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2021 NCI Drug Development Workshop
How to Advance A Therapeutic Candidate from Bench to Bedside
Goals*

- Historical perspective
- Re-familiarization of the principles of Cancer Immunology
- Range of Agents
- Creating a framework for IO clinical development

* These are personal perspective and does not represent NCI policy
Historical Perspective

- Observations
- Historical Narratives
Cancer Treatment Paradigms: Theory to Therapy

Hypothesis: Radiation damage can Treat Cancer 19-20th Century
1896: E. Grubbe: Rx of breast cancer
1909: C. Regaud: Chromatin target of XRT
1922: H. Coutard: Fractionated XRT

Hypothesis: Surgery as curative intent 19-20th Century
1882: W. Halsted: Sufficient Local Removal of the tumor to cure cancer
1954: O. Wangensteen: Rescue benefit with metastatectomy
1958: B. Fisher: Cancer’s metastatic behavior dictates outcome and thus less surgery is more
1995: S. Hellman & R. Weichselbaum: Oligometastasis hypothesis

Hypothesis: Unencumbered cell division causes cancer: Inhibit tumor cell division – Chemotherapy 20th Century
1940: L. Goodman & A. Gilman – Nitrogen Mustard
1948: S. Farber: Anti-Folate
1955: National Cancer Chemotherapy Service Center
1965: J. Holland, E. Freirech, E. Frei: Combination chemotherapy

1941: C. Huggins and C. Hodges: Testosterone inhibition CaP
1971: M. Cole: anti-estrogens in BrCa
2001: B. Druker: Inhibition of BCR-ABL by TKI in CML

Hypothesis: Immune therapy for cancer: Immune recognition and destruction of cancer– 18th - 21st Century
Immunotherapy for Cancer: Induce inflammation
A 18\textsuperscript{th} and 19\textsuperscript{th} Century Paradigm

1768: G. White: Use of poultice made from decaying toads for breast cancer

1844: S. Tanchou: Treatise on breast cancer: spontaneously or induced Gangrene as a therapeutic agent in cancer

1886: A. Verneuil: Suppuration after surgery; Congress of Surgery Paris

1891: W.B. Coley: Annals of Surgery describing Toxins: Initially used deliberate infection and in 1893 he began combining killed \textit{Streptococcus pyogenes} and \textit{Serratia marcescens} ---- 1985 mammalian TLR

Dr. William B. Coley (1862-1936)
Chief of the Bone Sarcoma Unit at Memorial Hospital in New York
Historical Development of Interferon

- First described by Yasu-ichi Nagano and Yasuhiko Kojima in 1954, reported in French (Seances Soc. Biol. Fil). - viral inhibitory factor

1960 Space Phase I Unit: Interferon
At year’s end, what’s new with interferon?

Last year the American Cancer Society (ACS) announced that it was spending $2 million to purchase 40 billion units of interferon from the Finnish Red Cross, enough for American oncologists to study interferon’s anticancer activity in about 150 patients. on widely available. The current price is high because all present methods involve collecting interferon from supernatant fluids of cells grown in culture, a time- and space-intensive procedure.  
This same problem, combined with inevitable losses

ACS commitment of $2M
Immunology captures the Public Interest
Principles of Cancer Immunology
Activation of a T Cell

Signal 1
MHC TCR

Signal 2
CD80/86 CD28

Signal 3
Cytokines

Adopted from SITC
Basic Immunology: Immune Response Kinetics

**Diagram:***

(A) Acute antigen/functional memory

(B) Chronic antigen/exhaustion

(C) Functional comparisons

**Table:***

<table>
<thead>
<tr>
<th></th>
<th>Memory</th>
<th>Exhausted</th>
<th>Reinvigorated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative potential</td>
<td>+++</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>Cytokine production</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Killing capacity</td>
<td>+++</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>Dependence on antigens for persistence</td>
<td>-</td>
<td>+++</td>
<td>?</td>
</tr>
<tr>
<td>Responsiveness to IL-21/15</td>
<td>+++</td>
<td>-</td>
<td>?</td>
</tr>
</tbody>
</table>

