Adriana Albini, PhD
Irccs MultiMedica – Milano (Italy),
**Immunoprevention: The tumor microenvironment as target**
Several studies have highlighted that immune cell activity is shaped by the tumor microenvironment (TME), resulting in the acquisition of pro-tumor and pro-angiogenic phenotype and function. In this scenario, the possibility to prevent the phenotype and functional alterations occurring within the TME that compromise immune tumor cell killing activities are urgently needed. Immunoprevention refers a new approach to cancer prevention, based on the stimulation of the immune system, even before tumor onset. Although cancer vaccines are considered an approach to cancer immunoprevention, increasing attention has been addressed to the identification and validation of diverse molecules that, along with cytokines or chemo/radio therapy, can enhance immune cell efficiency to kill cancer cells.

Several targets for immunoprevention within the TME have been proposed. IL-6-gp130-STAT3 axis has been reported to be fundamental for the orchestration of the inflammatory response in cancer. TH17 cells are increased in the TME during tumor development and several manuscripts have reported the role of IL-17 in promoting tumorigenesis. Programmed cell death-1 (PD-1), a CD28 family receptor, has a key role in tumor tolerance. Clinical studies have demonstrated significant clinical activity of monoclonal antibodies toward CTLA-4, PD-L1 and PD-1 in patients with advanced solid tumors. Given the success of these agents in melanoma and non-small cell lung cancer, immunoprevention targeting PD-1/PD-L1 might be of high relevance in patients with preneoplastic lesions associated with disease. However, use of these agents has been associated with induction of autoimmune disease.

Finally the angiogenic switch occurring in tumor-infiltrating and tumor-associated inflammatory cells (tumor associated macrophages-TAM, tumor associated neutrophils-TAN, and the recently discovered tumor infiltrating/associated NKs-TINK/TANKs) also represent a crucial issue in cancer biology. In addition to targeted therapies, diverse preventive diet-derived agents, including many phytochemicals (resveratrol, green tea catechins, quercetin, curcumin) and their derivatives, non-steroidal anti-inflammatory drugs (NSAIDs, eg. Aspirin) and biguanides (metformin, phenformin), have been shown to inhibit inflammation and angiogenesis (angioprevention) as well as the NFκB, Akt and STAT3 pathways and activate AMP-activated protein kinase (AMPK). Phytochemicals have been shown to efficiently target several components of the TME, including inflammatory cells (neutrophils, Natural Killer cells), and interfere with the acquisition of pro-tumor/pro-angiogenic features. These compounds show very low or no toxicity and can therefore be administered to phenotypically healthy individuals.

All together, in accordance with the success of clinical studies employing immune therapeutic interventions, these advances clearly suggest that cancer immunoprevention is ready to progress into the clinical care even for patients with relatively low risk for cancer.
MODALITIES OF CANCER PREVENTION AND THERAPY

REVIEW
Emerging strategies for cancer immunoprevention
JC Rowe1, SD Leach1 and FM Allsopp1

The crucial role of the immune system in the formation and progression of tumors has been widely accepted. On one hand, the surveillance role of the immune system plays an important role in endogenous tumor prevention, but on the other hand, in some special circumstances such as in chronic inflammation, the immune system can actually contribute to the formation and progression of tumors. In recent years, there has been an explosion of novel targeted immunotherapies for advanced cancers. In this present manuscript, we explore known and potential various types of cancer prevention strategies and focus on nonvaccine-based cancer preventive strategies targeting the immune system at the early stages of tumorgenesis.

Oncogene advance online publication, 14 September 2015; doi:10.1038/onc.2015.99
Tumor Progression

Transformation → Tumor cell Proliferation → Quiescent Hyperplastic foci (proliferation = death)

Host interactions (angiogenesis, stromal support, inflammation)

Lymphatic Metastases → Local Invasion → Extravasation → Hematic metastases
Appearance of endothelial surface viewed by scanning EM

VEGF-VEGF receptor inhibition for antiangiogenesis

Anti-VEGF antibodies

Soluble VEGF receptors

Aptamers

Anti-VEGF-1 antibodies

VEGFR-1

VEGFR-2

Endothelial cell

Small-molecule VEGFR TK inhibitors

Anti-VEGFR-2 antibodies

INSIGHT REVIEW NATURE 438: 967
Napoleone Ferrara & Robert S. Kerbel
The tumor microenvironment

Johanna A. Joyce & Jeffrey W. Pollard
Nature Reviews Cancer 9, 239-252 (April 2009)
doi:10.1038/nrc2618
Chemokines and other inflammatory agents induce neutrophil dependent angiogenesis in matrigel sponges

Inflammatory chemokines induce invasion of neutrophils and macrophages followed by endothelial cells. No angiogenesis by these agents occurs in neutropenic mice

Albini A, Tumor inflammatory angiogenesis and its chemoprevention. Cancer Res. 65:10637-41; 2005
Inflammation and Cancer - Angiogenesis and Inflammation


Rudolf Virchow
In inflammation and in tumor progression similar cellular activations occur in the microenvironment.
Phenotype Switching in Tumor Angiogenesis

Polarization of native immunity cells


Noonan, Albini et al, Cancer Metastasis Reviews
The intricate interplay between the innate and adaptive immune systems

2011 Nobel for Medicine
- Ralf M. Steinman
- Bruce A. Beutler
- Jules A. Hoffman

Adaptive Immunity
- Th1 cell
- Th2 cell
- Tcyto cell
- B cell

Innate Immunity
- M1/N1 <-> Polarization -> M2/N2
- Macrophage
- Neutrophil
- Dendritic cell
- NK cell
- Mast cell
The tumor microenvironment

What about NK cells?
NK cells were first described in the early 1970’s by R. Herberman and R. Kiessling. Later Reinold et al, (1981) termed them Large Granular Lymphocytes (LGLs)

They make up part of the Innate immunity

While they are lymphocytes, they are not T cells (they have no TCR)

NK cells show toxicity toward virus-infected and tumor cells yet do not require sensitization to antigens

They are 5-10% of total peripheral blood lymphocytes, 1-2% splenic lymphocytes

Regulated by a balance of inhibitory receptors (KIRs) specific for MHC class I antigens and activating signals (KARs)

Killer function exerted by cytotoxicity (GRANZIME and PERFORIN) or cytokine production
Emerging role for NKs as regulatory cells

Nature Immunology 9, 503 - 510 (2008)
Natural Killer cells (NKs) subsets

CD45+ CD3-

CD56\textsuperscript{dim} CD16+

Adapted from: Fehniger et al, Cytokine Growth Factor Rev. 2002;13(2):169-83

CD56\textsuperscript{bright} CD16-


Adapted from: Vacca et al, Trends in Immunology 2011; 32:517-23

CD56\textsuperscript{superbright} CD16-

Adapted from: Fehniger et al, Cytokine Growth Factor Rev. 2002;13(2):169-83
Killers become builders during pregnancy

Philippe Le Bouteiller & Julie Tabiasco

Circulating natural killer cells might be best known for their ability to disable and maintain target cells. But in the pregnant uterus of humans these cells seem to have a positive effect, regulating placental development and angiogenesis (pages 1065–1074).

