An Introduction to Cancer Treatment

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Lecture Outline

• Introduction to Cancer and Cancer Therapy
• Measuring the Response to Cancer Therapy
• General Principles of Cancer Pharmacology
  – Chemotherapy
  – Monoclonal Antibody Therapy
  – Immunotherapy
  – Molecularly Targeted Therapy
  – Hormonal Therapy
  – Stem Cell Therapy
• Mechanisms of Treatment Resistance
Introduction to Cancer and Cancer Therapy
What is Cancer?

- 100 different diseases
- Arises from any cell type
## Tumor Characteristics

<table>
<thead>
<tr>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Some degree of growth control</td>
<td>• Uncontrolled growth is hallmark</td>
</tr>
<tr>
<td>• Encapsulated</td>
<td>• Invasive</td>
</tr>
<tr>
<td>• Localized</td>
<td>• Metastatic</td>
</tr>
<tr>
<td>• Typical of cell of origin (&quot;differentiated&quot;)</td>
<td>• Atypical of cell of origin (&quot;undifferentiated&quot;)</td>
</tr>
<tr>
<td>• Indolent</td>
<td>• Anaplastic</td>
</tr>
<tr>
<td>• Non-recurrent</td>
<td>• Recurrent</td>
</tr>
</tbody>
</table>
Cancer: Scope of the Problem

• Cancer is a public health threat in the U.S.
  – Number 1 killer of Americans < 85
  – 1,400,000 new cases annually
  – 600,000 deaths annually
  – 1 patient dies every minute

• Cancer is increasingly common
  – 1 of 3 women
  – 1 of 2 men

• Cancer is expensive
  – $190 Billion per year in cancer care costs
Annual Estimated US Cancer Cases

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>33%</td>
<td>32%</td>
</tr>
<tr>
<td>Lung and bronchus</td>
<td>13%</td>
<td>12%</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>10%</td>
<td>11%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>7%</td>
<td>6%</td>
</tr>
<tr>
<td>Melanoma of skin</td>
<td>5%</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Kidney</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Oral Cavity</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>All Other Sites</td>
<td>17%</td>
<td>21%</td>
</tr>
</tbody>
</table>

33% Breast
13% Lung and bronchus
11% Colon and rectum
6% Uterine corpus
4% Non-Hodgkins lymphoma
4% Melanoma of skin
3% Ovary
3% Thyroid
2% Urinary bladder
2% Pancreas
21% All Other Sites

*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.
Trends in Cancer Incidence
1950-2008

SEER Data, 1950–2000, Table I–3
Trends in U.S. Cancer Incidence

QuickTime™ and a decompressor are needed to see this picture.
Change in the US Death Rates* by Cause, 1950 & 2005

* Age-adjusted to 2000 US standard population.
5-Year Rate Changes - Mortality
United States, 2000-2004
All Ages, Both Sexes, All Races (incl Hisp)

All Cancer Sites
Prostate -4.3#
Non-Hodgkin Lymphoma -3.7#
Cervix -3.5#
Colon & Rectum -3.3#
Stomach -3.2#
Breast (Female) -2.1#
Leukemia -1.5#
Brain & ONS -1.1#
Lung & Bronchus -1.1#
Oral Cavity & Pharynx -1.0#
Thyroid -0.9
Kidney & Renal Pelvis -0.7
Ovary -0.5
Esophagus -0.3
Uterus -0.1
Bladder
Melanoma of the Skin 0.0
Pancreas 0.1
Liver & Bile Duct 2.6#

Annual Percent Change

Created by statecancerprofiles.cancer.gov on 04/06/2008 11:19 am.
Annual Percent Change (APC) over the 5-year period calculated by SEER*Stat.
Source: Death data provided by the National Vital Statistics System public use data file. Death rates calculated by the National Cancer Institute using SEER*Stat. Death rates (deaths per 100,000 population per year) are age-adjusted to the 2000 US standard population (19 age groups: <1, 1-4, 5-9, ..., 80-84, 85+). Population counts for denominators are based on Census populations as modified by NCI.

