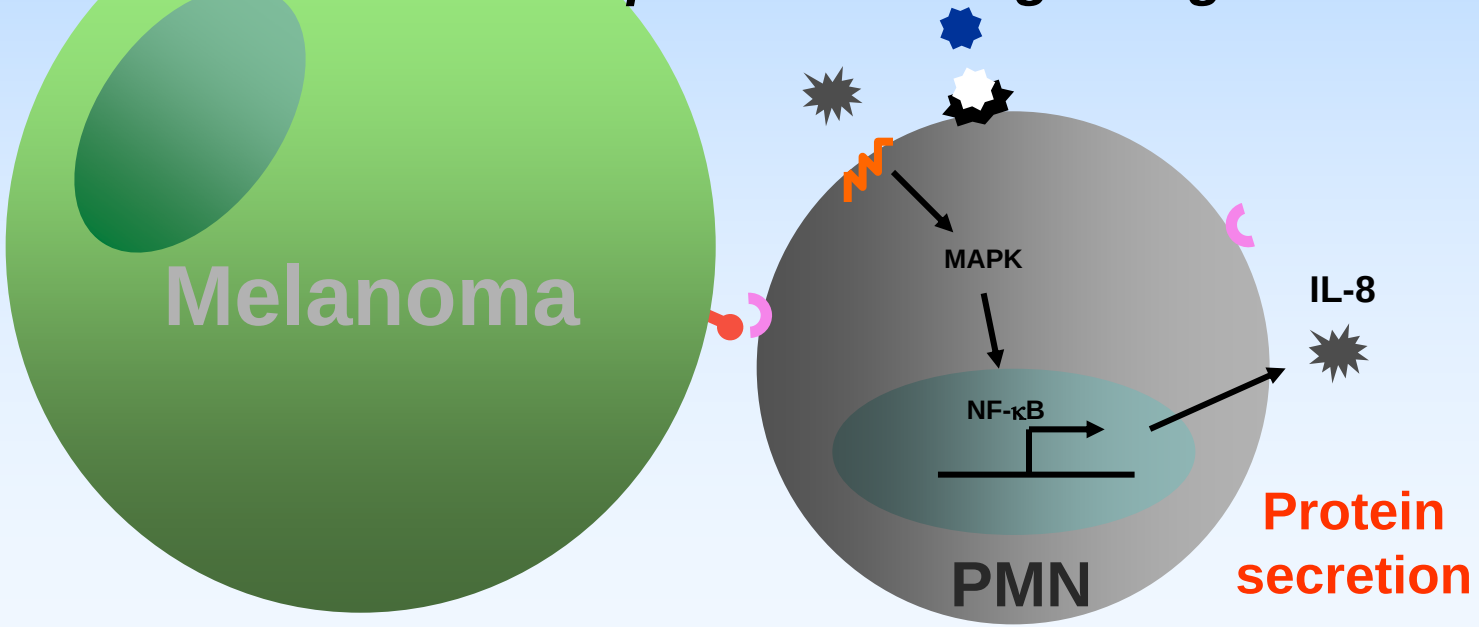


Dynamic: shear stress = 0.4 dyn/cm^2

To Clarify The Signaling Events Of Cytokine/Chemokine Induction Mediated By PMN-melanoma Interaction

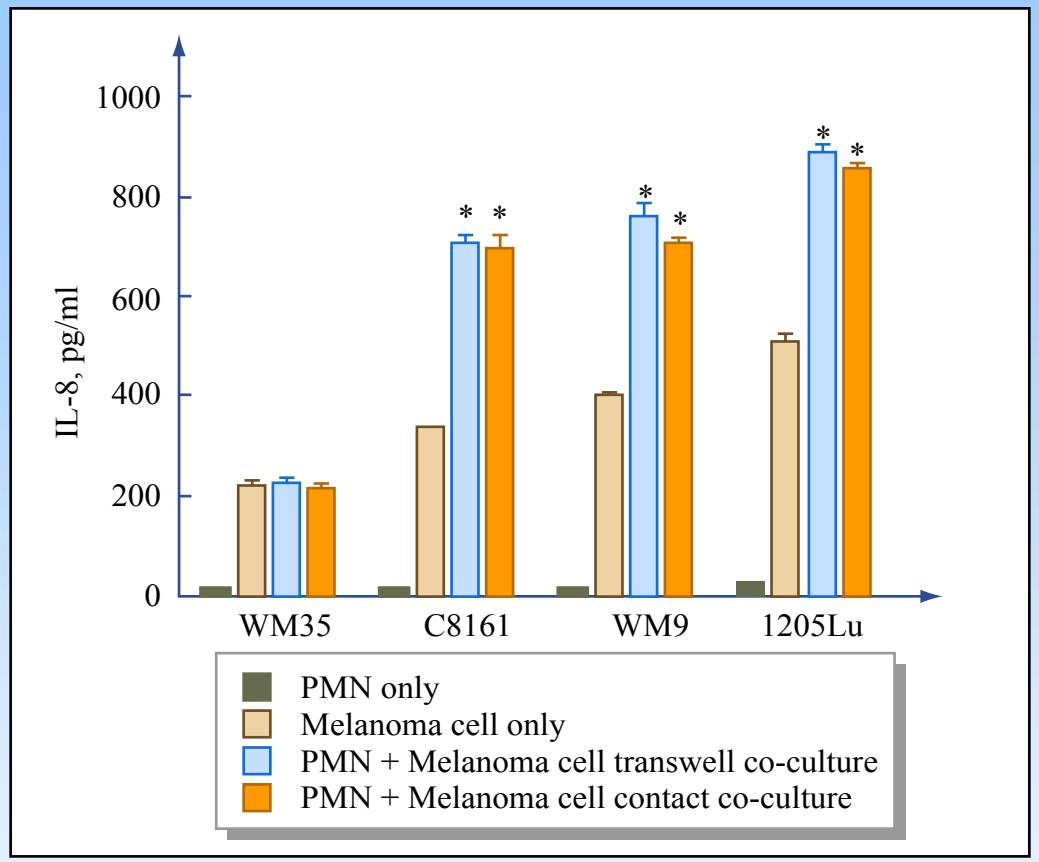
Hypothesis :