Figure 1 Human decidual NK cells in the early weeks of pregnancy promote trophoblast cell invasion and vascular growth; figure not to scale. (a) The human placenta is made of a villous tree (fetal part) floating in maternal blood supplied by spiral arteries or anchoring to the pregnant uterus (decidua). Fetal trophoblast cells located at the tips of anchoring villi stream into the decidua, where they encounter various types of maternal cells (fetal-maternal interface). (b) Hanna et al. provide evidence that decidual NK cells release IL-8 and IP-10 chemokines that bind to their specific receptors expressed by trophoblast cells, promoting invasion toward the spiral arteries. They also show that decidual NK cells produce proangiogenic factors (VEGF and PIGF) inducing vascular growth (endothelial cell proliferation), thus allowing the establishment of the placental circulation. Such decidual NK cell secretions are likely to be triggered by the activating Nkp44 and Nkp46 receptors upon interaction with their specific ligands (as yet unidentified). These ligands are expressed by maternal stromal cells (both ligands) and trophoblast cells (Nkp44 ligand).

dNK cells produce high levels of VEGF, PIGF, IL-8 and are involved in spiral artery development
NSCLC-infiltrating NK cells: phenotypic distribution

The CD56$^{\text{bright}}$CD16$^{-}$ subset predominates in the tumor samples, $P<0.0001$.

The CD56$^{\text{dim}}$CD16$^{+}$ predominates in blood and tissues from both NSCLC patients and controls that did not have oncologic disease.

Bruno et al, Neoplasia, 2013
NK cells from squamous cell NSCLC patients show significantly higher levels of VEGF and PLGF production.
Induction of endothelial morphogenesis and chemotaxis by tumor-derived NK cells

Bruno et al, Neoplasia, 2013
TGFβ1 is involved in tumor infiltrating NKs polarization towards a proangiogenic subset

The polarization of immune cells in the tumour environment by TGFβ

Richard A. Flavell*, Shomyesh Sanjabi**, Stephen H. Wrzesinski*** and Paula Licona-Limón*
Anti-angiogenesis in multistage tumorigenesis
Some Chemoprevention trials

- NSAID-Cox2 inhibitors (?)
- Various retinoids
- Fenretinide*
- Lycopene
- SERM*/Aromatase Inhib
- Finasteride
- Curcumin
- Isoflavones: genisteine
- Isoflavones: green tea
- Metformin

- Colon - (Others?)
- Lung, Head and Neck
- Breast
- Prostate
- Breast
- Prostate
- Colon
- various
- Prostate
- Breast
Effect of Celecoxib on Cardiovascular Events in two trials for the prevention of colorectal adenomas

<table>
<thead>
<tr>
<th></th>
<th>APC, n (%)</th>
<th></th>
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<th>PreSAP, n (%)</th>
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</thead>
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<tr>
<td></td>
<td>Placebo</td>
<td>200 mg BID</td>
<td>400 mg BID</td>
<td>Placebo</td>
</tr>
<tr>
<td></td>
<td>(n=679)</td>
<td>(n=685)</td>
<td>(n=671)</td>
<td>(n=628)</td>
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<tr>
<td>Total cardiovascular deaths</td>
<td>1 (0.1)</td>
<td>5 (0.7)</td>
<td>6 (0.9)</td>
<td>4 (0.6)</td>
</tr>
<tr>
<td>Total noncardiovascular deaths</td>
<td>5 (0.7)</td>
<td>3 (0.4)</td>
<td>3 (0.4)</td>
<td>3 (0.5)</td>
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<tr>
<td>Total deaths</td>
<td>6 (0.9)</td>
<td>8 (1.2)</td>
<td>9 (1.3)</td>
<td>7 (1.1)</td>
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<tr>
<td>Fatal and nonfatal myocardial infarction</td>
<td>3 (0.4)</td>
<td>12 (1.8)</td>
<td>10 (1.5)</td>
<td>4 (0.6)</td>
</tr>
<tr>
<td>Fatal and nonfatal stroke</td>
<td>3 (0.4)</td>
<td>3 (0.4)</td>
<td>6 (0.9)</td>
<td>7 (1.1)</td>
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<tr>
<td>Nonfatal events</td>
<td></td>
<td></td>
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<tr>
<td>Myocardial infarction</td>
<td>3 (0.4)</td>
<td>9 (1.3)</td>
<td>9 (1.3)</td>
<td>4 (0.6)</td>
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<tr>
<td>Stroke</td>
<td>3 (0.4)</td>
<td>3 (0.4)</td>
<td>5 (0.7)</td>
<td>5 (0.8)</td>
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<tr>
<td>Heart failure</td>
<td>2 (0.3)</td>
<td>1 (0.1)</td>
<td>4 (0.6)</td>
<td>0</td>
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<tr>
<td>Resuscitated sudden death</td>
<td>0</td>
<td>0</td>
<td>1 (0.1)</td>
<td>0</td>
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<tr>
<td>Thromboembolic event</td>
<td>1 (0.1)</td>
<td>3 (0.4)</td>
<td>4 (0.6)</td>
<td>1 (0.2)</td>
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<td>Hospitalization for unstable angina</td>
<td>5 (0.7)</td>
<td>6 (0.9)</td>
<td>2 (0.3)</td>
<td>5 (0.8)</td>
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<tr>
<td>Arrhythmia</td>
<td>9 (1.3)</td>
<td>4 (0.6)</td>
<td>7 (1.0)</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>Cardiovascular procedure</td>
<td>7 (1.0)</td>
<td>10 (1.5)</td>
<td>7 (1.0)</td>
<td>2 (0.3)</td>
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<tr>
<td>Other cardiovascular</td>
<td>9 (1.3)</td>
<td>11 (1.6)</td>
<td>14 (2.1)</td>
<td>5 (0.8)</td>
</tr>
</tbody>
</table>

Green Tea? Yes please!