# - The annual percent change is significantly different from zero (p<0.05).
What Causes Cancer?

- Spontaneous Mutation
  - DNA replication error rate (1 base per 1 billion base pairs copied per cell cycle)
- Genetic Predisposition
- Environmental/Chemical
  - Geophysical (radon, UV exposure)
  - Toxins (arsenic, asbestos, benzene, etc.)
- Infection
  - HPV, HIV, Hepatitis B and C, *H. pylori*
- Lifestyle
  - Tobacco
  - Alcohol
  - Diet
Approach to Cancer

20th Century
• Generic approach to diagnosis and treatment
• Diagnostic classification based on morphology and pathology
• Lack of systematic connection between research and clinical care
• Slow progress and analysis of data

21st Century
• Personalized, specific approach to patient and disease
• Molecular characterization and biologic processes
• Seamless translation between lab and clinic
• Rapid implementation through bioinformatics technology
History of Cancer Treatment

- Surgery: 1600 BC
- Surgical Oncology: 1809
- Radiation Therapy: 1895
- Chemotherapy: 1945
- Immunotherapy: 1984
- Targeted Therapy: 2001
- Stem Cell Therapy: ?
Measuring Responses to Cancer Therapy
Measuring Therapeutic Response

QuickTime™ and a decompressor are needed to see this picture.
Response Evaluation Criteria In Solid Tumors

• Method
  – to standardize treatment responses
  – to compare different drugs
  – to evaluate efficacy of drugs across different tumors

• Some problems with RECIST
  – Depends on ability to accurately measure tumor
  – Depends on timing of evaluation
  – Depends on the kinetics of response
RECIST Criteria

- **CR** = disappearance of all target lesions
- **PR** = 30% decrease in the sum of the longest diameter of target lesions
- **PD** = 20% increase in the sum of the longest diameter of target lesions
- **SD** = small changes that don’t meet above criteria

**CR** = complete response
**PR** = partial response
**PD** = progressive disease
**SD** = stable disease
World Health Organization (WHO) Criteria

• Similar classification to RECIST with four categories of response
  – CR
  – PR
  – SD
  – PD

• Major difference is use of bidirectional tumor measurements
  – No cap on number of target lesions
  – PR is > 50% regression
RECIST vs. WHO criteria: bi-linear measurement

Baseline

8 Weeks
Problems with RECIST

Baseline                                   Post-treatment
Other Measures of Efficacy

• Survival
  – Overall
  – Progression-free
  – Disease-free

• Benefit
  – Clinical
  – Palliative

• Quality of life
Biomarker

- A measurable characteristic that predicts a clinical endpoint
- “surrogate marker” is a biomarker that substitutes for a clinical endpoint
  - “surrogate marker” is a special case biomarker, i.e., not just a predictor of a clinical endpoint, but a reliable substitute for a clinical endpoint
    - the distinction has regulatory implications
- Outcome data is needed to establish validity of a surrogate marker
- About 39 FDA approved biomarkers at present
General Principles of Cancer Therapy
Six Characteristics of Cancer
Pathways to Cancer

- Loss of function ("tumor suppressor")
- Gain of function ("oncogene")
- Can occur in various sequences
- All converge to deregulate
Mutation Acquisition in Colon Cancer

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>5q</th>
<th>12q</th>
<th>18q</th>
<th>17p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alteration</td>
<td>Loss</td>
<td>Activation</td>
<td>Loss</td>
<td>Loss</td>
</tr>
<tr>
<td>Gene</td>
<td>APC</td>
<td>K-ras</td>
<td>DCC</td>
<td>p53</td>
</tr>
</tbody>
</table>
Development of Heterogeneity in Cancer

Mutations may continue to occur after malignant transformation.
Metastasis: A Lethal Effect of Cancer

- Growth of primary neoplasm
- Loss of adherence
- Neo-angiogenesis
- Invasion of lymphatics and/or blood vessels
- Transport
- Immune escape
- Capillary arrest
- Adherence
- New growth
- Angiogenesis
Tumor Perfusion and Resistance

- The further away from blood vessel the more hypoxia, nutrient starved and acidic
- Cells in this state may not be able to divide
- These cells are resistant to chemotherapy
- These cell survive treatment and begin to divide later
The Cell Cycle

Synthesis of cellular components for mitosis
G_2
19%

G_1

Mitosis
2%

Differentiation

Synthesis of cellular components needed for DNA synthesis

G_0

DNA synthesis
39%

Replication of DNA genome

THE CELL CYCLE

S
Some chemotherapy drugs depend on the status of the cell cycle.

