The Secret Killer

‘Chronic inflammation may be the engine that drives many of the most feared illnesses of middle and old age’. TIME magazine March 2004
Rudolf Ludwig Karl Virchow 1821-1902

He noticed the infiltration of leukocytes in malignant tissues and suggested that cancers arise at site of chronic inflammation.

Cells are the basic unit of structure in all organisms and also the basic unit of reproduction.
Balkwill and Mantovani, Inflammation and Cancer: back to Virchow. Lancet 357, 539, 2001
Current view of tumor-associated myeloid cells (TAMCs) differentiation in cancer
The Cancer-Immunity Cycle (based on antigen recognition)

Chemo- and radio-therapy can promote: Immunogenic cell death (calreticulin, HMGB1, ATP)

Chen DS and Mellman I, Immunity 2015
CANCER IMMUNO-EDITING and MACROPHAGE POLARIZATION

Schreiber R. *Nature Immunology* 2002

1) Initiation/promotion

2) Elimination

3) Equilibrium

4) Escape

Macrophage polarization during tumor progression

- CpG
- IFN-γ
- anti-IL-10
- anti-CSF-1
- anti-CCL2
- CD40 agonists
- inhibition of: p50 NF-κB, STAT3, STAT6

Restoration of:
- cytotoxicity against initiating cancer cells, vessels
- Treg
- TAM
- MDSC

Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment

*Cancer Cell* 2006

*Lancet Oncol.* 2012

-RNI ROI

- NF-κB

Proliferation

Apoptosis
**Immunoscore:** has become a valuable prognostic indicator of cancer progression, as it depicts immune reactions active at the tumor microenvironment, that critically control tumor development (more than TNM).
Immunotherapy

Check point inhibitors (e.g. anti-CTLA4, anti-PD-1)
cytokine-based immunotherapy (e.g. GM-CSF, IFNα, IL-2)

Unbalance towards immune response
Activation of benign autoimmunity in cancer

Natural “protective autoantibodies” against tumor-antigens (anti-thyroblobulin, anti-melanocytes, anti-survivin-breast) were isolated from patients and healthy donors (as well as from newborns)

The clinical benefit of healthy autoimmunity can be inferred by the finding that the administration to cancer patients of antibodies to immune suppressor molecules (CTLA4, PD-1/PD-L1) can unleash an autoimmune attack on the patient's tumor
William Coley (1862-1936)

1891: William Coley (Memorial Sloan Kettering Cancer Center-MSKCC, NY). Used the Coley toxin containing live or inactivated bacteria like *Serratia Marcescens* and *Streptococcus pyogenes* to treat over 1000 sarcoma patients by intratumor injections. Reproducibility was limited but some patients showed a benefit.

Albert Calmette (1863-1933) and Camille Guèrin (1872-1961)

*BCG* is a vaccine used to prevent tuberculosis (TB). Is composed of mycobacterium Bovis that causes inflammation-dependent immunotherapy of superficial bladder cancer; it has been used for over 30 years. The most effective immunotherapy against a human tumor (ladder).

Paul Ehrlich (1854-1905)

Microbiologo tedesco (fondatore della chemioterapia) 1900: suggerisce che alcune molecole all’interno dell’organismo possono essere in grado di combattere i tumori.

Sir Frank Macfarlane Burnet (1899-1989)

Suggerisce che le cellule tumorali possono causare una risposta immunitaria in grado di distruggere il tumore senza alcuna manifestazione clinica (1957: teoria dell’Immunosorveglianza).
CANCER TREATMENTS

1850
Surgery
Harvey Cushing (1869 -1939) neurochirurgo

1900
Bacterial products (Wiliam Coley, Calmette-Guerin)

1950
Radiotherapy

2000
Chemotherapy – Hormonal Therapy
1946 mostarde azotato

Targeted Therapy
Imatinib (Gleevec), 2001

Epigenetic Therapy
Azacytidine (Vidaza), 2004

Immunotherapy
Ipilimumab, (Yervoy), 2011
Current anticancer immunotherapies

- Immunosuppressive chemotherapies:
  - bleomycin
  - bortezomib
  - cyclophosphamide
  - doxorubicin
  - oxaliplatin

