A Decade in the Life of Tumor Immunology

Olivera J. Finn and Michael T. Lotze

University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261 (O. J. F.), GlaxoSmithKline Pharmaceuticals, King of Prussia, Pennsylvania 19406

The role of the immune system in the recognition and prevention or therapy of cancer remains a fascinating and important topic of research. The first in the series of Keystone Symposia on Cellular Immunity and Immunotherapy of Cancer was held in 1990 in Park City, Utah (1). Progress in tumor immunology and new developments in immunology that could impact on this field have been featured and appraised in these meetings at 3- or 4-year intervals (2, 3). The fourth meeting in this series was held in January 2000 in Santa Fe, NM. This special issue of Clinical Cancer Research is devoted to the major topics discussed at the meeting. It features original articles by cytokine-activated T cells that were not antigen specific but could kill tumor cells, the identification of tumor antigens focused the field on specificity. Years of experiments with tumors in mice taught two important lessons: antitumor immune responses can be tumor specific, and tumor growth can be prevented by immunization. Identification of tumor antigens in human tumors foreshadowed the future of tumor-specific immunization in people. This goal was very appealing and every newly identified tumor peptide brought that goal closer to reality. Already by the time of the second symposium, Phase I clinical trials in peptide-based cancer vaccines occupied a large portion of the meeting agenda. Progress in identification of new tumor antigens and their use in cancer immunotherapy continues to generate excitement in the field, and papers by Kao et al., Geiger et al., Beatty et al., Santin et al., Rudolf et al., Gajewski et al., Meeker et al., Zier et al., Pittet et al., and Romero et al. featured in this meeting participate searches in cancer immunotherapy.

Michael T. Lotze, MD
Chief Cellular Therapy Officer (CCO); Nurix Therapeutics
Email: mlotze@nurixtx.com
Cell: 412-478-3316

DECEMBER 29, 1993

387,144
2.18.21
Disclosures-Company & Consultant

• Clinigen (IL-2)

• Nurix (NextACT) Chief Cellular Therapy Officer

• Checkmate, Inc. (TLR9/Checkpoints)

• iRepertoire, Inc. (Hudson Alpha Institute)

Before we begin, I would like to remind you that my comments and responses to questions reflect my views only and are not necessarily those of Nurix, Nurix’s management team or Nurix’s board of directors.

Moreover, my comments and responses are made only as of today, and may include statements related to Nurix, Nurix’s product candidates and Nurix’s business that are forward-looking statements under the federal securities laws. Actual results may differ materially from those contained in or implied by these forward-looking statements due to risks and uncertainties associated with Nurix’s business. For a discussion of the material risks and other important factors that could impact Nurix’s actual results, please refer to Nurix’s SEC filings, which can be found on the Nurix website.

Finally, any statements I may make about Nurix, Nurix’s product candidates and Nurix’s business are not intended to contradict or modify Nurix’s existing public disclosures.
Evidence that cytokines play a role in rheumatoid arthritis

Fionula M. Brennan, Iain B. McInnes

November 3, 2008


Rheumatoid Arthritis

- Pro-inflammatory
- Activates NF-kB
- Induces cell death

TNF-α

- Pro-inflammatory
- Increases production of neutrophil attractants
- Works synergistically with IL-1 and TNF-α

IL-17

- Pro-inflammatory
- Induces expression of MHC II
- Promotes Th1 differentiation

IL-6

VEGF
PDGF

- Pro-angiogenic factor
- Growth factor
- Pro-inflammatory
- Osteoclast stimulation

IL-8
RANTES
MCP-1

- Chemoattractant
- Recruitment of leukocytes to inflamed tissue

IFN-γ

- Bone remodeling
- Breakdown of extracellular matrix

MMP3
MMP1
MMP9

IL-10

- Anti-inflammatory
- Blocks NF-kB signaling
- Alternative activation of macrophages

TGFβ

- Anti-inflammatory
- Blocks activation of lymphocytic and monocytic phagocytes
- Treg/Th17 cell differentiator

T cell

IL-21

Plasma cell differentiation

IL-6

- Pro-inflammatory
- Osteoclast stimulation

Auto-antibodies

IL-1β

- Enhances IL-17 expression
- Pro-inflammatory

Mac

IL-6
+TGFβ

IL-12

IL-23

- Enhances cytotoxic function of CD8 and NK cells

VEGF
PDGF

- Pro-inflammatory
- Activates NF-kB
- Induces cell death

TNF-α

- Pro-inflammatory
- Increases production of neutrophil attractants
- Works synergistically with IL-1 and TNF-α