- Doubling time: time to double the cell population
- Growth fraction: fraction of cells in the cell cycle

**Diagram:**
- S: DNA synthesis
- G\(_1\): G\(_0\) resting phase
- G\(_2\): premiotic interval
- M: M-phase (mitosis)

**Cell Cycle Phases:**
- S-phase: specific
  - Cytosine arabinoside, hydroxyurea
  - S-phase-specific, self-limited: 6-mercaptopurine, methotrexate
- G\(_0\) phase: non-specific
  - Alkylating drugs, nitrosoureas, antitumor antibiotics, procarbazine, cisplatinum, dacarbazine
Doubling Times of Tumors Vary

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Doubling Time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt’s lymphoma</td>
<td>1-1.5</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>3-4</td>
</tr>
<tr>
<td>Hodgkin’s Disease</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>80-90</td>
</tr>
<tr>
<td>Lung</td>
<td>90-150</td>
</tr>
<tr>
<td>Breast</td>
<td>100-150</td>
</tr>
</tbody>
</table>
Cell Cycle Dependent Drugs

- **S phase**
  - Capecitabine, cytarabine, doxorubicin, fludarabine, fluoxuridine, fluroruracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, prednisone, procarbazine, thioguanine

- **M phase**
  - Docetaxel, etoposide, paclitaxel, teniposide, vinblastine, vincristine, vinblastine

- **G2 phase**
  - Bleomycin, irinotecan, mitoxantrone, topotecan

- **G1 phase**
  - Asparaginase, corticosteroids
Principles of Chemotherapy

- Pharmacokinetics - study of the time course of drug and metabolite levels in body compartments
- Pharmacodynamics - study of drug effects at cellular level
- Bioavailability - amount of drug able to act
  - Patient compliance
  - GI tract absorption
  - First-pass metabolism in liver
Principles of Pharmacokinetics

- AUC - area under the plasma concentration-time curve (used as measure of total drug exposure)
- $C(t)$ - Drug concentration in plasma at time $t$
- $T_{1/2}$ - Half-life (time required for plasma drug concentration to decrease by half)
- $V_d$ - Volume of distribution (hypothetical volume required to dissolve total amount of drug at the same concentration)
- $Cl$ - Clearance (rate of elimination from body)
Gompertzian Growth

Why do tumors slow down?

- Local hypoxia
- Lack of nutrients
- Accumulation of toxic metabolites
- Lack of cell-cell communication

Norton et al. Nature 1976
Most chemotherapy drugs have a narrow therapeutic index.

- Therapeutic index is the degree of separation between therapeutic and toxic doses.
Most chemotherapy drugs are thought to work through First Order Kinetics.
Relationship of drug-induced cell kill and lifespan

- No lag phase in drug action
- Constant generation time in surviving cells (i.e. Gompertzian growth)
- No resistance
- No compartments
- No drug-induced adverse events
Purpose of Chemotherapy

- **Curative** – elimination of all known tumor
- **Adjuvant** - elimination of micrometastatic disease after primary treatment
- **Neoadjuvant** – elimination of some tumor prior to primary treatment
- **Palliative** – elimination of symptoms and improvement in quality of life; may control tumor growth
Chemotherapy Terminology

- **Induction**
  Treatment to induce complete remission

- **Consolidation**
  Treatment of sub-clinical residual disease with the intent of increasing cure rate or survival

- **Maintenance**
  Long-term, usually low-dose therapy to decrease recurrence/progression rate

- **Salvage**
  Treatment of early relapse or persistent disease
Sites of Chemotherapy Activity

- **6-MERCAPTOPURINE**
  - 6-THIOGUANINE
  - Inhibit purine ring biosynthesis
  - Inhibit nucleotide interconversions

- **METHOTREXATE**
  - Inhibit dihydrofolate reductase, block TMP and purine synthesis

- **CAMPTOTHECINS**
  - **ETOPOSIDE**
  - **TENPOSIDE**
  - **DAUNORUBICIN**
  - **DOXORUBICIN**
  - Block topoisomerase function

- **PROTEIN TYROSINE KINASE INHIBITORS**
  - **BORTEZOMIB**
  - **ANTIBODIES**
  - Block activity

- **DNA**
  - (transfer, messenger, ribosomal)

- **HNA**
  - (transfer, messenger, ribosomal)

- **PROTEINS**
  - Enzymes (etc.)
  - Microtubules

- **RNA**
  - Deoxyribonucleotides
  - Ribonucleotides
  - Purine synthesis
  - Pyrimidine synthesis

- **PALA**
  - Inhibits pyrimidine biosynthesis

- **HYDROXYUREA**
  - Inhibits ribonucleoside reductase

- **5-FLUOROURACIL**
  - Inhibits TMP synthesis

- **GEMCITABINE**
  - CYTARABINE
  - FLUDARABINE
  - 2-OHRODEOXYADENOSINE
  - Inhibit DNA synthesis

- **PLATINUM ANALOGS**
  - **ALKYLATING AGENTS**
  - **MITOMYCIN**
  - **CISPLATIN**
  - **TEMOCOLAMIDE**
  - Form adducts with DNA