- Checkpoint blockers:
  - anti-CTLA-4 (ipilimumab)
  - anti-PD-1 (nivolumab, pembrolizumab)
  - anti-PD-L1 (avelumab, atezolizumab)

- Co-stimulatory mAbs:
  - anti-4-1BB (4-1BBL, 4-1BB agonist)
  - anti-OX40 (OX40L, OX40 agonist)
  - anti-CD137 (CD137L, 4-1BBL agonist)

- Antigen vaccines:
  - NKG2D ligands (poliovirus E1, vaccines)
  - GS-9873 (GSK)

- Artificial T cell receptors (TCRs)
  - Anti-CD3/CD19 TCR fusion protein
  - Anti-CD19/CD20 TCR fusion protein

- CAR-T cell therapy:
  - Anti-CD19 CAR-T cells

- Adoptive T cell transfer:
  - Autologous T cells
  - Allogeneic T cells

- MDSC inhibitors:
  - CpG, IFNγ, anti-IL-10
  - Adenosine inhibitors
  - IDO inhibitors

- Inhibitors of immune-checkpoint signaling:
  - mTOR inhibitors
  - PI3K inhibitors

- Immune checkpoint inhibitors:
  - Anti-LAG-3 (BMS-986016)
  - Anti-TIM-3 (MEDI4736)

- Adjuvants:
  - Lipopolysaccharide (LPS)
  - CpG oligodeoxynucleotides (ODNs)

- Immunomodulatory drugs:
  - Interferon-α (IFNα)
  - Interleukin-2 (IL-2)
  - Interleukin-12 (IL-12)

- Immunostimulatory cytokines:
  - Interferon-γ (IFNγ)
  - Tumor necrosis factor-α (TNFα)

- Anticancer vaccines:
  - Oncorine®: non-pathogenic viral strains that specifically infect cancer cells, triggering their demise (China 2005)

- T cell receptor (TCR) therapy:
  - Anti-CD19 CAR-T cells

Galluzzi et al Oncotarget 2014
# Cytokine-mediated immunotherapy

## Table 1. Clinical trials recently launched to evaluate the safety and efficacy of immunostimulatory cytokines in cancer patients