Pauken & Wherry
Trends in Immunology
2015
CD4 Differentiation to Effector Cells

- **Th1**
  - T-bet
  - IFN-γ, TNF
  - Antiviral, bacterial immunity

- **Th2**
  - GATA-3, STAT-6
  - IL-4, IL-13
  - Immunity to extracellular parasites

- **Treg**
  - Foxp3
  - TGF-β
  - Regulation/tolerance

- **Th17**
  - RORγT
  - IL-17
  - Inflammation, fungal immunity

- **T_{FH}**
  - Bcl-6
  - IL-21
  - T cell help for B cells

IL-12, IL-4, TGF-β, IL-6, IL-23, CXCR3, CCR5, CCR4, CCR5

- **Naive T**
  - CCR7

- **Primed T**
  - CXCR5

- **Adopted from SITC**
Hierarchical Expression of CD8 T Cell Immune Checkpoints

Adapted from Kishton RJ, Sukumar M, Restifo NP, Cancer Metabolism Review, 2017.
Exhausted CD8⁺ T Cell

- Reduced proliferative capacity
- Reduced production of effector cytokines
- Reduced cytotoxicity
- Elevated and sustained expression of multiple inhibitory receptors

Gene expression and chromatin changes

Induction of the exhausted CD8⁺ T cell phenotype

Immune Response to Chronic Viral Infections
Anti-Tumor Immune Response
CAR T Cell Functions in Solid Tumors

IL-2 ↓  TNF-α ↓  IFN-γ ↓  Granzyme B ↓
Heterogeneity Model of CD8 T cells

SLEC: Short lived effector cells
Tmp: T memory precursors

Yuki Muroyama & E. John Wherry 2021
MHC = Major Histocompatibility Complex

Lipid antigens presentation to T cells through CD1 (related to the class I MHC molecules)
Central and Peripheral Tolerance

**Central Tolerance**
- For T cells it occurs in the thymus
- Some survive as regulatory (suppressor) T cells while others escape to peripheral tissues

**Peripheral Tolerance**
- Self-reactive T cells are suppressed by regulatory T cells
- CTLA-4 and PD-1, among other molecules play a role in maintaining self-reactive T cells from becoming activated (anergic)

Adopted from SITC
Immune Cycle
Locations of Immune Checkpoint Control

From Chen & Mellman

Central

Peripheral

CTLA4

PD1-PDL: other IC
Tumor Immungenicity: Immune Surveillance & Immunoediting

Ehrlich 1909
Burnet and Thomas 1957
Schreiber 2002

BM, bone marrow; iDC, immature dendritic cell; Mj, macrophage; SLN, sentinel lymph node;
TAM, tumor-associated macrophage; TA, tumor antigens; TDSFs, tumor-derived soluble factors;
TE, effector T cell; TiDC, tumor-associated iDC; Tregs, regulatory T cells.

From FS Hodi
Range of Agents
## Range of Agents:
The Landscape of Immunotherapy Targets & Agents for Cancer in 2021