- **L-ASPARAGINASE**
  - Deaminate asparagine
  - Inhibits protein synthesis

- **PACLITAXEL**
  - **VINCA ALKALOIDS**
  - **COLCHICINE**
  - **ESTRAMUSTINE**
  - Inhibit function of microtubules

**PALA** = *N*-phosphonoacetyl-L-aspartate; **TMP** = thymidine monophosphate.
Why does chemotherapy fail?

- Patient status may limit therapy
  - Overall health status and co-morbidities
  - Toxicity of chemotherapy drug
- Cancer growth fractions may be low
- Cancer cells may not be in a “sensitive” stage of the cell cycle
- Cancer cells are not homogeneous
- Tumor perfusion is not uniform
- Cancer may spread to different body compartments
- Cancer cells can display intrinsic resistance or develop resistance over time
Mechanisms of Drug Resistance

- Decrease in cell uptake or increased efflux of drugs (MDR, P-glycoprotein)*
- Increased proficiency of cell/DNA repair
- Increased levels of “target” enzyme
- Alterations in “target” enzyme
- Decreased drug activation
- Sanctuary sites (e.g. CNS)

- Actinomycin D, Doxorubicin, Melphalan, Vinca alkaloids
- Cis-platinum, mitomycin C, cyclophosphamide
- methotrexate, imatinib
- 5-fluorouracil
- Cytosine arabinoside, 5-FU, doxorubicin
Bone marrow toxicity also limits chemotherapy administration

- Common with most agents
- Degree of suppression and rate of recovery depends on drug and dose
- Rapid recovery: cyclophosphamide, methotrexate, vinblastine, cytosine arabinoside
- Delayed recovery: BCNU, melphalan, XRT
Combination Chemotherapy

Why: Drug resistance and toxicity
Combination of agents

• Concurrent
• Sequential
• each with single-agent activity but alternative mechanisms and non-overlapping toxicities

Rationale: Allow maximum tumor kill while minimizing side-effects
Goldie-Coldman Model: minimize treatment failure due to acquired drug resistance

• Treatment should begin as early as possible when the malignant cell population is at its smallest.

• To avoid selection of doubly resistant mutants by sequential chemotherapy, multiple mutually non-cross-resistant drugs should be used together.

• To achieve maximal kill of both sensitive and moderately resistant cells, cytotoxic drugs should be administered as frequently as possible and in doses well above the minimally cytotoxic doses.

Goldie JH et al., Cancer Treat Rep 1982; 66: 439-449
R.S. Day (1986): The “worst-drug rule”

• If two treatments (A and B) are available, and B is known to be superior, the physician is likely to use B first.

• Cells that are resistant to B must be eliminated then by the weaker drug A.

• In some settings, a better outcome is reached if drug A is used first in a sequential regimen.

Day RS, Cancer Res 1986; 46: 3876
Molecular Pathways that Promote Malignant Phenotype
Chemotherapy
Types of Chemotherapy

- Alkylating Agents
  - Nitrogen mustards
  - Nitrosureas
  - Platinum compounds
- Antimetabolites
- Natural Products
  - Vinca alkaloids
  - Antibiotics
  - Anthracyclines
  - Taxanes
Alkylating Agents

- Form covalent bonds with sites on DNA, RNA, or proteins
  - Especially $N^7$ position of guanine on DNA
  - Not cell cycle specific (but does require proliferation)
  - Resistance via enhanced DNA repair or glutathione conjugation
- Toxicities
  - Myelosuppression, gonadal dysfunction
Alkylating Agents

- Largest class of antineoplastic drugs

**General Properties/Mechanism:**
- All are electrophilic molecules that covalently modify nucleophilic molecules in cells
- DNA most important adduct (N7 and O6 of Guanine) for anticancer properties

**General Types of Alkylating Agents**
- **Monofunctional**
  - Cause single strand DNA break
- **Bifunctional**
  - Inhibit DNA replication and transcription by crosslinking
Alkylating Agents

• **Nitrogen Mustards**
  – Cyclophosphamide, ifosfamide, chlorambucil, mechlorethamine
  – Bind to DNA

• **Nitrosureas**
  – Carmustine, lomustine
  – High lipid solubility
    - Rapid penetration of blood-brain barrier
  – Highly reactive intermediates (chloroethyl *diazohydroxide*, *isocyanate*)

• **Platinum agents**
  – Cisplatin, carboplatin, oxaliplatin
  – Produce intrastrand and interstrand DNA cross-links leading to DNA adducts
QuickTime™ and a decompressor are needed to see this picture.
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Antimetabolites

- Analogs of natural metabolites in RNA and DNA synthesis
- Compete with normal metabolites for enzymes or substitute for metabolite incorporated into DNA or RNA
- S-phase specific
- Toxicities
  - Stomatitis, diarrhea, myelosuppression
Antimetabolites