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Indication(s)</th>
<th>Status</th>
<th>Phase</th>
<th>Route</th>
<th>Notes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3L</td>
<td>Lymphoma</td>
<td>Recruiting</td>
<td>II</td>
<td>i.t.</td>
<td>Combined with radiotherapy and a TLR3 agonist</td>
<td>NCT01976585</td>
</tr>
<tr>
<td></td>
<td>Breast carcinoma</td>
<td>Recruiting</td>
<td>I/II</td>
<td>s.c.</td>
<td>Combined with a FOLR1-targeting vaccine</td>
<td>NCT02019524</td>
</tr>
<tr>
<td></td>
<td>Ovarian carcinoma</td>
<td>Completed</td>
<td>II</td>
<td>s.c.</td>
<td>Combined with rituximab</td>
<td>NCT01939730</td>
</tr>
<tr>
<td></td>
<td>Follicular B-cell lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF</td>
<td>GBM</td>
<td>Not yet recruiting</td>
<td>I/II</td>
<td>n.a.</td>
<td>Combined with multiple peptide vaccine and imiquimod</td>
<td>NCT02078648</td>
</tr>
<tr>
<td></td>
<td>GBM Gliosarcoma</td>
<td>Not yet recruiting</td>
<td>II</td>
<td>s.c.</td>
<td>Combined with a cell-based vaccine, bevacizumab and cyclophosphamide</td>
<td>NCT01903330</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>Completed</td>
<td>III</td>
<td>s.c.</td>
<td>As single agent or combined with TYR-targeting vaccine</td>
<td>NCT01989572</td>
</tr>
<tr>
<td></td>
<td>Mesothelioma</td>
<td>Recruiting</td>
<td>I/II</td>
<td>n.a.</td>
<td>Combined with ipilimumab</td>
<td>NCT02009397</td>
</tr>
<tr>
<td></td>
<td>NSCLC</td>
<td>Recruiting</td>
<td>II</td>
<td>n.a.</td>
<td>Combined with an autophagosome-derived vaccine and imiquimod</td>
<td>NCT01909752</td>
</tr>
<tr>
<td></td>
<td>AML</td>
<td>Recruiting</td>
<td>IV</td>
<td>n.a.</td>
<td>As single agent upon allogeneic stem cell transplantation</td>
<td>NCT02027064</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal neuroendocrine tumors</td>
<td>Not yet recruiting</td>
<td>III</td>
<td>s.c.</td>
<td>As single agent</td>
<td>NCT01860742</td>
</tr>
<tr>
<td></td>
<td>Anal intraepithelial neoplasia</td>
<td>Recruiting</td>
<td>I/II</td>
<td>s.c.</td>
<td>Combined with a HPV-16-targeting vaccine</td>
<td>NCT01923116</td>
</tr>
<tr>
<td></td>
<td>Childhood craniofacryngioma</td>
<td>Not yet recruiting</td>
<td>II</td>
<td>s.c.</td>
<td>As single agent</td>
<td>NCT01964300</td>
</tr>
<tr>
<td>IFN-α</td>
<td>CML</td>
<td>Not yet recruiting</td>
<td>II</td>
<td>n.a.</td>
<td>Combined with dasatinib</td>
<td>NCT01872442</td>
</tr>
<tr>
<td>IFN-α2b</td>
<td></td>
<td>Not yet recruiting</td>
<td>II</td>
<td>s.c.</td>
<td>Combined with imatinib and nilotinib</td>
<td>NCT02001818</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruiting</td>
<td>I</td>
<td>s.c.</td>
<td>Combined with imatinib</td>
<td>NCT01933906</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recruiting</td>
<td>II</td>
<td>s.c.</td>
<td>Combined with nilotinib</td>
<td>NCT01866553</td>
</tr>
<tr>
<td>IFNγ</td>
<td>Soft tissue sarcoma</td>
<td>Recruiting</td>
<td>n.a.</td>
<td>s.c.</td>
<td>Combined with anti-PDCD1 mAb</td>
<td>NCT02089685</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CML, chronic myeloid leukemia; FLT3L, fms-related tyrosine kinase 3 ligand; FOLH1, folate hydrolase 1; FOLR1, folate receptor 1; GBM, glioblastoma multiforme; GM-CSF, granulocyte macrophage colony-stimulating factor; HPV-16, human papillomavirus Type 16; IFN, interferon; IL, interleukin; i.t., intra tumorum; i.v., intra venam; mAb, monoclonal antibody; MRD, minimal residual disease; n.a., not available; NHL, non-Hodgkin lymphoma; NK, natural killer; NSCLC, non-small cell lung carcinoma; PBL, peripheral blood lymphocyte; PDCD1, programmed cell death 1; RCC, renal cell carcinoma; SABR, stereotactic ablative body radiotherapy; s.c., sub cutem; TNFα, tumor necrosis factor α; TYR, tyrosinase; WT1, Wilms tumor 1. *Between 2013, May 1st and the date of submission.
Immunopeptidome: T-cell development and function are regulated by MHC-associated self peptides, collectively referred to as the immunopeptidome.