Tumor cells modulate protein secretion through activation of transcription factor signaling in PMNs



PMN : polymorphonuclear neutrophils

IL-8 Secretions from PMN, Melanoma Cells and Co-cultures



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Western blot results.

Figure by MIT OpenCourseWare.

- ELISA data
- WM35 : low metastatic potential
- Increased IL-8 in co-cultures of
  C8161 - PMNs
  WM9 - PMNs
  1205Lu - PMNs

- Western blots
- Increased IL-8 from PMNs (Transwell) co-cultured with C8161, WM9, 1205Lu
- Constant IL-8 from melanoma cells

Mac-1 Expression after Melanoma-PMN Co-culture

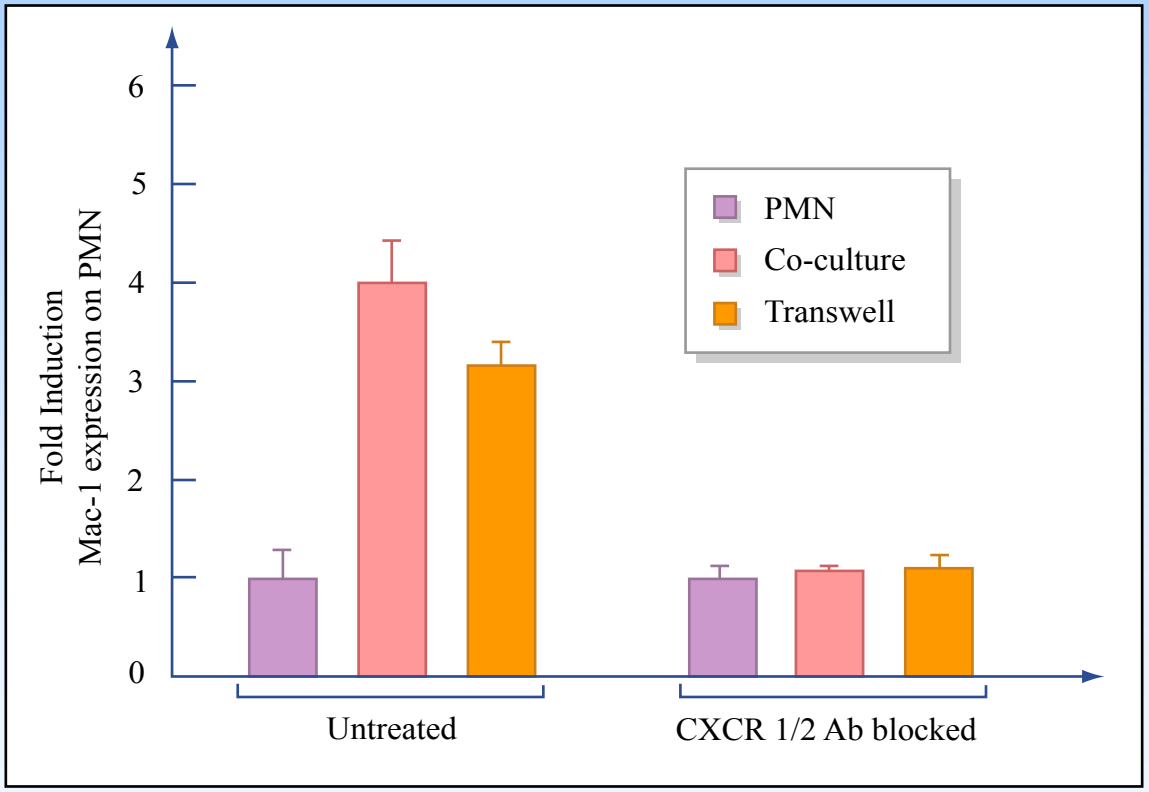

Figure by MIT OpenCourseWare.

Blocking Intercellular IL-8 Signaling Affects PMN-facilitated Melanoma Extravasation

- Melanoma cell extravasation increased in response to IL-8-stimulated PMNs compared with non-stimulated PMN groups.
- Melanoma extravasation decreased significantly in the presence of CXCR1/CXCR2-blocked PMNs or anti-IL-8 neutralizing antibody compared with unstimulated PMNs.

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Graph showing results of treatment with CXCR1/2- and IL-8-blockers.