<table>
<thead>
<tr>
<th>Adoptive</th>
<th>Depletion</th>
<th>Antibody</th>
<th>&quot;Kines&quot;</th>
<th>Metabolomes</th>
<th>Environ</th>
<th>Intratumoral</th>
<th>Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>Treg</td>
<td>ICI</td>
<td>IFN α, β, &amp; γ</td>
<td>Adenosine</td>
<td>Gut Microbio</td>
<td>TLRs</td>
<td>Targets Modification</td>
</tr>
<tr>
<td>NK cells</td>
<td>MDSC</td>
<td>BiTEs/TriTEs</td>
<td>IL-2</td>
<td>Tyrosine (IDO)</td>
<td>Other Microbio</td>
<td>oncolysates</td>
<td>Epigenetic Agents</td>
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<tr>
<td>Dendritic Cells</td>
<td>TAMs</td>
<td>Conjugate</td>
<td>Designed</td>
<td>Adrenergic Stress</td>
<td>Chemokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34 Cells</td>
<td>Lymphodeplete</td>
<td>Cytokine block</td>
<td>G- &amp; GM-CSF</td>
<td>Glucocorticoid Stress</td>
<td>Cytokines</td>
<td></td>
<td>Germline</td>
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<tr>
<td>Genetic Mod</td>
<td>CAFs</td>
<td>Chemo block</td>
<td>IL-7</td>
<td></td>
<td></td>
<td></td>
<td>Genes</td>
</tr>
<tr>
<td>Allo</td>
<td>Exosomes</td>
<td>Integrin Block</td>
<td>IL-12</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>ATP-idase (CD39, 73)</td>
<td>IL-15</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ADCC</td>
<td>TNFα</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-4</td>
<td></td>
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</tr>
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</table>
Engineered T cells

- Chimeric Antigen Receptor
  - Starting with Auto (Allo) T cells
  - CAR engineering
    - MHC independent
    - Receptor/Target Surface
    - Target specificity
    - Transmembrane bridge
    - Humanization of sVC domain
    - Signaling domains
  - Knockout (armored)
    - TCR
    - HLA
    - Other

- TCR T cells
  - Starting with Auto (Allo) T cells
  - Targets can be intracellular immune epitopes
  - MHC specific
  - Target specificity
  - Knockout (armored)
    - TCR
    - HLA
    - Other
Immune Checkpoints

Interactions with antigen-presenting cells that regulate T cell responses

The Immune Synapse

VISTA in the TME (Yum & Hong 2021)
Creating a framework for IO clinical development
Combination Drug Therapy

Doroshow JH and Simon RM 2017

Combination Principles

- Drugs used in combination should cause measurable tumor regressions when employed individually – **Not always the case in IO development**
- Each ought to demonstrate a different mechanism of action to minimize the development of resistance – **IO pathway dependent**
- The clinical toxicities of each compound should not overlap to permit their use in effective doses – **irAE are common across IO agents**
- Intensive intermittent treatment is preferred over continuous, low-dose therapy to enhance cytoreduction - **Maintenance of effector cell populations**
- Trial designed on systems biology: mechanistic understanding of drug action could facilitate a clinical-rial-design approach based on precise measures of biochemical heterogeneity from patient-derived materials - **Ditto for IO**
Meckel-Serres Law or Theory of Recapitulation
Ontogeny recapitulates Phylogeny (Haekel)
Applied to Cancer Immunology (Ernstoff 2000)

Antigen ➤ Presentation ➤ Activation ➤ Expansion ➤ Regulation ➤ Trafficking ➤ Exhaustion ➤ Escape ➤ Resistance

Key strategies to reinitiate the anti-tumour immune response according to each phenotype

## History of Combination IOs

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Indication</th>
<th>Author</th>
<th>Year</th>
</tr>
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<tbody>
<tr>
<td>IL-2, ATC</td>
<td>Mel. RCC</td>
<td>Rosenberg</td>
<td>1965</td>
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<tr>
<td>IL-2, IFNa</td>
<td>RCC</td>
<td>Atzopdien</td>
<td>1990</td>
</tr>
<tr>
<td>IFNa, IFNg</td>
<td>RCC</td>
<td>Ernstoff</td>
<td>1992</td>
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<tr>
<td>Biochemo</td>
<td>Mel</td>
<td>Ron</td>
<td>1994</td>
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<tr>
<td>IFNb, IL-2</td>
<td>RCC</td>
<td>Witte</td>
<td>1995</td>
</tr>
<tr>
<td>BiAb IFNg</td>
<td>Her2+</td>
<td>Lewis</td>
<td>2001</td>
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<tr>
<td>Ipi, IL-2</td>
<td>Mel</td>
<td>Maker</td>
<td>2005</td>
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<tr>
<td>IFNa, Vax</td>
<td>Mel</td>
<td>Mitchell</td>
<td>2007</td>
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<tr>
<td>IFNa, IL-2, Vax</td>
<td>RCC</td>
<td>Schwaab</td>
<td>2009</td>
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<tr>
<td>Ipi, gp100 Vax</td>
<td>Mel</td>
<td>Hodi</td>
<td>2010</td>
</tr>
<tr>
<td>Ipi, Nivo</td>
<td>Mel</td>
<td>Wolchock</td>
<td>2013</td>
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</table>
Dose Response Curve Considerations for IO agents
Tumor Microenvironment