IL-17

- Pro-inflammatory
- Induces expression of MHC II
- Promotes Th1 differentiation

IL-6

IFN-γ

- Bone remodeling
- Breakdown of extracellular matrix

MMP3
MMP1
MMP9

- Chemoattractant
- Recruitment of leukocytes to inflamed tissue

Auto-antibodies

IL-1β

- Enhances IL-17 expression
- Pro-inflammatory

Mac

IL-6
+TGFβ

IL-12

IL-23
Cancer Immunotherapy

Figure 1: Dual mechanism of action of interleukin-2

APC = antigen-presenting cell; HD-IL-2 = high-dose interleukin; NK = natural killer; MHC = major histocompatibility complex. TCR = T cell receptor. Source: Cancer Immunotherapy

CANCER IMMUNOTHERAPY WITH IL2 CURRENT STATUS AND FUTURE DEVELOPMENTS

Shilpa Gupta, Neeraj Agarwal

Oncology & Hematology Review, 2016;12

Nature Reviews Cancer 20 04DOI:10.1038/nrc1252

Cytokines in cancer pathogenesis and cancer therapy

• Glenn Dranoff
IL-2: The First Effective ImmunoRx for Human Cancer

Steven A. Rosenberg


DOI: https://doi.org/10.4049/jimmunol.1490019
Foundations of Cancer Therapy

Surgery
ChemoRx
Radiation
• Other Targets:
  • Signal Transduction
  • Autophagy
  • Oncogenes
  • Tumor Suppressor Genes

T Cells
• Immune Stimulants
  • IL2-1st ICI
  • Checkpt Inhibition
    • CTLA4, PD1, PDL1
  • Adoptive Cell Therapy
    (CARTs, TIL)
  • DC, Other Vaccines

Anti-VEGF
• Chloroquine
• Platelet Derived Growth Factor (PDGF)
• Fibroblast Growth Factor (FGF)
• Tie 2 Kinase stabilization
• ß-phosphatase inhibitors

Tumor Microenvironment
• Stromal Component
• Tumor Associated Macrophage
• MDSCs
• Neutrophils
Disturbance of function (*functio laesa*): the legendary 5th cardinal sign of inflammation, added by Galen to the four cardinal signs of Celsus=Autophagy.


---

*Calor* (warmth), *dolor* (pain), *tumor* (swelling) and *rubor* (redness), and (later) loss of function Celsus, *De Medicina*

Cancer is the endstage of chronic inflammation in adults
Evolution of Cancer Treatments: Surgery (ACS)

Ancient physicians and surgeons knew that cancer would usually come back after it was surgically removed. The Roman physician Celsus wrote, “After excision, even when a scar has formed, none the less the disease has returned.”

Successful Surgical Excision is Immunotherapy
Successful Radiation Therapy is Immunotherapy
Successful Hormonal Therapy is Immunotherapy
Successful Chemotherapy is Immunotherapy
A cold-blooded view of adaptive immunity
Nature Review Immunology (2018)
Martin F. Flajnik Department of Microbiology and Immunology
University of Maryland Baltimore

Innate immunity in single cell organisms (4byrs) evolved:
0) TLRs, NLRs, ALRs, RLRs, STING (STRANGER-Janeway)
1) engulf organisms by xenophagy;
2) develop ‘adaptive’ immunity involving CRISPR/Cas9;
3) toxic metabolites (yeast: alcohol; plants: salicylic acid and jasmonic acid) that limit pathogens;
4) SIRPα/CD47
5) PIR’s

NK cells and MHC-like molecules (Maternal/Fetal interface):
*Botryllus schlosseri* is a colonial

DANGER-Matzinger

DANGER- Non-self
Adapted from: Lesterhuis et al., Nature Reviews, 2011

History of Immunotherapy

- First report of allogeneic bone marrow transplant
- Hypothesis of cancer immunosurveillance by Burnet
- Description of immune infiltrates in tumors by Virchow
- Discovery of MHC class I-Restricted CD8+ T cell Recognition by Zinkernagel and Doherty
- First study with IL-2
- Rediscovery of the regulatory T cell by Sakaguchi
- BCG in bladder cancer
- HPV vaccine in VIN
- Sarcoma receptors
- Imiquimod used to treat VIN


Fraietta, Joseph A <jfrai@upenn.edu>
The lymphocyte as a factor in natural and induced resistance to transplanted cancer.