Inhibiting ^{V600E}B-Raf Reduced Melanoma Cell Extravasation *in vitro*

- **B-Raf** encodes a RAS-regulated kinase that mediates cell growth and malignant transformation kinase pathway activation.
- As the most mutated gene in malignant melanomas, B-Raf has raised questions whether targeting B-Raf might effectively reduce melanoma metastasis.

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- Inhibition of mutant ^{V600E}B-Raf greatly reduced melanoma extravasation compared with non-inhibited melanoma cells (untransfected, nucleofected with buffer only or scrambled siRNA) under both static and dynamic flow conditions.

Inhibiting ^{V600E}B-Raf reduced IL-8 production

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A, Inhibition of mutant ^{V600E}B-Raf significantly reduced the IL-8 production from melanoma cells (1205 Lu and UACC 903M) cultured alone compared with the control melanoma cells (untransfected melanoma and melanoma nucleofected with buffer only or scrambled siRNA).

B, IL-8 production from tumor microenvironment (including both PMN and melanoma cells) increased after PMN co-cultured with control melanoma cells (~1.5 fold), whereas IL-8 production either kept the same or even reduced after PMN co-culture with melanoma cells treated with siRNA against mutant ^{V600E}B-Raf. Values were normalized to the summed background level of PMN and melanoma cell cultured alone.

Mac-1 expression on PMN after co-culture with melanoma cells

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Mac-1 expression on PMN increased significantly after PMN co-cultured with control melanoma cells (untransfected melanoma and melanoma nucleofected with buffer only or scrambled siRNA). However, the co-culture between PMN and melanoma cells treated with siRNA against ^{V600E}B-Raf did not significantly increase Mac-1 expression on PMN.

Disruption of ICAM-1/β_2 integrin binding inhibited melanoma cell extravasation

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A, ICAM-1 expression on melanoma cells (1205 Lu and UACC 903M) was reduced after knockdown of mutant ^{V600E}B-Raf and ICAM-1 using siRNA; ***B***, knockdown of mutant ^{V600E}B-Raf and ICAM-1 inhibited PMN-mediated melanoma extravasation, values are mean $\pm$ SEM;