Blood Vessel  TAF  Soluble Factors  Exosomes  Nerves  ECM

THE ROOM WHERE IT HAPPENS

DIRECT AE

EFFECCTOR CELL

REGULATORY CELLS

ORGAN TOXICITY  EXHAUSTION/DEATH

ANTIBODIES  MEMORY

EPITOPE SPREAD  CYTOKINES/CHIMOKINES RELEASE

TREATMENT

CYTOKINES/CHIMOKINES  DRUGS

ANTIBODY  ADOPTIVE CELL THERAPY (CAR, TCR)

ENGINEERED ANTIBODY (BITES, etc.)  SMALL MOLECULES

ARMED ANTIBODY

EFFECT

DIRECT AE

Blood Vessel  TAF  Soluble Factors  Exosomes  Nerves  ECM

NATIONAL CANCER INSTITUTE
Dose Response Considerations for IO agents

- Agent "infusion" Rate
- "Cellular Response" Rate 1
- Plasma Kinetics
- Plasma Kinetics 1
- Effector site Equilibrium
- Effector site Equilibrium 1
- Normalization constant
- Normalization constant 1
- Dose Response Curve 1
- Cellular Dose Response Curve 2

\[ r(t) = \text{Infusion rate} \]
\[ C_p(t) = [\text{Plasma}] \]
\[ C_e(t) = [\text{Apparent effector compartment}] \]
\[ C_{e,n}(t) = \text{normalized [Apparent effector compartment]} \]
\[ E(t) = \text{Effect} \]

\[ t = \text{time} \]
Biomarker Approaches to TME

a

Stable state | Pre-transition state | New stable state

Tissue state

Therapeutic response

Dynamic biomarkers

Tipping point

b

Non-responder

Immune checkpoint antibody

Responder

Dynamic biomarker associated with response
## Future Directions for Checkpoint Inhibition
**Single Agent Activity, in what setting, Recapitulate Immune pathways**

<table>
<thead>
<tr>
<th>Checkpoint Blockers &amp; Agonists</th>
<th>Small Molecules</th>
<th>Vascular Targets</th>
<th>Traditional Rx</th>
<th>Cytokines</th>
<th>Intratumor</th>
<th>Cellular &amp; Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination</td>
<td>Epigenetic</td>
<td>Vascular “Zip Code”</td>
<td>Immunogenic death</td>
<td>Inflammation</td>
<td>△ TME</td>
<td>TAg presentation</td>
</tr>
<tr>
<td>Sequence</td>
<td>IO Pathway targets</td>
<td>Normalization</td>
<td>IO impacts</td>
<td>Regulation</td>
<td>Abscopal</td>
<td>Starting Product</td>
</tr>
<tr>
<td>Resistance</td>
<td>△ TME</td>
<td>△ TME</td>
<td>△ TME</td>
<td>Migration</td>
<td></td>
<td>Product Survival</td>
</tr>
<tr>
<td>PK/PD/Toxicity</td>
<td>PK/PD/Toxicity</td>
<td>PK/PD/Toxicity</td>
<td>TKI regulation of IO pathway</td>
<td>PK/PD/Toxicity</td>
<td></td>
<td>PK/PD/Toxicity</td>
</tr>
<tr>
<td>Non classical CPs Micorbiome Adrenergic R</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Future Directions: Defining and Overcoming Resistance