**Immunopeptidomes analysis:** proteogenomic approaches that combine mass spectrometry and next-generation sequencing.

**Genome sequencing**

Given the great progress of genome sequencing technology (whole genome NGS) during the last years, the possibility of identifying in tumor cells new somatic Mutations-derived tumor-specific neo-antigens provided an opportunity to construct **individualized therapeutic cancer vaccines** or to select T cells to be used in **adoptive immunotherapy** of cancer patients.

Clinical activity of cancer vaccines (2010-2014)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Tumor</th>
<th>Phase</th>
<th>N patients</th>
<th>Stage</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE-3</td>
<td>NSCLC</td>
<td>II R</td>
<td>183</td>
<td>IB-II</td>
<td>Trend</td>
</tr>
<tr>
<td>MUC-1 L-BLP25</td>
<td>NSCLC</td>
<td>II</td>
<td>34</td>
<td>IIB</td>
<td>P=0.016</td>
</tr>
<tr>
<td>Provenge</td>
<td>Prostate cancer</td>
<td>III</td>
<td>341/171</td>
<td>HR</td>
<td>P&lt;0.03</td>
</tr>
<tr>
<td>DC</td>
<td>gp100</td>
<td>III</td>
<td>185</td>
<td>IV</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td>IL-2 +/- gp100</td>
<td>Melanoma</td>
<td>III</td>
<td>185</td>
<td>IV</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td>E75/Her2/neu</td>
<td>Breast cancer</td>
<td>IIR</td>
<td>101/75</td>
<td>IV</td>
<td>P&lt;0.04</td>
</tr>
<tr>
<td>BiovaxID</td>
<td>Follicular Lymphoma</td>
<td>III</td>
<td>76/41</td>
<td></td>
<td>P&lt;0.04</td>
</tr>
<tr>
<td>IMA901+Cyclo</td>
<td>RCC</td>
<td>IIR</td>
<td>96</td>
<td>III</td>
<td>P&lt;0.023</td>
</tr>
</tbody>
</table>
Costimulatory and coinhibitory receptors in the immune synapse

Ipilimumab
Nivolumab, Pembrolizumab

Monoclonal antibodies: tools for diagnosis and cancer immunotherapy
Failure of Immunotherapy

• the anti–PD-1/PD-L1 therapy has substantial clinical activity in advanced-stage non–small cell lung cancer (NSCLC) and small cell lung cancer, with an overall response rate (ORR) of 15%–20%. Despite this, many patients do not benefit from anti-PD-1/PD-L1.

• In analogy, IFNγ, originally termed “macrophage activating factor” (Adams and Hamilton, 1984), was paradoxically shown to be equally necessary for melanoma development and rejection (Zaidi and Merlino, 2011).
Major issues in cancer immunotherapy: Immunosuppressive micro- and macroenvironments

Challenge: breaking of immunosuppression in cancer
Constitutive and inducible suppressive mechanisms in cancer

David H Munn et al.  Current Opinion in Immunology
A vicious link between “inflammation” and “immunosuppression”
Emergency Hematopoiesis in Cancer

HSC

BM

g-CSF

neutrophil

HSC

CSFs

TUMOR

TAM

G-SCF

Arginase I

INOS

M-CSF

monocyte

MDSC/TAM

Strauss L et al Cancer Cell 2015
induction of Treg cells, ROS) and NO, Arg-1, IL-10, ADAM17(CD62L), galectin 9(TIM3), iNOS/NOS2, Arginase I, Indoleamine 2,3 Dioxygenase)

PD-1, CTLA-4, TIM3, BTLA, adenosine A2AR; interleukin-10, TGF)-β, adenosine, IDO, and arginase induction of Treg cells, ROS) and NO, Arg-1, IL-10, ADAM17(CD62L), galectin 9(TIM3), iNOS/NOS2, Arginase I, Indoleamine 2,3 Dioxygenase