The green tea flavonoid EGCG inhibits MMP activity Garbisa... *Albini*, Nature Medicine, 5:1216, 1999.

Genes regulated by NAC and EGCG

Green tea epigallocatechin-gallate

<table>
<thead>
<tr>
<th>EGCG and NAC Responsive Angiogenesis Related Genes</th>
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<td>Probe set</td>
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<td>39790_at</td>
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<td>1211_s_at</td>
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<td>37844_at</td>
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<tr>
<td>39734_at</td>
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<tr>
<td>35414_s_at</td>
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</table>

Correlations

EGCG

NAC

NF-κB
ANGIOPREVENTION: Chemoprevention of angiogenesis

Quiescent Tumor (proliferation=death)

Angiogenic Switch

Inflammation

Normal Cell
Tumor Cell
Apoptotic Cell

PROGRESSION

Local Invasion

Metastatic Lymphatic Colonization

Vascular Metastatic Dissemination

Tosetti F, Ferrari N, De Flora S, Albini A. Angioprevention': angiogenesis is a common and key target for cancer chemopreventive agents. Faseb J. 16:2-14.; 2002
Inhibition of NF-κB Translocation by Beer hop isoflavone Xantohumol

Xantohumol from bier hop: A new “angiopreventive” agent
Xanthohumol inhibits angiogenesis in matrigel pellets in vivo

Xanthohumol is a chalcone found in beer hops; it has been shown to be a candidate chemopreventive agent and to have anti-angiogenic properties.
The triterpenoid CDDO-Me inhibits angiogenesis

Vannini et al, MCT
Chemopreventive agents that possess antiangiogenic properties

- Alpha-difluoromethyl-ornithine (DFMO)
- Aspirin
- Brassinin
- Celecoxib
- Curcumin
- 1α,25-dihydroxyvitamin D3
- Ellagic acid
- Epigallocatechin 3-gallate
- Finisteride
- Genistein
- N-acetylcysteine (NAC)
- Naringenin
- Oltipraz
- Resveratrol
- Retinoids
- Selenium
- Silymarin
- Statins
- Statins
- Sulindac
- Tamoxifen

Li, WW et al, Tumor angiogenesis as a target for dietary cancer prevention J Oncol. 2012; 2012: 879623
Selected Key Signaling Pathways that are Targets for Angioprevention

Clinical Trials Of Chemoprevention With Anti-Angiogenic Molecules

### Table 1: Clinical trials of chemoprevention with antiangiogenic principles *

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular target or mechanism</th>
<th>Condition (ClinicalTrials.gov Identifier)</th>
<th>Phase</th>
<th>Study type or design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>VEGF, mTOR, AMPK, MMP-2, MMP-9, VEGF RE, Met, Stat3, NF-kB</td>
<td>Barrett’s metaplasia (NCT01447927)</td>
<td>I, II</td>
<td>R, DB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colorectal adenoma or high BMI (NCT01312467)</td>
<td>II</td>
<td>R, DB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prostate cancer (NCT01439913)</td>
<td>II</td>
<td>R, DB</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>PPARγ agonist, bFGF, VEGF, IL-8, CXCL5, CXCL1</td>
<td>Lung cancer (NCT00780234)</td>
<td>II</td>
<td>I, R, DB</td>
</tr>
<tr>
<td>Aspirin and tea polyphenols</td>
<td>COX inhibitor, VEGF, COX-independent</td>
<td>Oesophagel squamous cell carcinoma (NCT01490521)</td>
<td>III</td>
<td>I, R, DB</td>
</tr>
<tr>
<td>Aspirin and esomeprazole</td>
<td>COX inhibitor, VEGF, COX-independent</td>
<td>Barrett’s metaplasia (NCT00357682)</td>
<td>III</td>
<td>I, R, DB</td>
</tr>
<tr>
<td>Aspirin and efomithine</td>
<td>COX inhibitor, VEGF, COX-independent</td>
<td>Familial adenomatous polyposis (NCT00983580)</td>
<td>II</td>
<td>I, R, DB</td>
</tr>
<tr>
<td>Sulindac*</td>
<td>COX-2 inhibitor</td>
<td>Oral premalignant lesions (NCT00299195)</td>
<td>P</td>
<td>I, R, DB</td>
</tr>
<tr>
<td>Sulindac and efomithine</td>
<td>COX-2 inhibitor</td>
<td>Colorectal premalignant or nonmalignant lesions (NCT00118365)</td>
<td>III</td>
<td>I, R, DB</td>
</tr>
<tr>
<td>Celecoxib*</td>
<td>COX-2 inhibitor</td>
<td>Cervical intraepithelial neoplasia (NCT00081263)</td>
<td>II</td>
<td>I, R, DB</td>
</tr>
</tbody>
</table>