Hence, it would seem fair to conclude that the lymphocyte is a necessary factor in cancer immunity – James B. Murphy and John J. Morton (Murphy and Morton 1915)
Rearranged Receptors in B and T Cells

Susumu Tonegawa Professor, Nobel Laureate
(1987 Nobel Prize In Physiology or Medicine)
Massachusetts Institute of Technology
Massachusetts, US


A Convergent Immunological Holy Trinity of Adaptive Immunity in Lampreys: Discovery of the Variable Lymphocyte Receptors

Martin F. Flajnik

doi: 10.4049/jimmunol.1800965

*J Immunol* 2018; 201:1331-1335
Zeev Pancer and Max Cooper With a Larval Lamprey at UAB
Strictly Private & Confidential

Inflamma™ Therapeutics

>500 Million Years of Adaptive Immunity-Wu Xing
The Immunologic Big Bang

Antibody-B cells
(1986-Melanoma)
Shape/FcR

NK Cells (1980-LAK)-Stress Ligands/MHC−/−

NKT Cells (2009)
Lipids/CD1

αβ T cells (1988-TIL)
Linear Peptides/MHC

γδ T cells (2007)
Metabolites

"Wǔ zhǒng liúxíng zhī qì" (五種流行之氣) or "the five types of chi dominating at different times".
The Hellström Paradox

- A paradox lies at the heart of cancer.
- Coursing through many tumors are legions of immune cells, including the T cells that should be fighting the cancer.
- Yet these T cells are typically dysfunctional — they stop working and let the tumor grow with abandon.
- Scientists have a name for this conundrum: the Hellström paradox, after Ingegerd and Karl Hellström, the immunologists who first drew attention to it more than 50 years ago.
Original Hallmarks of Cancer

Therapeutic Targeting Of the Hallmarks

Emerging Hallmarks

Enabling Characteristics

Aerobic glycolysis inhibitors

Deregulating cellular energetics

Resisting cell death

Genome instability & mutation

Inducing angiogenesis

Activating invasion & metastasis

Tumor-promoting inflammation

Avoiding immune destruction

Enabling replicative immortality

Selective anti-inflammatory drugs

Immune activating anti-CTLA4 mAb

Telomerase Inhibitors

Proapoptotic BH3 mimetics

Resisting cell death

Proapoptotic BH3 mimetics

Inhibitors of VEGF signaling

Inhibitors of HGF/c-Met

PARP inhibitors

Proliferative signaling

Sustaining growth suppressors

Evading growth suppressors

Hallmarks of Cancer: The Next Generation.
But Cantell and the Finnish Red Cross, now producing 250 billion units (5,285 quarts) a year, have provided the great bulk of pure interferon used for clinical studies on humans, including a $2 million batch bought last year by the American Cancer Society. “Production is the bottleneck,” says Cantell, who finds it “stupid and irritating” that until recently nobody else has tried to produce the substance in large-scale volume.
History of SITC


• 1985 Cytokine Therapeutics – 1st Annual Meeting of SBT (1986) in Williamsburg

• 1990’s Antibody (Her2, CD20, VEGF, etc.) Therapeutics – First Primer on Tumor Immunology (1998, Pittsburgh)

• 2000’s Cancer Vaccines - iSBTc (2002); SITC (2010)

• 2010’s Cell Therapies (TIL, CART, DC, NK/NKT, etc.) - Journal for ImmunoTherapy of Cancer (2013)

• 2015 Checkpoint Inhibitors; Oncolytic Viruses/Cytokines

• 2021+ The Future Just Ain’t What it Used to be (Yogi Berra) – 1st Cancer Immunotherapy Winter School (2019); 1st SCION Workshop (2022)
The First Winter Course SITC 2019 Mesa AZ

The First SCION 2022
Cancer Immunotherapy

2013

2014
In a major win for Boston-based Dana-Farber Cancer Institute, a federal court ruled that one of its researchers should be listed as an inventor on six patents that are believed to be worth billions of dollars.
The Critical Need for More Effective Immunotherapies for Solid Tumors