Myeloid Suppressor Cells in Cancer

(oxygen levels, glucose levels, pH)
Pathways of macrophage polarization

FUNCTIONS

- Th1 RESPONSES
- TYPE I INFLAMMATION; DTH
- KILLING OF INTRACELLULAR PARASITES
- TUMOR RESISTANCE

- Th2 RESPONSES; TYPE II INFLAMMATION;
- ALLERGY;
- KILLING and ENCAPSULATION of PARASITES;
- MATRIX DEPOSITION and REMODELING;
- ANGIogenesis and TUMOR PROMOTION


MN/MCA1 (Saccani A Cancer Res 2006); HCC; CRC
p50 NF-κB is a prognostic indicator of CRC progression

Blue → DAPI
Green → CD68
Gray → p50

Porta C et al. Submitted
### Strategies targeting TAM accumulation

#### Recruitment
- **CCL2 inhibitor (bindarit)**
  - Melanoma and vascular pathology
  - Glioma (mouse and human)
- **Anti-CCL2 (Ab)**
  - Breast, Prostate cancer
- **Anti-CSF-1 (Ab and antisense-ODN)**
  - Breast, Prostate, lung,
- **Anti-CSF1R mAb (RG7155)**
  - Mouse colon carcinoma, human diffuse-type giant cell tumor
- **fms-tyrosine kinase inhibitors**
  - Acute myeloid leukemia and bone metastasis

#### Depletion
- **Aptamers targeting CD124 (IL-4Rα)**
  - Mammary tumor (mouse)
- **Trabectedin (Allavena P. Cancer Cell 2013)**
  - Liposarcoma
- **Biphosphonates**
  - Mammary tumor (mouse)

#### Switch to M1
- **CpG-ODN + anti-IL-10**
  - Mammary tumor (mouse)
- **anti-CD40 (agonist)**
  - Pancreatic cancer
- **STAT6 inhibitors**
  - Mammary tumor (mouse)
- **p50 inhibition**
  - fibrosarcoma, B16, CRC (mouse)

Overview of MDSC involvement in myeloid cell differentiation in cancer

### Table 1 | Minimal phenotypic characteristics necessary to identify cells as MDSC.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Phenotype</th>
<th>Human (in PBMC fraction)</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MDSC (not sufficient for MDSC characterization)</td>
<td>Gr-1&lt;sup&gt;+&lt;/sup&gt; CD11b&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Total (mixed) MDSC</td>
<td>Not clearly determined</td>
</tr>
<tr>
<td>PMN-MDSC</td>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt; Ly6C&lt;sup&gt;+&lt;/sup&gt;Ly6G&lt;sup&gt;+&lt;/sup&gt;</td>
<td>PMN-MDSC</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;CD11b&lt;sup&gt;+&lt;/sup&gt;CD15&lt;sup&gt;+&lt;/sup&gt; (or CD66b&lt;sup&gt;+&lt;/sup&gt;)</td>
</tr>
<tr>
<td>M-MDSC</td>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt; Ly6C&lt;sup&gt;+&lt;/sup&gt;Ly6G&lt;sup&gt;-&lt;/sup&gt;</td>
<td>M-MDSC</td>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;CD14&lt;sup&gt;+&lt;/sup&gt;HLA-DR&lt;sup&gt;low&lt;/sup&gt;/CD15&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>eMDSC</td>
<td>Not clearly determined</td>
<td>e-MDSC</td>
<td>Lin&lt;sup&gt;-&lt;/sup&gt;(CD3/CD19/CD14/CD16)/HLA-DR&lt;sup&gt;-&lt;/sup&gt;/CD33&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

eMDSC, early-stage MDSC; MoMDSC, myeloid-derived suppressor cell; M-MDSC, monocytic-MDSC; PMN-MDSC, peripheral blood mononuclear cell; PMN-MDSC, polymorphonuclear-MDSC.

Although phenotype is the first necessary step for defining MDSC, please note that, it cannot be used as the sole parameter for distinction between PMN-MDSC and neutrophils and M-MDSC and monocytes.