- Primary Resistance
- Secondary Resistance
- Resistance after treatment discontinuation
Primary vs. Secondary Resistance
Clinical Data observations

PFS Landmark analysis of the most important studies in advanced melanoma
Resistance Mechanisms

A: No immune response
B: Immune response
   No Clinical Response
C: Resistant clones
D: Acquired immune resistance
Turan et al.
JITC 2018

**Color Key**

-4 -2 0 2 4
Row Z-Score

**Immune-active**

**Immune-silent**

**Ubiquitous:**
- Mesenchymal Transition
- SHC1/STAT3
- Barrier Molecules
- TAM receptors
- Hypoxia

**Immune enriched:**
- Immunogenic Cell Death
- IL23/Th17 axis
- Regulatory T Cells
- Checkpoints Cluster
- Myeloid Suppressor Cells
- IDO/INOS

**Ubiquitous***
- MAPK
- β-catenin

**Immune depleted:**
Integrated Approach to Overcome Resistance

Trafficking of T cells
The Median is not the Message: Stephen Jay Gould 1991

When diagnosed with abdominal mesothelioma he read that the 'median mortality' was eight months and concluded that most people would read such a statement as 'I will probably be dead in eight months.'

Dr. Gould’s observation:
Firstly that, biologically, it is variation that is the hard reality rather than imperfect measures for a central tendency.

Secondly, that even with knowledge of prognostic features it is often difficult at diagnosis to know whether any individual is going to be to the left or right of the median. As many curves are right-skewed some patients will survive a long time, which was the case with Gould who died of another cause some years later.
Contact Marc S. Ernstoff, MD: marc.ernstoff@nih.gov
Extra slides
PK Interferon by route

Kirkwood, Ernstoff et al 1985
PD of Interferon

Ernstoff et al 1984

Ernstoff et al 1985

Figure 2. Human bone marrow colony-forming granulocyte-macrophage cell assay for each patient and as mean ± SE (n = 11).
Fig. 1. Changes in lymphocyte number after 24-h IL2 infusions. The absolute lymphocyte count was determined for patients pre- and 24 h post-, IL2 treatment (Rx). The absolute number of lymphocytes staining with OKT3 (T-lymphocytes) or Leu 12 (B-lymphocytes) is shown, as well as the OKT3:OKT8 ratio. ○, 1 x 10^6 units of IL2 over 24 h; ●, 10 x 10^6 units of IL2 over 24 h.

Thompson JA et al 1987
IFNs acute phases

Lee and Ashkar 2018

Level of cytokine production

Hours post-infection

24

48

72

Antiviral state

IFN-β

CCL2

IL-18

IFN-α/β

IFN-γ

NK cell recruitment

Inflammatory monocyte recruitment

Th1 adaptive response

Ag expression

Ag processing

Co-stimulatory molecules

MHC expression

ILC2

immunopathology

IL-5, IL-6

IL-13
## Future Directions for Checkpoint Inhibition: Small Molecules

### Therapeutic monoclonal antibodies versus small molecule therapies

<table>
<thead>
<tr>
<th>Monoclonal antibodies</th>
<th>Small molecule therapies</th>
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<tbody>
<tr>
<td>Larger (~150kD); mainly extracellular</td>
<td>Smaller (&lt;1 kD); able to enter cells and cross blood-brain barrier</td>
</tr>
<tr>
<td>Target-specific</td>
<td>Less specific</td>
</tr>
<tr>
<td>Parenteral administration</td>
<td>Oral administration possible</td>
</tr>
<tr>
<td>Longer dosing interval (half-life: days to weeks)</td>
<td>Shorter dosing interval (half-life: hours)</td>
</tr>
<tr>
<td>Not eliminated via hepatic, renal or biliary routes</td>
<td>Elimination via hepatic, renal and/or biliary routes</td>
</tr>
<tr>
<td>Lower risk of drug-drug interactions</td>
<td>Drug-drug interactions possible</td>
</tr>
</tbody>
</table>