Adapted from NEJM Dec 21 2017
Before There were Checkpoints

Virchow: Immune infiltrates
Burnet: Immune-surveillance
Rosenberg: IL-2 and LAK cells
IDEC
Maloney: Rituximab
Bendani: Anti-idiotype vaccination


Coley’s toxin
Morales: BCG
Lejeune: Isolated limb perfusion
Slaman: Trastuzumab
Complete remission of a sarcoma in a patient after 2 episodes of erysipelas caused by *streptococcus pyogenes*

William Coley, 1893
E. Donnall Thomas
The Nobel Prize, 1990

first successful HSCT in treatment of acute leukemias

First 40 Yrs of Cancer Immunotherapy

IL-2 Activate NK/LAK Cells Infused

IL-2 Given to Patients - The First Checkpoint Inhibitor

Tumor Infiltrating Lymphocytes (TILs) And gene therapy (PD-1/PD-L1)

IL-4 given to patients; MART-1/Melan A

IL-12 given to patients

IL-12 Gene Therapy

Dendritic Cells Given To Patients

HMGB1 as the Ur-Cytokine; regulates autophagy and apoptosis


T-cells & TCGF | Tumor Vaccines | Innate Immunity Checkpoints

Pubmed: > 387,294 articles - Tumor Immunology

2.19.21
Unpacking Current Immunotherapies to Drive T-cell Responses

Identify potential neoantigens

Induce or expand neoantigen specific T cells

Provide checkpoint blockade

Interleukin 2

Identify reactive subcultures

Clone 1-5 TCR into lentiviral vectors;
confirm reactivity to neoAg

Transfect Peripheral Blood T-cells

Deliver to Patient

A

Induce tumor cell destruction
Cytokines are medically relevant endogenous small (~15kDa) proteins

Cytokine-based therapies in human disease

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Brand name</th>
<th>Status</th>
<th>Indication</th>
<th>Year of 1st FDA Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>Proleukin</td>
<td>Approved</td>
<td>Cancer</td>
<td>1992</td>
</tr>
<tr>
<td>IL-11</td>
<td>Neumega</td>
<td>Approved</td>
<td>Thrombocytopenia</td>
<td>1994</td>
</tr>
<tr>
<td>EPO</td>
<td>Epogen</td>
<td>Approved</td>
<td>Anemia</td>
<td>1989</td>
</tr>
<tr>
<td>GCSF</td>
<td>Neupogen</td>
<td>Approved</td>
<td>Myelosuppression from chemo</td>
<td>1991</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Leukine</td>
<td>Approved</td>
<td>Myelosuppression from chemo</td>
<td>1991</td>
</tr>
<tr>
<td>IFN-α</td>
<td>Intron-A</td>
<td>Approved</td>
<td>Hepatitis, Cancer</td>
<td>1991</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Betaseron</td>
<td>Approved</td>
<td>Multiple sclerosis</td>
<td>1993</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Actimmune</td>
<td>Approved</td>
<td>Granulomatosis</td>
<td>1990</td>
</tr>
<tr>
<td>IL-7</td>
<td>Clin dev</td>
<td>Clin dev</td>
<td>Cancer, anti-viral</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>Clin Dev</td>
<td>Clin Dev</td>
<td>Cancer, anti-inflammatory</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>Clin dev</td>
<td>Clin dev</td>
<td>Cancer, anti-viral</td>
<td></td>
</tr>
<tr>
<td>IL-15</td>
<td>Clin dev</td>
<td>Clin dev</td>
<td>Cancer</td>
<td></td>
</tr>
<tr>
<td>IL-21</td>
<td>Clin dev</td>
<td>Clin dev</td>
<td>Cancer</td>
<td></td>
</tr>
</tbody>
</table>
Dupont 1983
Taniguchi, T 1983
Roche 9/84
Cetus-PEG
Chiron 1990
Novartis
Prometheus
Nestle
Clinigen

Return of Jonah
John Wehrle 1980

Paging Dr. Crick
John Wehrle
Proof of Principle:
Deep responses produce remissions

High Dose IL-2 Immunotherapy

- Approved in patients with melanoma and kidney cancer.
- Significant ‘toxicity’.
- Associated with ‘cytokine storm’.
- iNOS blockers, sTNF-R or IL-1Ra have yielded limited reduction in side effects.
- IL-2 treatment is associated with a ‘systemic autophagic syndrome’ and temporally limited tissue dysfunction.