Inhibiting ^{V600E}B-Raf disrupts NF-κB activity

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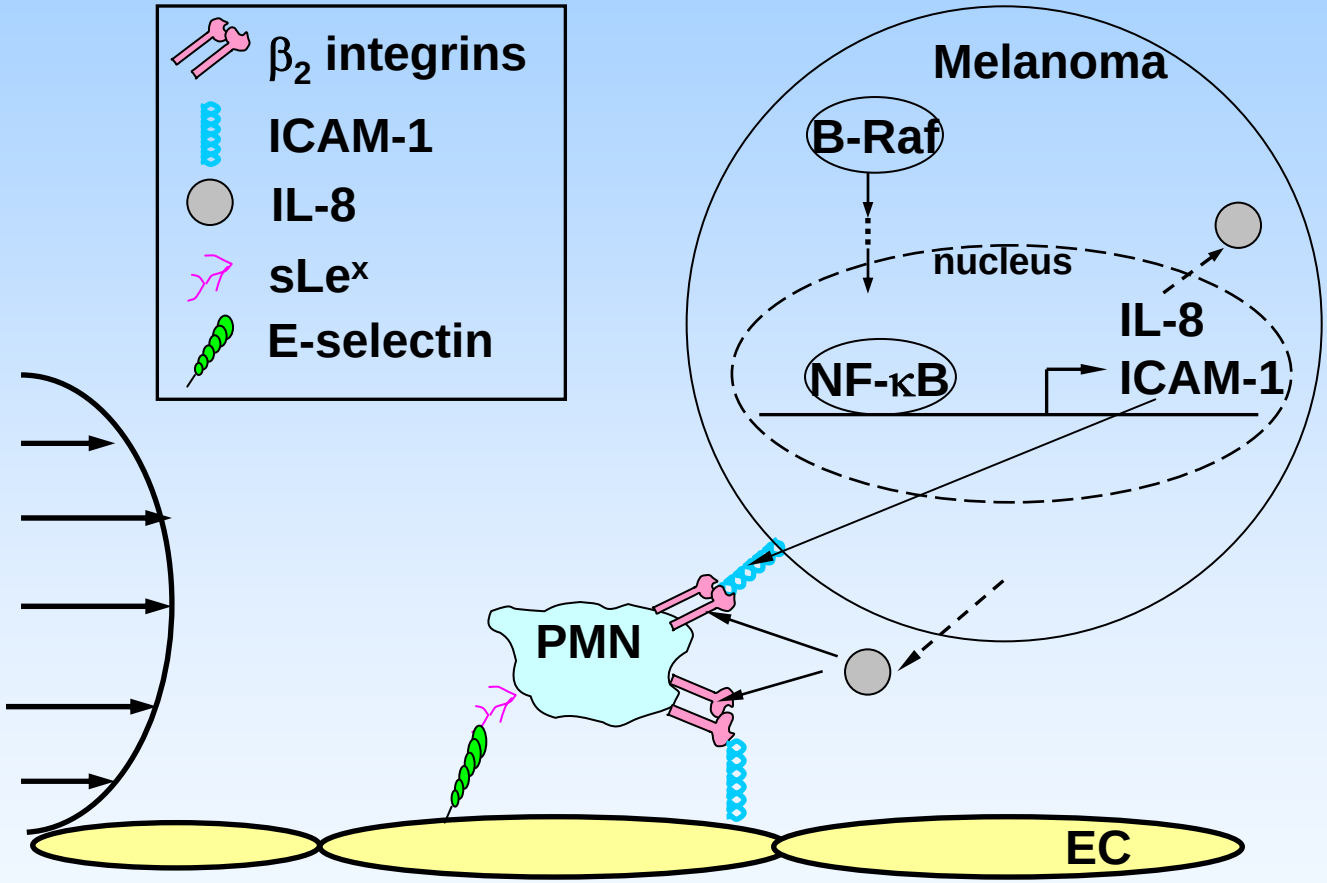
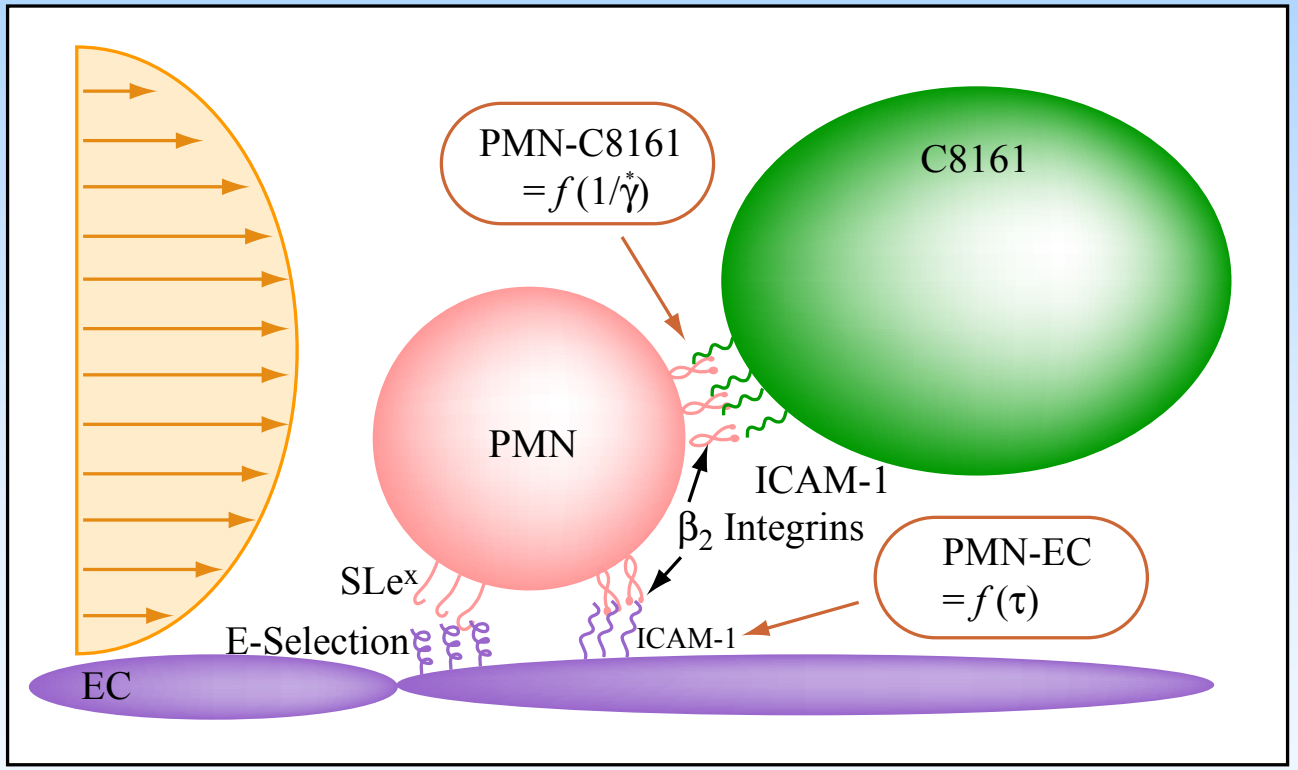
A, NF-κB activity is reduced after inhibition of mutant ^{V600E}B-Raf in 1205 Lu (lane 10) compared with control cases (untransfected 1205 Lu, lane 1; 1205 Lu nucleofected with scrambled siRNA, lane 4 or buffer only, lane 7). The complexes were supershifted by polyclonal antibodies against p50 (lanes 2, 5, 8 and 11) and p65 (lanes 3, 6, 9 and 12). ***B***, PDTC treatment reduced IL-8 secretion and ICAM-1 expression on melanoma cells. 1205 Lu cells were treated with PDTC (100μM) for 1hr. After twice washing, 1205 Lu cells were cultured using fresh culture media. Left panel: supernatant after 4h was collected and IL-8 secretion was detected by ELISA; right panel: ICAM-1 expression on 1205 Lu was examined by flow cytometry.

Mechanism of inhibiting ^{V600E}B-Raf to disrupt melanoma extravasation



PMN-facilitated Melanoma Extravasation



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Parallel-Plate Flow Assay

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Please see: http://www.biomedcentral.com/content/figures/1471-2172-2-9-1.jpg.

FMLP-stimulated neutrophils were injected together with tumor cells at a very low flow rate (0.004 ml/min) for 2 min to accumulate cells within the flow chamber. After the initial accumulation, a step increase in flow rate was applied to the cells.

BMC Immunology. 2:9, 2001.