### Phytochemicals and derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular target or mechanism</th>
<th>Condition (ClinicalTrials.gov Identifier)</th>
<th>Phase</th>
<th>Study type or design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>NF-κB, VEGF, mTOR, miR-21, miR-17-92, miR-15a, miR-16a</td>
<td>Familial adenomatous polyposis (NCT00927485)</td>
<td>–</td>
<td>I, R, DB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colorectal cancer (NCT01331517)</td>
<td>I</td>
<td>I, OL, SG, Ph</td>
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<tr>
<td>Polyphenon E</td>
<td>MMP3, STAT3, VEGF, miR-34a, miR-125b</td>
<td>Prostate intraepithelial neoplasia (NCT00596011)</td>
<td>II</td>
<td>I, R, DB</td>
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<tr>
<td></td>
<td></td>
<td>Duodenal carcinoma in situ (NCT01063485)</td>
<td>P</td>
<td>I, R, SG, OL</td>
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<td></td>
<td></td>
<td>Bronchial dysplasia (NCT00611650)</td>
<td>II</td>
<td>I, R, DB</td>
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<tr>
<td></td>
<td></td>
<td>Cervical intraepithelial neoplasia (NCT00308323)</td>
<td>II</td>
<td>I, R, DB</td>
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<tr>
<td>Resveratrol</td>
<td>NF-κB, IKK α/β, EGF, VEGF</td>
<td>Healthy adult smokers (NCT01492114)</td>
<td>III</td>
<td>I, R, DB, CS</td>
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<tr>
<td></td>
<td>VEGF</td>
<td>Impaired glucose tolerance (NCT01375959)</td>
<td>P</td>
<td>R, DB, CS</td>
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<tr>
<td>Genistein</td>
<td>NF-κB, AP-1, p53, FAK, VEGF, HIF-1α, PTEN</td>
<td>Type II diabetes mellitus (NCT00951912)</td>
<td>–</td>
<td>I, R, DB</td>
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<tr>
<td></td>
<td>VEGF</td>
<td>High risk for breast cancer (NCT00290768)</td>
<td>–</td>
<td>IIb</td>
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<tr>
<td>Genistein and vitamin D</td>
<td>NF-κB, AP-1, p53, FAK, VEGF, HIF-1α, PTEN</td>
<td>Early stage prostate cancer (NCT01325311)</td>
<td>II</td>
<td>I, R, DB</td>
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<tr>
<td>Black raspberry</td>
<td>COX-2, NOGS</td>
<td>Head and neck cancer (NCT01469429)</td>
<td>I-II</td>
<td>I, R, OL</td>
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<tr>
<td>Sulfurophane</td>
<td>HDAC, NF-κB, VEGF, HIF-1α, MMP-2, MMP-9</td>
<td>Prostate cancer (NCT01265958)</td>
<td>–</td>
<td>I, R, DB</td>
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<tr>
<td></td>
<td>VEGF, HIF-1α, MMP-2, MMP-9</td>
<td>Prostate cancer (NCT00946309)</td>
<td>I-II</td>
<td>I, R, DB</td>
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### Other compounds

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<th>Study type or design</th>
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<tr>
<td>Propranolol</td>
<td>VEGF, MMP-2, MMP-9, NF-κB</td>
<td>Infantile haemangiomata (NCT01074437)</td>
<td>II</td>
<td>I, R, DB</td>
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<tr>
<td>Imiquimod</td>
<td>MMP-9, interferons</td>
<td>Lentigo maligna (NCT01088737)</td>
<td>II-III</td>
<td>I, NR, SG, OL</td>
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<td>Everolimus</td>
<td>mTOR</td>
<td>Skin cancer (NCT00709188)</td>
<td>III</td>
<td>I, R, SG, OL</td>
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<td>Farneside</td>
<td>IGF-1, IGF-2, IGF-binding protein 3-9,</td>
<td>High risk for breast cancer (NCT01479192)</td>
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<td>I, R, DB</td>
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<tr>
<td>Tamoxifen</td>
<td>Oestrogen receptors, angiogenes, VEGF, endostatin</td>
<td>Breast cancer (NCT01357772)</td>
<td>III</td>
<td>I, R, DB</td>
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</tbody>
</table>

The Four Levels of Angioprevention

PROBLEMS ....
from angiogenesis and the microenvironment in therapy

Tumor Heterogeneity
Resistance to anti-angiogenic therapy


Bevacizumab failed adjuvant trials in colon cancer

Hypoxia as angiogenesis stimulator
Escape pathways-redundancy
Cancer stem cells- endotelial cell precursors
A way to resist
Toxicity
Lack of biomarkers
NEW ROLES FOR CHEMOPREVENTIVE DRUGS

Shusuke Toden, Yoshinaga Okugawa, Thomas Jascur, Dominik Wodarz, Natalia L. Komarova, Constanze Buhrmann, Mehdi Shakibaei, C. Richard Boland, and Ajay Goel

Curcumin mediates chemosensitization to 5-fluorouracil through miRNA-induced suppression of epithelial-to-mesenchymal transition in chemoresistant colorectal cancer

Summary Curcumin appears to sensitize chemotherapy drugs to cancer, but the mechanism is unclear.
We demonstrated in 5FU resistant colorectal cell lines that curcumin attenuates drug resistance through inhibition of EMT via upregulation of EMT suppressive miRNAs.
Phytochemicals ability to re-polarize tumor pro-angiogenic NKs

Figure 2: Exposure of NK cells to TGFβ leads to a reduction of perforin content and an increase in VEGF production. Preliminary data suggest that phytochemicals (triterpenoids) are able to counteract these effects.
Targeting metabolism

CELL BIOLOGY

Ancient Sensor for Ancient Drug
Reuben J. Shaw¹ and Lewis C. Cantley²
A common drug has an unexpected effect on a metabolic enzyme that stimulates fat utilization.

Simon A. Hawley et al.
The Ancient Drug Salicylate Directly Activates AMP-Activated Protein Kinase
Science 336, 918 (2012)

Targeting a metabolic sensor. Many compounds that activate AMPK were first identified as active components of herbal medicines. Several (purple) probably act on mitochondria to increase concentrations of AMP and ADP, which bind the γ subunit. The small molecule A769662 and salicylate (blue), the active derivative of aspirin, activate AMPK through a mechanism involving the AMPK β subunit.
LKB1-AMPK-mTOR

†Translation, †Cell growth, †Ribosome biogenesis, †Metabolism, †Proliferation, ▼Autophagy
A decreased risk of breast cancer was observed in female patients with type 2 diabetes using metformin on a long-term basis.  

Epidemiological studies have confirmed that metformin, but not other anti-diabetic drugs, significantly reduces cancer incidence and improves cancer patients’ survival in type 2 diabetics.