AR. Chavez, X Liang, MT Lotze.
Cytokine Working Group
CWG: The Abbreviated History (SITC 2018)

David McDermott, MD
Beth Israel Deaconess Medical Center
Dana Farber/Harvard Cancer Center
Harvard Medical School
Second Randomized Treatment with Interleukin 2 as Immunotherapy for Cancer

Fig 2. Survival of patients completely responding to high-dose versus low-dose intravenous interleukin-2.


Induction of Systemic and Therapeutic Antitumor Immunity Using Intratumoral Injection of Dendritic Cells Genetically Modified to Express Interleukin 12

Yasuhiro Nishioka, Motohiro Hirao, Paul D. Robbins, Michael T. Lotze, and Hideaki Tahara

Departments of Surgery [Y.N., M.H., M.T.L., H.T.] and Molecular Genetics and Biochemistry [Y.N., M.H., P.D.R., M.T.L., H.T.], School of Medicine, University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, Pennsylvania 15213
Cheever, Greenberg, Fefer

The “Early Years” (1970’s-80’s): Developing T cell therapy in mouse models

- Having very good colleagues early in your career really makes a difference

Mac Cheever
Tumor Infiltrating Lymphocyte (TIL) Therapy – Iovance (Instil, Myst, NxACT-Nurix, Achilles...)

Lotze, US Patent 20190083539 2018
FROM THE ANALYST’S COUCH

The global pipeline of cell therapies for cancer

Jia Xin Yu, Vanessa M. Hubbard-Lucey and Jun Yang

822 | NOVEMBER 2019 | volume 18

www.nature.com/nrd

<table>
<thead>
<tr>
<th>Therapy type</th>
<th>Year</th>
<th>2019</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR-T cell</td>
<td></td>
<td>264</td>
<td>194</td>
</tr>
<tr>
<td>NK cell and NKT cell</td>
<td></td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Novel T cell technology</td>
<td>2019</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2018</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>TAA/TSA-targeted T cell</td>
<td></td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>TCR-T cell</td>
<td>2019</td>
<td>58</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2018</td>
<td>45</td>
<td>13</td>
</tr>
<tr>
<td>TIL cell</td>
<td>2019</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2018</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Other cell therapies</td>
<td>2019</td>
<td>85</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2018</td>
<td>56</td>
<td>14</td>
</tr>
</tbody>
</table>

Development stage:
- Marketed
- Phase III
- Phase II
- Phase I
- Preclinical
## Molecular Targets for Cell Therapy

<table>
<thead>
<tr>
<th>Target</th>
<th>Year</th>
<th>2019</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>TAA</td>
<td></td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>BCMA</td>
<td></td>
<td>5</td>
<td>34</td>
</tr>
<tr>
<td>HER2</td>
<td></td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>GD2</td>
<td></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td></td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>CD20</td>
<td></td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Mesothelin</td>
<td></td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>EBV</td>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>CD33</td>
<td></td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

### Diagram Description

- **X-axis**: Number of active agents
- **Y-axis**: Year
- **Legend**:
  - CAR-T cell
  - NK and NKT cell
  - Novel T cell technology
  - TAA/TSA-targeted T cell
  - TCR-T cell
  - TIL cell
  - Other cell therapies

---

822 | NOVEMBER 2019 | volume 18 www.nature.com/nrd
Ipilimumab/CTLA-4 Antibody for melanoma patients-2010

**Graph:**
- **X-axis:** Months
- **Y-axis:** Proportion alive
- **Data Points:**
  - 12 months: 31%
  - 24 months: 26%
- **Legend:**
  - X = censored data

**References:**
- The NEW ENGLAND JOURNAL of MEDICINE
  - Improved Survival with Ipilimumab in Patients with Metastatic Melanoma
  - Authors: [List of authors]
  - Publication Date: [Date]

**Note:**
- The graph shows the survival rates of patients treated with Ipilimumab, with a significant improvement at 24 months compared to earlier points in the study.
**PD1 AB RESULTS: RCC PATIENTS**

*Patients treated at the 10 mg/kg dose*
Immunotherapy Drugs Slow Skin Cancer That Has Spread to the Brain

NYT August 22, 2018
Operational Taxonomic Units

Merck Pembro Anti-PD1 + *Bifidobacterium longum*