TC-PMN Collision and Aggregation Near the Endothelium in a Shear Flow

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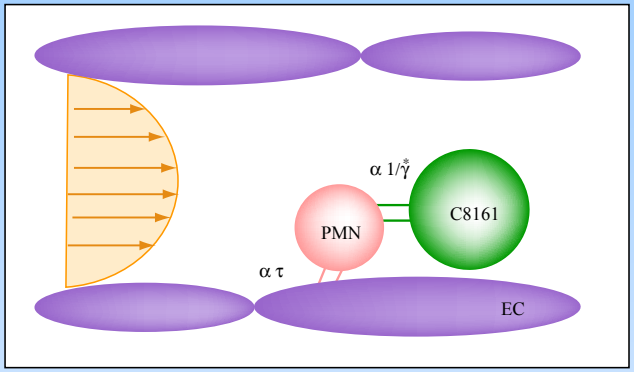
The following parameters were measured and found to be influenced by shear conditions:

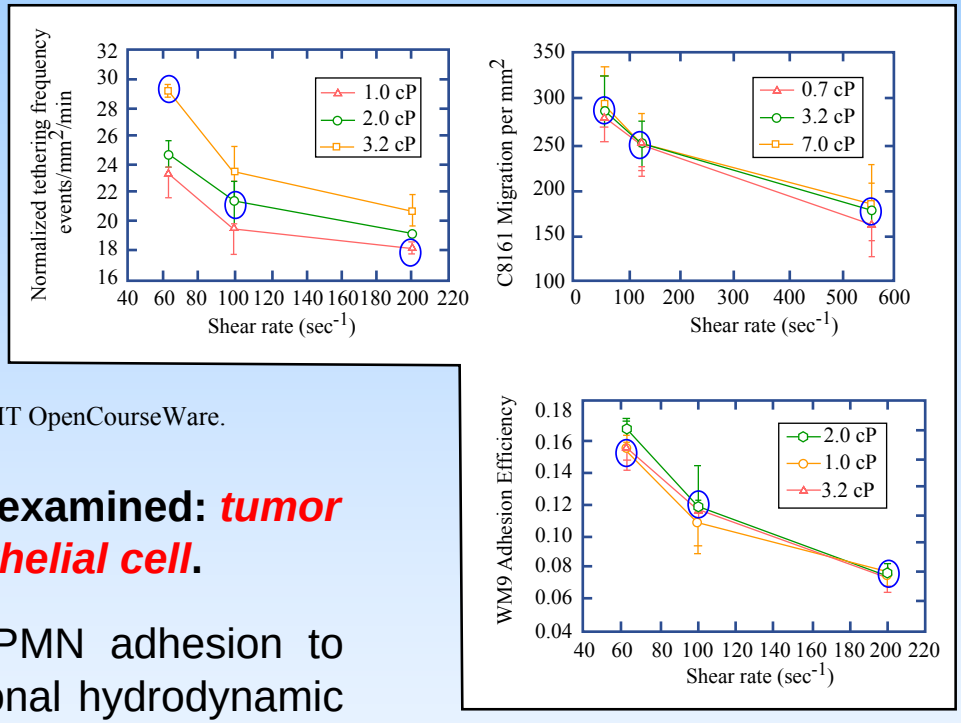
- the number of TC-PMN collisions;
- the number of TCs captured by PMNs; and
- the number of TCs adhered to ECs as a result of TC-PMN collision/aggregation.

$$\text{Adhesion Efficiency} = \frac{\text{Number of TC adhered to EC monolayer as a result of collisions}}{\text{Total number of collisions}}$$

Two Different Types of Cell Aggregation Are Examined:
Tumor Cell to PMN & PMN to Endothelial Cell



$$\tau = \mu\dot{\gamma}$$



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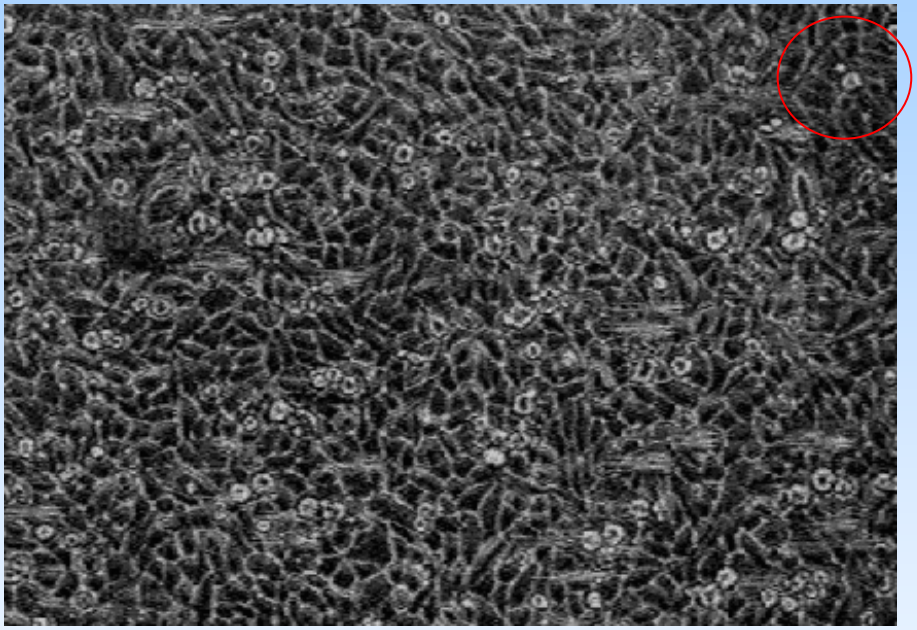
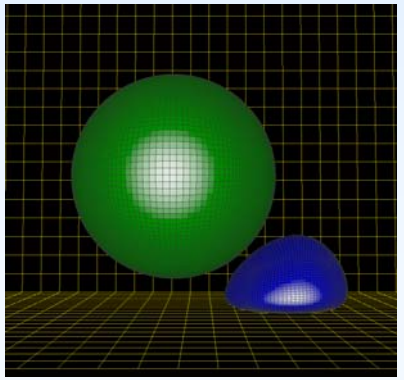
Two different cell aggregations are examined: *tumor cell to PMN* and *PMN to endothelial cell*.