Bodmer M et al, Diabetes Care 2010
Evans JM et al. BMJ 2005
Landman GW et al. Diabetes Care 2010
Metformin inhibits angiogenesis in vivo
Inhibition of network formation by metformin is AMPK dependent

Knock-down of AMPKα1 by siRNA

- AMPK α1
- β-act
- AMPK α1/β-act

Master Segment Length

- Metf 0 0 1 1 10 10 50 50
- Metf siRNA c α1 c α1 c α1 c α1

Segments

- Metf 0 0 1 1 10 10 50 50
- Metf siRNA c α1 c α1 c α1 c α1
Metformin modulates several angiogenesis associated genes

Microarray data:

VEGFA
PTGS2
F3
ERG
FLT1
SAT1
PRKX1
CYPIB1
WARS
ADAMTS1
CXCR4
CCL2
Metformin modulates several angiogenesis associated genes

qRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ctrl</th>
<th>6h</th>
<th>24h</th>
</tr>
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<tbody>
<tr>
<td>VEGFA</td>
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<td>PTGS2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1B1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>β-act</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXC2 (PTGS2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td></td>
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<td>ADAMTS1</td>
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<td>CCL2</td>
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</table>

Protein (WB)

<table>
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<tr>
<th>Protein</th>
<th>Ctrl</th>
<th>6h</th>
<th>24h</th>
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<tbody>
<tr>
<td>CXCR4</td>
<td>-</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>-</td>
<td>-</td>
<td>Down</td>
</tr>
<tr>
<td>CXC2 (PTGS2)</td>
<td>-</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>VEGF</td>
<td>-</td>
<td>-</td>
<td>Up</td>
</tr>
<tr>
<td>ADAMTS1</td>
<td>-</td>
<td>Up</td>
<td>Down</td>
</tr>
</tbody>
</table>

Fold change over control
Tumor cell supernatants increase the angiogenesis-associated cytochrome CYP1B1 isoform, metformin blocks this effect.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10mM Metformin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Huvec + NC medium</td>
<td>Huvec + NC medium</td>
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<tr>
<td>CYP1B1</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>β-act</td>
<td>2.9</td>
<td>0.86</td>
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<td>CYP1B1/β-act</td>
<td>3.43</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Huvec + MDA-MB231 CM</td>
<td>Huvec + MDA-MB231 CM</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>4.5</td>
<td>0.52</td>
</tr>
<tr>
<td>β-act</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1B1/β-act</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AMPK also modulates CYP1B1.

 boosts CYP1B1
effects of metformin on endothelial cells proangiogenic factors…
and on tumor cells...
Metformin inhibits angiogenesis in vivo particularly in the context of obesity.

- WAT weight (gr)
  - ND
  - ND Metf
  - HFD
  - HFD Metf

- MVD (% of Control)
  - ND
  - ND Metf
  - HFD
  - HFD Metf
Cancer Cell derived exosomes (CCE) have important roles in the intercellular communication between cancer and stromal cells that will result in the maturation of the tumour microenvironment and tumour growth and proliferation.

Exosomes and immunity

Exosomes from mature DCs (mDCs) can stimulate innate immune responses in various immune and non-immune cells and promote a pro-inflammatory response leading to pro-tumour effects.

Jeffrey S. Schorey, EMBO reports (2014)
Antibody-dependent cellular cytotoxicity (ADCC)

Mediated by either NK cells or CTL

The action of ADCC is dependent on the recognition of the objective cell by antibodies attached on the surface of the effector cell (terminally differentiated leukocyte)

The process is part of the adaptive immune response due to the dependence on antibodies

A former antibody response is required for this mechanism to take effect and be effective against an invading cells/pathogen

ADCC-mediating therapeutic antibodies
FDA approved for cancer therapy

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
<th>Cancer indication</th>
<th>Mechanisms of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>CD20+ B cell NHL, CD20+ follicular NHL, CLL</td>
<td>ADCC, CDC, direct induction of apoptosis</td>
</tr>
<tr>
<td>Ofatumumab</td>
<td>CD20</td>
<td>CLL</td>
<td>ADCC, CDC</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Her2/neu</td>
<td>Breast cancer</td>
<td>ADCC, abrogation of tumor cell signaling</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>EGFR</td>
<td>colorectal cancer, SCCHN</td>
<td>ADCC, abrogation of tumor cell signaling</td>
</tr>
<tr>
<td>Alemtuzumab*</td>
<td>CD52</td>
<td>CLL</td>
<td>ADCC, CDC, direct induction of apoptosis</td>
</tr>
</tbody>
</table>

NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; SCCHN, squamous cell carcinoma of the head and neck.
*Withdrawn from the market in August, 2012.

The mechanisms of action include:

- immune-mediated effects as CDC and ADCC
- effects on the tumor microenvironment
- direct anti-tumor effects
- blocking receptor signaling or acting as an agonist
- delivery of a cytotoxic agent
**Immune Checkpoint blockade PD-1/PD-L1**

**Figure 4** Two general mechanisms of expression of immune-checkpoint ligands on tumour cells. The examples in this figure use the programmed cell death protein 1 (PD1) ligand, PDL1 (also known as B7-H1), for illustrative purposes, although the concept probably applies to multiple immune-checkpoint ligands, including PDL2 (also known as B7-DC).

**a** | Innate immune resistance. In some tumours, constitutive oncogenic signalling can upregulate PDL1 expression on all tumour cells, independently of inflammatory signals in the tumour microenvironment. Activation of the AKT and signal transducer and activator of transcription 3 (STAT3) pathways has been reported to drive PDL1 expression.

**b** | Adaptive immune resistance. In some tumours, PDL1 is not constitutively expressed, but rather it is induced in response to inflammatory signals that are produced by an active antitumour immune response. The non-uniform expression of PDL1, which is commonly restricted to regions of the tumour that have tumour-infiltrating lymphocytes, suggests that PDL1 is adaptively induced as a consequence of immune responses within the tumour microenvironment. Adaptive induction may be a common mechanism for the expression of multiple immune-checkpoint molecules in tumours. IFNγ, interferon-γ; MHC, major histocompatibility complex; TCR, T cell receptor.

Pardoll et al., Nat Rev Cancer 2012
Figure 2 Therapeutic interventions being investigated that target immune inhibitory pathways in the tumor microenvironment. In melanoma, T cell–infiltrated tumors show the highest expression of PD-L1, IDO and T_{reg} cells, and indirect evidence suggests T cell–intrinsic anergy as well. Monoclonal antibodies blocking PD-1–PD-L1 interactions, small-molecule inhibitors of IDO, CD25-targeting agents to deplete T_{reg} cells and γc-binding cytokines to promote homeostatic proliferation of T cells and anergy reversal are all being investigated clinically.