It is important, whenever possible, to use cells from control mice or healthy donors as controls.
Myeloid-derived suppressor cells (MDSCs) can inhibit efficient antitumour T cell responses through a number of mechanisms.

## Pharmacological regulation of MDSC in cancer

<table>
<thead>
<tr>
<th>Therapeutic treatment</th>
<th>Type of cancer*</th>
<th>Molecular events</th>
<th>Effects on myeloid cells</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroaspirin</td>
<td>Colon carcinoma</td>
<td>Downregulation of ARG1, INOS and peroxynitrite in MDSCs</td>
<td>Inhibition of MDSC suppressive effects</td>
<td>172</td>
</tr>
<tr>
<td>Phosphodiesterase-5</td>
<td>Mammary carcinoma, colon carcinoma and fibrosarcoma</td>
<td>Downregulation of ARG1, INOS, and CD124 (IL-4Ra) in MDSCs</td>
<td>Inhibition of MDSC suppressive effects</td>
<td>96</td>
</tr>
<tr>
<td>Inhibitors (ildenaftil and rilastil)</td>
<td>Fibrosarcoma and thymoma</td>
<td>Downregulation of ARG1, INOS, and peroxynitrite in MDSCs; expression of nitrated or nitrosylated CCL2</td>
<td>Inhibition of MDSC suppressive effects; increased CD8+ T cell/MDSC ratio in tumours</td>
<td>102</td>
</tr>
<tr>
<td>ATR (an NO donor based on the furoxan molecule)</td>
<td>Colon carcinoma, lung carcinoma and thymoma</td>
<td>Inhibition of ROS production by MDSCs</td>
<td>Inhibition of MDSC suppressive effects</td>
<td>173</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Fibrosarcoma and colon, breast, lung and kidney cancer; human renal cell carcinoma</td>
<td>Possible KIT blockade; STAT3 inhibition; GM-CSF confers resistance by activating STAT5 in intratumoral MDSCs</td>
<td>Inhibition of MDSC expansion in lymphoid organs but not in the tumour stroma; modest inhibition of MDSC population expansion in patients</td>
<td>174–178</td>
</tr>
<tr>
<td>Tyrosine kinase inhibitor (sunitinib)</td>
<td>Mammary carcinoma, mesothelioma, lung carcinoma and gliona</td>
<td>Downregulation of PGE2, ARG1, ROS and CCL2 and increase in the expression of CXCL10 by MDSCs</td>
<td>Inhibition of MDSC suppressive effects</td>
<td>137,162, 179</td>
</tr>
<tr>
<td>Cyclooxygenase 2 inhibitors (SC58236, SC58125 and celecoxib)</td>
<td>Colon carcinoma</td>
<td>Blockade of the KIT–SCF interaction</td>
<td>Inhibition of MDSC population expansion</td>
<td>180</td>
</tr>
<tr>
<td>KIT-specific antibody</td>
<td>Mammary carcinoma</td>
<td>Blockade of CSF1R and Kit</td>
<td>Inhibition of TAM recruitment</td>
<td>181</td>
</tr>
<tr>
<td>CSF1R and KIT receptor tyrosine kinase inhibitor (PLX3397)</td>
<td>Mammary carcinoma</td>
<td>Interference with CCL2 binding to CCR2 and with VEGF-A upregulation</td>
<td>Inhibition of metastatic spread by targeting inflammatory monocytes and macrophages</td>
<td>50</td>
</tr>
<tr>
<td>CCL2-specific monoclonal antibody</td>
<td>Mammary carcinoma</td>
<td>Reduction in VEGF and pro-MMP9 serum levels</td>
<td>Inhibition of MDSC population expansion</td>
<td>182,183</td>
</tr>
<tr>
<td>Amino-bisphosphonate (zoledronate)</td>
<td>Lymphomas and sarcoma</td>
<td>NOS downregulation</td>
<td>Changes in MDSC subset distribution</td>