- PMN tethering data indicates that PMN adhesion to endothelial cells follows a more traditional hydrodynamic relationship and is proportional to shear stress and contact duration.
- The adhesion and migration data reveal that tumor cell to PMN adhesion varies inversely to shear rate and is dependant on contact duration while independent of shear stress.

Effects of Shear Rate and Shear Stress on PMN-Melanoma Interactions

- Experimental and Computational Approach
  - Focus on interactions between PMN and melanoma cell
  - Model second step: melanoma cell colliding and adhering to a tethered PMN on EC by capturing:
    - Deformation
    - Collision
    - Adhesion kinetics





Side-View PIV System

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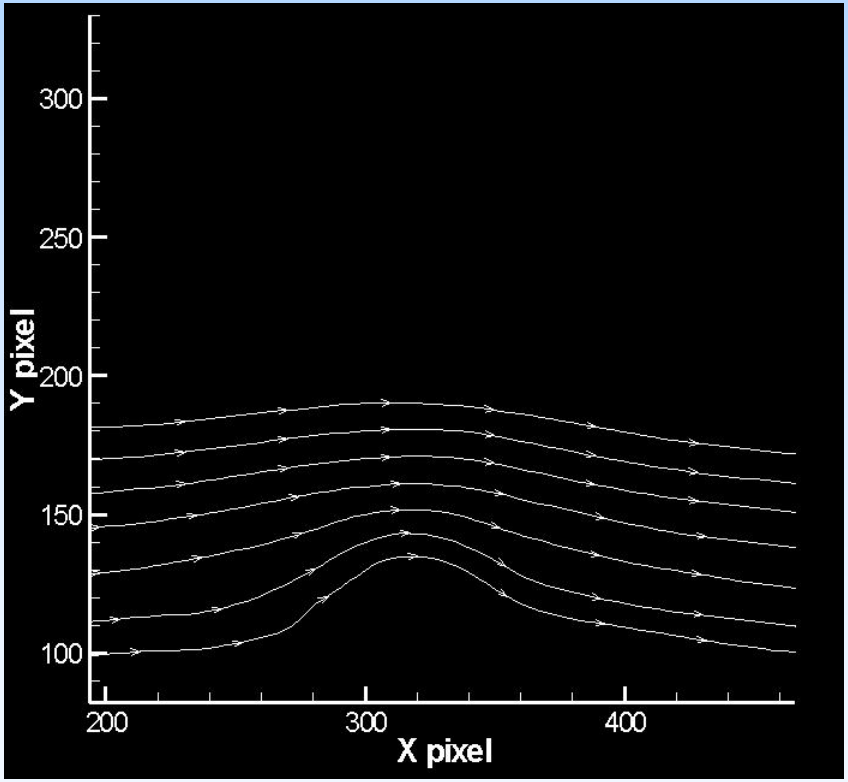
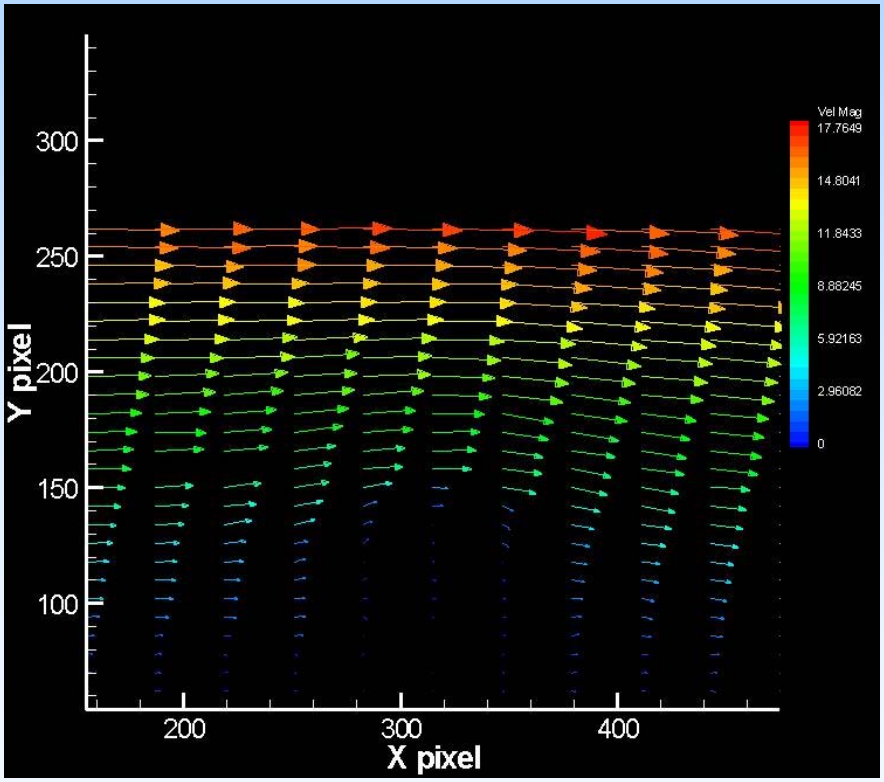
Velocity Profiles