Gajewski, Schreiber and Fu, Nat Immunol 2013
Table 1 | The clinical development of agents that target immune-checkpoint pathways

<table>
<thead>
<tr>
<th>Target</th>
<th>Biological function</th>
<th>Antibody or Ig fusion protein</th>
<th>State of clinical development*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTLA4</td>
<td>Inhibitory receptor</td>
<td>Ipilimumab</td>
<td>FDA approved for melanoma, Phase II and Phase III trials ongoing for multiple cancers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tremelimumab</td>
<td>Previously tested in a Phase III trial of patients with melanoma; not currently active</td>
</tr>
<tr>
<td>PD1</td>
<td>Inhibitory receptor</td>
<td>MDX-1106 (also known as BMS-936558)</td>
<td>Phase I/II trials in patients with melanoma and renal and lung cancers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MK347</td>
<td>Phase I trial in multiple cancers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT-011†</td>
<td>Phase I trial in multiple cancers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMP-224§</td>
<td>Phase I trial in multiple cancers</td>
</tr>
<tr>
<td>PDL1</td>
<td>Ligand for PD1</td>
<td>MDX-1105</td>
<td>Phase I trial in multiple cancers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple mAbs</td>
<td>Phase I trials planned for 2012</td>
</tr>
<tr>
<td>LAG3</td>
<td>Inhibitory receptor</td>
<td>IMP321‖</td>
<td>Phase III trial in breast cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple mAbs</td>
<td>Preclinical development</td>
</tr>
<tr>
<td>B7-H3</td>
<td>Inhibitory ligand</td>
<td>MGA271</td>
<td>Phase I trial in multiple cancers</td>
</tr>
<tr>
<td>B7-H4</td>
<td>Inhibitory ligand</td>
<td></td>
<td>Preclinical development</td>
</tr>
<tr>
<td>TIM3</td>
<td>Inhibitory receptor</td>
<td></td>
<td>Preclinical development</td>
</tr>
</tbody>
</table>

CTLA4, cytotoxic T-lymphocyte–associated antigen 4; FDA, US Food and Drug Administration; Ig, immunoglobulin; LAG3, lymphocyte activation gene 3; mAbs, monoclonal antibodies; PD1, programmed cell death protein 1; PDL, PD1 ligand; TIM3, T cell membrane protein 3. *As of January 2012. †PD1 specificity not validated in any published material. §PDL2–Ig fusion protein. ‖LAG3–Ig fusion protein.
Table 1. Durable responses with CTLA-4, PD-1, and PD-L1 inhibition in individual clinical trials

<table>
<thead>
<tr>
<th>Trial and reference</th>
<th>$N$</th>
<th>Median response duration (mo)</th>
<th>RR (%)</th>
<th>Complete response (%)</th>
<th>Partial response (%)</th>
<th>SD (%)</th>
<th>Median OS (mo)</th>
</tr>
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<tbody>
<tr>
<td><strong>CTLA-4 Inhibition (ipi)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lpi + gp 100</td>
<td>56</td>
<td>88</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>NR</td>
<td>14</td>
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<tr>
<td>lpi + IL-2</td>
<td>36</td>
<td>79</td>
<td>25</td>
<td>17</td>
<td>8</td>
<td>NR</td>
<td>16</td>
</tr>
<tr>
<td>lpi DE +/- gp100</td>
<td>85</td>
<td>42</td>
<td>20</td>
<td>6</td>
<td>14</td>
<td>NR</td>
<td>13</td>
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<td>Phase II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>lpi 10 mg/kg (PT)</td>
<td>155</td>
<td>NR</td>
<td>5.8</td>
<td>0</td>
<td>5.8</td>
<td>21</td>
<td>10.2</td>
</tr>
<tr>
<td>lpi 10 mg/kg (PT)</td>
<td>71</td>
<td>NR</td>
<td>11.1</td>
<td>2.7</td>
<td>8.3</td>
<td>18.1</td>
<td>11.4</td>
</tr>
<tr>
<td>lpi 3 mg/kg (PT)</td>
<td>71</td>
<td>NR</td>
<td>4.2</td>
<td>0</td>
<td>4.2</td>
<td>22.2</td>
<td>8.7</td>
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<tr>
<td>lpi 10 mg/kg (PT + TN) + budenoside</td>
<td>58</td>
<td>NR</td>
<td>12</td>
<td>2</td>
<td>10</td>
<td>19</td>
<td>17.7</td>
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<tr>
<td>lpi 10 mg/kg (PT + TN) + placebo</td>
<td>57</td>
<td>NR</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>19</td>
<td>19.3</td>
</tr>
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<td>Phase III</td>
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<tr>
<td>lpi + dacarbazine</td>
<td>250</td>
<td>19.3</td>
<td>15.2</td>
<td>1.6</td>
<td>13.6</td>
<td>18</td>
<td>11.2</td>
</tr>
<tr>
<td>lpi + gp100</td>
<td>403</td>
<td>11.5</td>
<td>5.7</td>
<td>0.2</td>
<td>5.5</td>
<td>14.4</td>
<td>10</td>
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<tr>
<td>lpi + placebo</td>
<td>137</td>
<td>Not reached</td>
<td>11</td>
<td>1.5</td>
<td>9.5</td>
<td>17.5</td>
<td>10.1</td>
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<tr>
<td><strong>CTLA-4 Inhibition (treme)</strong></td>
<td></td>
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<td>Phase II</td>
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<tr>
<td>Treme (10–15 mg/kg)</td>
<td>84</td>
<td>NR$^a$</td>
<td>9.5</td>
<td>2.4</td>
<td>7.1</td>
<td>31</td>
<td>10</td>
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<td>Phase III</td>
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<tr>
<td>Treme (15 mg/kg)</td>
<td>328</td>
<td>35.8</td>
<td>11</td>
<td>3</td>
<td>8</td>
<td>NR</td>
<td>12.6</td>
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<td><strong>PD-1 Blockade</strong></td>
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</tr>
<tr>
<td>Nivolumab (0.1–10 mg/kg)</td>
<td>94</td>
<td>12.0 (73.1% ongoing)</td>
<td>28</td>
<td>0</td>
<td>28</td>
<td>6</td>
<td>16.8</td>
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<tr>
<td>Lambrolizumab (2–10 mg/kg)</td>
<td>135</td>
<td>81% ongoing$^b$</td>
<td>38</td>
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<tr>
<td><strong>PD-L1 Blockade</strong></td>
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<tr>
<td>Phase I</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMS-936559 (0.3–10 mg/kg)</td>
<td>52</td>
<td>16.2 (55.5% ongoing)</td>
<td>17</td>
<td>0</td>
<td>17</td>
<td>27</td>
<td>NR</td>
</tr>
</tbody>
</table>

Abbreviations: lpi, ipilimumab; Treme, tremelimumab; NR, not reported; TN, treatment naïve; PT, prior treatment; OS, overall survival.