<td>184</td>
</tr>
<tr>
<td>Very small size proteoliposomes</td>
<td>Breast cancer</td>
<td>Interference with the chemokines CXCL12 and CXCL5</td>
<td>Altered recruitment of immature myeloid cells to the tumour</td>
<td>134</td>
</tr>
<tr>
<td>Antagonists of CXCR2 (S-265610) and CXCR4 (AMD3100)</td>
<td>Various mouse and human tumours in nude mice</td>
<td>Interference with prokineticin 2 pleiotropic activity</td>
<td>Inhibition of polymorphonuclear MDSC population expansion and recruitment to tumour and pre-metastatic niches</td>
<td>68,69</td>
</tr>
<tr>
<td>Prokineticin 2-specific antibody</td>
<td>Various human and mouse tumours in nude mice</td>
<td>Interference with prokineticin 2 pleiotropic activity</td>
<td>Inhibition of polymorphonuclear MDSC population expansion and recruitment to tumour and pre-metastatic niches</td>
<td>68,69</td>
</tr>
<tr>
<td>CSF1R antagonist (GW2580)</td>
<td>Lung carcinoma and prostate cancer</td>
<td>CSF1R interference; downregulation of ARG1 in MDSCs; reduction in VEGF and MMP9 levels in the tumour</td>
<td>Inhibition of the expansion of MDSC and macrophage populations and their recruitment to the tumour</td>
<td>185</td>
</tr>
<tr>
<td>VEGF-Trap (afiblercept), VEGF-specific antibody (bevacizumab (Avastin; Genentech/Roche))</td>
<td>Various human solid tumours and human metastatic renal cell cancer</td>
<td>VEGF interference blocks tumour growth</td>
<td>Increased functional maturation of DCs</td>
<td>186,187</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>Lung cancer and breast cancer</td>
<td>MDSC apoptosis</td>
<td>Inhibition of MDSC population expansion</td>
<td>127,188</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>Thymoma</td>
<td>MDSC apoptosis</td>
<td>Inhibition of MDSC population expansion</td>
<td>189</td>
</tr>
<tr>
<td>Doxorubicin–cyclophosphamide</td>
<td>Human breast cancer</td>
<td>May induce MDSC apoptosis</td>
<td>Weak inhibition of MDSC population expansion</td>
<td>88</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>Mammary carcinoma</td>
<td>MDSC apoptosis; differentiation of surviving cells to M1 macrophages</td>
<td>Inhibition of MDSC population expansion; macrophage polarization to M1 phenotype</td>
<td>100</td>
</tr>
<tr>
<td>All-trans retinoic acid</td>
<td>Sarcoma and colon carcinoma; human metastatic renal cell carcinoma</td>
<td>Differentiation of immature myeloid cells to mature leukocytes</td>
<td>Inhibition of MDSC accumulation</td>
<td>191,192</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Human head and neck cancer</td>
<td>Forced differentiation of CD34+ immature myeloid cells</td>
<td>Moderate inhibition of MDSC population expansion</td>
<td>193</td>
</tr>
<tr>
<td>Combined treatment with IL-12, CCL16, CpG DNA and an IL-10 receptor-specific monoclonal antibody</td>
<td>Lung cancer and breast cancer</td>
<td>Decreased levels of IL-10, CCL2 and TGFβ; increased levels of TNF, IL-15 and IL-18</td>
<td>TAM reprogramming</td>
<td>194,195</td>
</tr>
</tbody>
</table>
Combining immunotherapy with epigenetic drugs to tackle cancer