- Interrogation windows:

  30 x 20 pixels (W x H), 0.23 μm/pixel

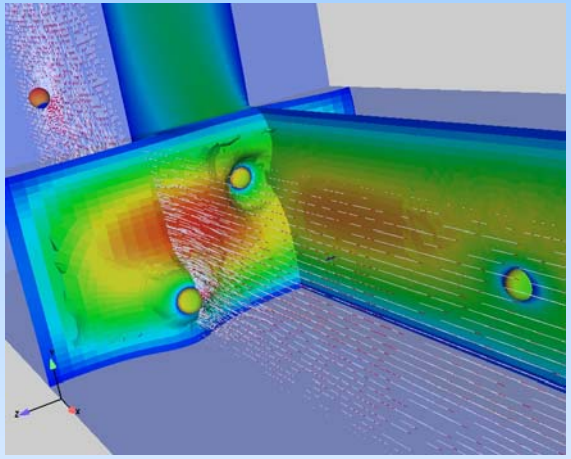


Stream lines over an adhered 16-μm bead near the microslide wall.

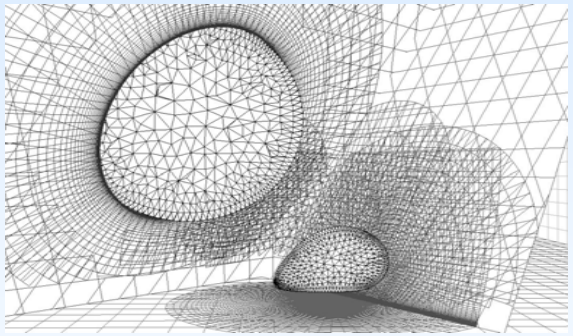


Velocity vectors over an adhered 16-μm bead near the microslide wall.