$^a$Seventy-five percent of objective responses ongoing at 15+ to 28+ months.

$^b$At 11 months median follow-up.
Multiple strategies to target the TME

- **Tumor vasculature**
  - Bevacizumab (anti-VEGF-A)
  - S-265610 (anti-CXCR2)
  - Sunitinib (RTK inhibitor)
  - VEGF-Trap (decoy receptor)

- **Immune activation**
  - Ipilimumab (anti-CTLA-4)
  - Nivolumab (anti-PD1R)
  - Lambrolizumab (anti-PD-L1)

- **Repolarization and re-education**
  - BLZ945 (anti-CSF-1R)
  - CD40 mAb

- **Metastasis and/or outgrowth**
  - MLN1202 (anti-CCR2)

- **Altered immune cell recruitment, expansion and depletion**
  - PLX3397 (anti-CSF-1R and anti-KIT)
  - AMD3100 (anti-CXCR4)
  - S-265610 (anti-CXCR2)
  - GW2580 (anti-CSF-1R)
  - Trabectedin (chemotherapy)

---

Daniela F Quail & Johanna A Joyce
IMMUNOSCORE (I)

Figure 1 Immunoscore definition and method.

Galon et al., J Translat med 2012
Example:
Immunoscore in Rectal Cancer

Anitei et al., Clin Cancer Res 2014
Depletion of Carcinoma-Associated Fibroblasts and Fibrosis Induces Immunosuppression and Accelerates Pancreas Cancer with Reduced Survival

Berna C. Ozdemir,⁎,1,2 Tsvetolina Pantcheva-Hoang,3 Julienne L. Carstens,1 Xiaofeng Zheng,1 Chia-Chin Wu,1 Tyler R. Simpson,2 Hanano Lalakia,1 Hikaru Sugimoto,1,2 Christoph Kahlert,1,4 Sergey V. Novitsky,5 Ana De Jesus-Acosta,7 Padmavati Sharma,1 Podram Holdar,3,4,5 Umair Mahmood,1,2 Lynda Chin,1 Harold L. Moses,1,2 Valerie M. Weaver,1,2 Anirban Maitra,1,5 James P. Allison,2 Valerie S. LeBlu̇e,1,2 and Raghu Kalluri1,2,7,*

1Department of Cancer Biology, Mechanobiology Research Center, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
2Division of Matrix Biology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02115, USA
3Department of Immunology, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
4Department of Genomic Medicine, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
5Department of Surgery, University of California, San Francisco, San Francisco, CA 94143, USA
6Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA
7Department of Medical Oncology, Johns Hopkins Hospital, Baltimore, MD 21287, USA

SUMMARY

Pancreatic ductal adenocarcinoma (PDAC) is associated with marked fibrosis and stromal myofibroblasts, but their functional contribution remains unknown. Transgenic mice with the ability to delete αSMA myofibroblasts in pancreatic cancer were generated. Depletion starting at either noninvasive precursor (pancreatic intraepithelial neoplasia) or the PDAC stage led to invasive, undifferentiated tumor with enhanced hypoxia, epithelial-to-mesenchymal transition, and cancer stem cells, with diminished animal survival. In PDAC patients, fewer myofibroblasts in their tumors also correlated with reduced survival. Suppressed immune surveillance with increased CD4+Foxp3+ Tregs was observed in myofibroblast-depleted mouse tumors. Although myofibroblast-depleted tumors did not respond to gemcitabine, anti-CTLA4 immunotherapy reversed disease acceleration and prolonged animal survival. This study underscores the need for caution in targeting carcinoma-associated fibroblasts in PDAC.
Tumor Heterogeneity in the microenvironment(I)

CSC/CIC-RESISTANCE MECHANISMS
- Vasculogenic mimicry (VE-cadherin)
- Epithelial-to-mesenchymal transition (Snail, Twist, Vimentin)
- Increased resistance to hypoxia (HIF1-α, VEGF)
- Increased angiogenesis (PLGF, FGF-2, VEGF, IL-8)
- Immune evasion (loss of tumor antigens, TGFβ, IL-10, VEGF, PGE2)

STEM MARKERS/PATHWAYS:
- PI3K/AKT
- Hedgehog/GLI
- HIF2α/p53
- Notch-1

INFLAMMATION:
- TNFα, IL1-α, IL1-β

ANGIOGENESIS:
- VEGF, bFGF
- PDGF, HDGF
- HIF1-α, Epsin-1, -2

ECM:
- Integrins
- MMP-1, -2, -9

CSC/CIC

STROMA:
- WNT16B
- HGF/MET

Cancer Associated Fibroblasts (CAF)

Inflammatory cells

Extracellular Matrix

Drugs targeting CSC & TUMIC

Standard therapeutic options

Endothelial cells

TUMOR ERADICATION

Albini et al, Connective Tissue Res, 56, 414-25
The difference between immunoprevention and immuno-therapy is in the timing and the expected outcome.

Immuno-prevention uses a vaccine in an individual at risk for developing cancer, to generate immunologic memory that prepares the immune system to detect and eliminate future premalignant lesions.

Immunotherapy addresses the disease after it is already diagnosed through vaccines or monoclonal antibody therapies.

Sean O. Ryan et al, Allergy and Immunology 2006
PROS and CONS OF CANCER IMMUNOPREVENTION

Cancer Immunoprevention: A New Approach to Intercept Cancer Early

Asad Umar
Emerging strategies for cancer immunoprevention

The crucial role of the immune system in the formation and progression of tumors has been widely accepted. On one hand, the surveillance role of the immune system plays an important role in endogenous tumor prevention, but on the other hand, in some special circumstances such as in chronic inflammation, the immune system can actually contribute to the formation and progression of tumors. In recent years, there has been an explosion of novel targeted immunotherapies for advanced cancers. In the present manuscript, we explore known and potential various types of cancer prevention strategies and focus on nonvaccine-based cancer preventive strategies targeting the immune system at the early stages of tumorigenesis.