HDACs inhibitors
(e.g. trichostatin, suberoylanidine hydroxamic acid)

Table 1  Approved drugs that target epigenetic mechanism

<table>
<thead>
<tr>
<th>Company</th>
<th>Target</th>
<th>Agent</th>
<th>Indications</th>
<th>Development stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merck</td>
<td>HDAC</td>
<td>Zolinza (vorinostat) (suberoylanidine hydroxamic acid, aka SAHA)</td>
<td>Cutaneous T-cell lymphoma (CTCL)</td>
<td>FDA approved Oct. 2006</td>
</tr>
<tr>
<td>Celgene</td>
<td>HDAC</td>
<td>Isto &lt;sub&gt;d&lt;/sub&gt; (romidepsin) (formerly FK228, a cyclic peptide principally active against class 1 HDACs), nanomolar pot</td>
<td>CTCL</td>
<td>FDA approved Nov. 5, 2009</td>
</tr>
<tr>
<td>Celgene</td>
<td>DNMT</td>
<td>Vidaza (5-aza-dC)</td>
<td>MDS</td>
<td>FDA approved May 2004</td>
</tr>
<tr>
<td>Eisai Tokyo, sub licensed to Johnson &amp; Johnson</td>
<td>DNMT</td>
<td>Dacogen (decitabine)</td>
<td>MDS</td>
<td>FDA approved May 2006</td>
</tr>
</tbody>
</table>

DNA hypomethylating agents (DHA)
- 1° generation: Zebularine, 5-azacytidine, 5-aza-2’-deoxycytidine
- 2° generation: SGI-110 (guadecitabine)

HDACs inhibitors
(e.g. trichostatin, suberoylanidine hydroxamic acid)

“Combining immunotherapy with epigenetic drugs to tackle cancer”
PGE2-induced p50 NF-κB-mediated reprogramming of M-MDSC suppressive functions

Cytokine-mediated immunotherapy (IFNγ, IL-12)

IFNγ owns immunostimulatory (e.g. MHC, antiviral, antitumor) and immunoregulatory activities (IDO, NOS2, PD-L1)
Entinostat was approved as HDAC inhibitor by the FDA, for the treatment of rare T cell lymphomas.

Azacitidine and Decitabine, both of which affect DNA methylation have already been approved by the FDA for use in myelodysplastic syndrome.
Emergency Hematopoiesis in Cancer

CSFs

BM

neutrophil

HSC

G-CSF (eIF4EBP, STAT3)

Arginase I (STAT6)

INOS (STAT1)

M-CSF (PU.1, IRF8)

TUMOR

Host macroenvironment

PMN-MDSC

M-MDSC

Tumor microenvironment

monocyte

(M2) TAM

Strauss L et al Cancer Cell 2015
Role of RORC1 in the Expansion of MDSCs and TAMs During Tumor-Driven Emergency Hematopoiesis

A

![Graph showing tumor volume (cm³) over days for MN/MCA1 and Rorc1+/- > WT models.](Image)

B

![Bar graph showing number of metastasis (N) and tumor volume (cm³) over days for Chem-induced fibrosarcoma and Mammary carcinoma (MMTV-PyMT) models.](Image)

C

![Chart showing percentage of MDSCs (M-MDSC, PMN-MDSC) for WT and Rorc1+/- models, with significance bars.](Image)

D

![Images of H&E stained spleens showing IL-17A, IL-4R expression for WT and Rorc1+/- models, with scale bars.](Image)

E

![Bar graph showing percentage of cells and number of metastasis over days for PBS and SR1078 treated groups.](Image)

RORC agonist SR1078
RORC1+ myeloid cells promote tumor development

**Tumor promotion**

RORC1/RORγ

→

M-MDSC → PMN-MDSC

→

TAM (M2) → CD4+/CD8+

→

Lymphocyte → Cancer cell

**Tumor rejection**

RORC1/RORγ

→

Mono/Macro → Mature Neu

→

Lymphocyte → CD8+

→

CD8+ → Cancer cell

RORC1 Inhibitors

Strauss L et al Cancer Cell 2015
Reciprocal influence between anticancer therapies and tumor-associated myeloid cells