Computational Fluid Dynamics and Statistic Population Balance Model



Cell population studies



CFD studies

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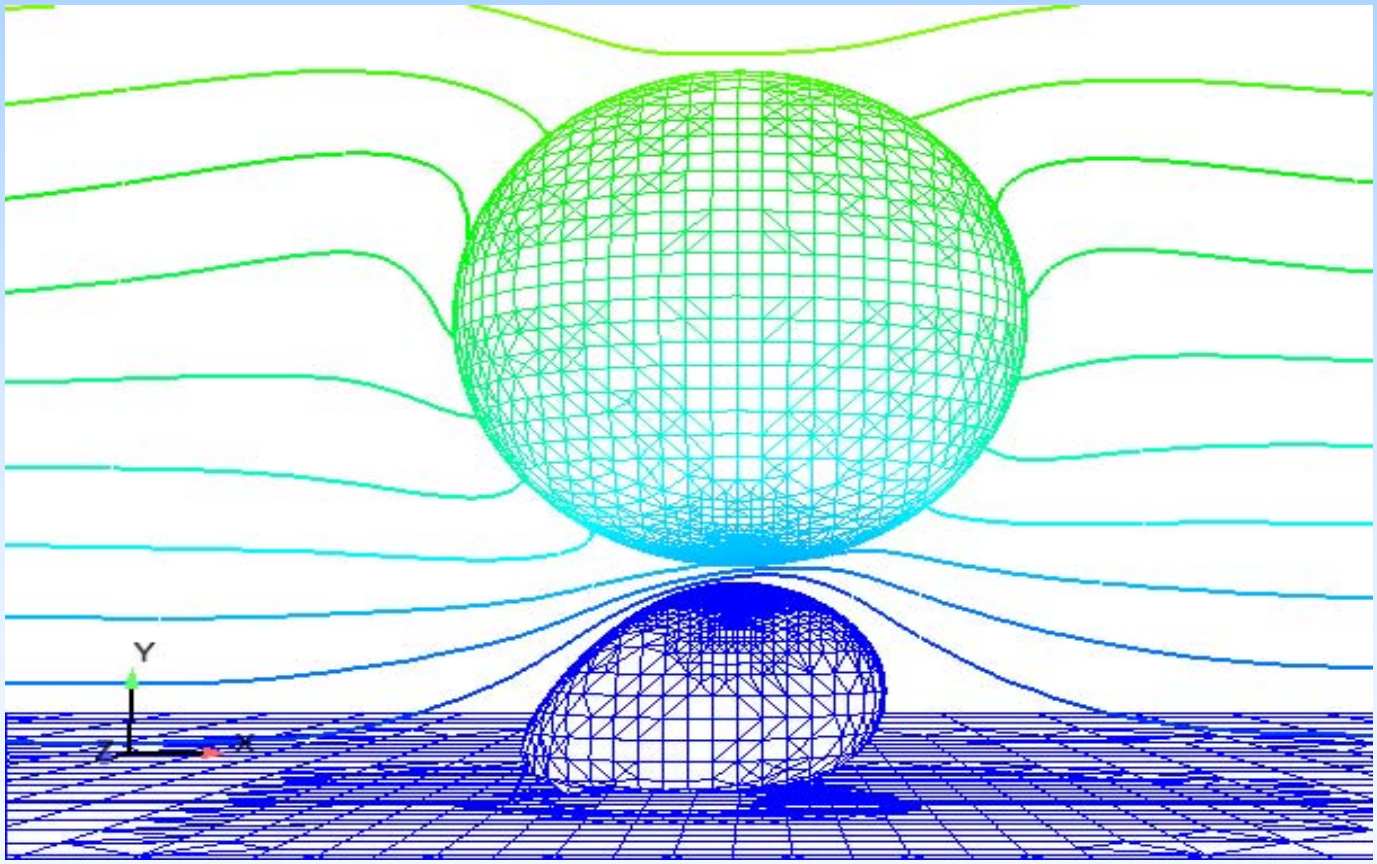
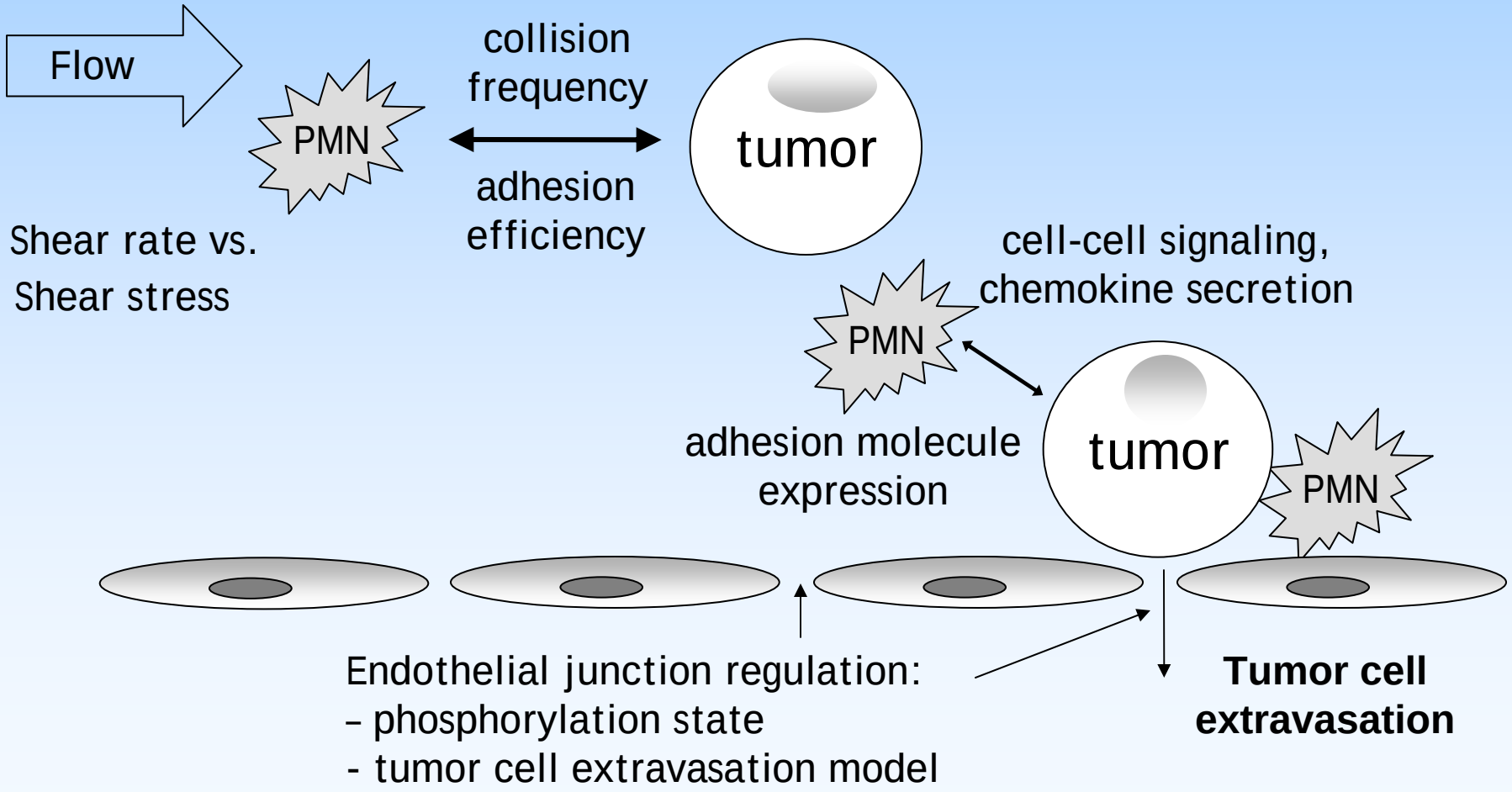
Micro-PIV experimental studies

Simulation of a melanoma cell binding to a PMN



Cancer Immunoediting in Leukocyte-mediated Melanoma Extravasation



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