Onco gene advance online publication, 14 September 2015; doi:10.1038/onc.2015.98

STRATEGIES FOR CANCER IMMUNOPREVENTION
# Overview of recent non-viral cancer immunoprevention studies


## Table 1. Overview of recent nonviral cancer immunoprevention studies

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Vaccine type</th>
<th>Adjuvant</th>
<th>Target</th>
<th>Treatment effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer prevention</td>
<td>Peptide vaccine</td>
<td>Toll-like receptor 3 agonist (poly-ICLC)</td>
<td>MUC1</td>
<td>44% MUC1-specific antibody response. Vaccine induces long-term memory. Nonresponders showed higher percentage of immunosuppressive mediators. No clinical endpoints.</td>
<td>9</td>
</tr>
<tr>
<td>Melanoma</td>
<td>DC vaccine</td>
<td>KLH</td>
<td>Melanoma-associated antigens (tyrosinase, Melan-A/MART-1, gp100, MAGE-A, and MAGE-3) or autologous tumor cell lysate</td>
<td>3-year DFS 40.9% vs. 14.5% (historical controls, P = 0.0016). 3-year OS 68.2% vs. 25.7% (historical controls, P = 0.0016).</td>
<td>10</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Ipilimumab + peptide vaccine</td>
<td>Montanide ISA 51 VG</td>
<td>Tyrosinase, gp100, MART-1</td>
<td>23% stopped treatment due to toxicity related to ipilimumab. Median RFS and OS not reached at 29.5 months.</td>
<td>11</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Peptide vaccine</td>
<td>GM-CSF, HER2 peptide E75</td>
<td></td>
<td>2-year DFS 94.3% vs. 86.6% (controls, P = 0.08). Subset analysis: Improved DFS in node-positive, low HER2-expressing and low grade tumors. Improved DFS in HER2-high-expressing patients receiving vaccine plus trastuzumab compared with vaccine alone.</td>
<td>12</td>
</tr>
</tbody>
</table>

## Table 1. Overview of recent nonviral cancer immunoprevention studies

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Vaccine type</th>
<th>Adjuvant</th>
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<tbody>
<tr>
<td>Follicular lymphoma</td>
<td>Protein vaccine</td>
<td>KLH</td>
<td>Tumor isotype matched IgM and IgG</td>
<td>Median DFS 44.2 vs. 30.6 months (controls, P = 0.047). Median OS not reached. Subset analysis: Improved DFS with IgM isotype vaccine.</td>
<td>15</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Protein vaccine</td>
<td>AS02a</td>
<td>MAGE-A3</td>
<td>Trial done pre-rituximab. 44-month recurrence rate 35% vs. 43% (controls, not significant). DFS or OS not significantly improved.</td>
<td>21</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Peptide vaccine</td>
<td>KLH plus QS-21</td>
<td>GME ganglioside</td>
<td>Trial stopped after median follow-up of 18 months due to increased survival in control group (HR 1.66; P = 0.02).</td>
<td>22</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Viral vector vs. DC vaccine</td>
<td>GM-CSF (viral vector)</td>
<td>CEA, MUC1, CD80, CD54, and CD58</td>
<td>RFS 22.9 vs. 28.9 months (DC vs. viral vector). Median OS not reached in either group vs. 44.1 months (historical controls, P &lt; 0.0001).</td>
<td>16</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Whole cell vaccine (3V/A)</td>
<td>GM-CSF</td>
<td>Allogenic pancreatic cancer cell line antigens (incl. mutated k-ras, macrophilin)</td>
<td>OS 24.8 vs. 20.3 months (historical controls, not significant). Induction of macrophilin-specific CD6 T cells correlated with increased DFS.</td>
<td>17</td>
</tr>
</tbody>
</table>
Cancer Immunoprevention—The Next Frontier

Marie-Anne D. Smith1,2, Elizabeth M. Jaffe3,4, and Eric R. Lutz1,2,3,4

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2Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland
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4The Skip Viragh Pancreatic Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland

First genetic changes, e.g., mutated Kras, BRAF, P53

Progressive genetic changes, e.g., cyclin D1, mesothelin

Primary prevention

Secondary prevention

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PGE2 can inhibit the ability of maturing DCs to produce IL-12 during priming, biasing the resulting adaptive immune response toward a Th2 profile.

COX-2 inhibitors such as aspirin and celecoxib can reverse this effect, allowing generation of Th1.

PGE2 facilitates differentiation of monocytes into immunosuppressive MDSCs, which function to inhibit the adaptive immune response and promote Treg populations through depletion of environmental arginine, expression of nitric oxide synthase, production of reactive oxygen species, and elaboration of Th2 cytokines IL-10 and TGF-B.

COX-2 inhibitors aspirin and celecoxib can reverse this effect.

Curcumin administration can enhance the Th1 and decrease Th2 immune response.

Metformin and curcumin increase effector CD8+ T cell populations and resulting memory cells, both of which are critical for an effective adaptive immune response.

Metformin may increase MHC-I expression on tumor cells, increasing visibility to effector CD8+ T cells.

Bexarotene inhibits apoptosis in T cells by increasing expression of BCL2.

ZA and other bisphosphonates increase phosphoantigens in PBMCs and on cancer cells themselves, resulting in activation of anti-tumor gamma delta T cells

PGE2 can increase immunosuppressive Treg, MDSC, and M2 macrophage populations.

COX inhibitors aspirin, celecoxib, and meloxicam can reverse this effect.

Both the AI letrozole and curcumin have been shown to reduce Treg populations.

The retinoid ATRA can differentiate MDSCs into immature DCs, which may account for its ability to enhance proliferation of both effector and memory CD8+ T cells.

Curcumin can inhibit MDSCs and differentiate them toward a M1-like phenotype; (D)ZA decreases populations of M2 macrophages and may re-polarize them to the anti-tumor M1 phenotype.
IMPRECISION MEDICINE?
Redundant Pathways: New drugs
Different populations: Combination and sequential therapy
Bypassing cancer cell heterogeneity: Addressing angiogenesis, microenvironment and inflammation
Targeted delivery
Killing stem cells
Continuation beyond progression
Angioprevention (or prevention + cytotoxic)
Metronomics
Biomarkers-Genomics-Single cell analysis
Modeling
CANCER AS A CONSPIRACY OF MANY “GOOD GUYS” TURNED BAD: THE MICROENVIRONMENT CAN BE “CHEMOPREVENTED” FROM HELPING THE TUMOR TO GROW AND PROGRESS
Laboratorio di Biologia Vascolare ed Angiogenesi.

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