- **Folate Analogs**
  - Methotrexate, pemetrexed
- **Purine Analogs**
  - Fludarabine, mercaptopurine, thioguanine
- **Adenosine Analogs**
  - Cladribine, pentostatin
- **Pyrimidine Analogs**
  - Fluorouracil, cytarabine, gemcitabine
Pyrimidine Synthesis

5-FU → FdUMP → dUMP → dUMP

De Novo Pathway

Thymidylate Synthase

THF → DHF → DHF

Dihydrofolate Reductase

MTX

Cell Membrane

dTTP → DNA

dTDP → dTMP

Thymidylate Synthase Inhibitors

THF

Salvage Pathway

Thymidine

TK

 Salvage Pathway
Methotrexate

- Folate Analog – inhibits dyhydrofolate reductase
- Crosses blood-brain barrier
- Excreted renally
  - Impaired renal excretion with ASA, NSAID’s, penicillins, probenecid, cephalosporins
- 3rd space accumulation
- Leucovorin
  - Repletes folate preferentially in normal cells
5-FU: Mechanism of Action

Diagram showing the mechanism of 5-FU action:
- 5-FU converts to FdUrd, which is then converted to FdUMP.
- FdUMP is then converted to dUMP and dTMP.
- dTMP is converted to Thymidine.
- FdUMP can also be converted to FdUTP, which is involved in DNA synthesis.
- FdUrd, FUMP, FUDP, and FUTP are involved in RNA synthesis.
- Uridine can be converted to FUTP.
Leucovorin (LV) potentiates 5-FU

Catabolism (85%)

FUH₂

DPD

FUPA

FBAL

FU

FUréd

FUMP

FUDP

FUTP

RNA

RNA

FUdR

FdUMP

FdUDP

FdUTP

TS

dUMP

dTMP

DNA

5,10-CH₂THF

DHF

LV

Metabolized to 5-10 methylene THF (Replete intracellular folate)
Leucovorin (Folinic acid)

- Inactive on its own as a chemotherapeutic agent
- In combination with (after) methotrexate
  - “Leucovorin rescue”
  - Decrease toxicity of (high-dose) methotrexate when given 24-48 hours after dose
- In combination with 5-FU
  - Potentiates effect by increasing intracellular 5,10-methylene THF
  - Ternary complex comprising 5,10-methylene THF, FdUMP, and thymidylate synthase
QuickTime™ and a decompressor are needed to see this picture.
Natural Products

• Natural products with antitumor activity
• Current drugs may be synthetic versions or semi-synthetic analogs of the full parent compound or its active structure
  – Vinca alkaloids
  – Antibiotics
  – Anthracyclines, anthracenediones
  – Taxanes
  – Topoisomerase inhibitors
QuickTime™ and a decompressor are needed to see this picture.
Antibiotics

- Bleomycin
- DNA intercalation
- Spontaneous oxidation and free-radical formation leading to strand breakage

Toxicity:
- Pulmonary fibrosis
- Desquamation of fingers, elbows
Antibiotics

- Mitomycin C
- Derived from *Streptomyces* species
- DNA alkylation
- Superficial bladder cancer, anal, head & neck, esophageal cancers
- Toxicity:
  - Delayed myelosuppression
  - Hemolytic uremic syndrome
  - Interstitial pneumonitis
  - Cardiomyopathy
Antibiotics

• Dactinomycin
• Inhibition of RNA and protein synthesis
• Toxicity:
  – Myelosuppression
  – Nausea, vomiting, mucositis
Anthracyclines and anthracyclenediones

- Daunorubicin, doxorubicin, idarubicin, epirubicin
- Products derived from fungus streptomyces percutus var caesius
- Cause intercalation between DNA base pairs and inhibit DNA topoisomerase II, formation of free radicals
- Toxicity:
  - Cardiotoxicity, myelosuppression, stomatitis, alopecia, nausea, vomiting
Vinca Alkaloids

- Vincristine, vinblastine, vinorelbine
- Derived from periwinkle plant (*vinca rosea* or *cantharanthus roseus*)
- Bind in S phase to tubulin
- Block polymerization of microtubules (M phase arrest)
- Toxicities
  - Neuropathy (including paralytic ileus), myelosuppression
QuickTime™ and a decompressor are needed to see this picture.
Taxanes

- Paclitaxel, docetaxel
- Semi-synthetic derivatives of yew plant
- Microtubule agents that promote microtubular assembly and stability, leading to M phase arrest
- Toxicities:
  - Neuropathy, myelosuppression
QuickTime™ and a decompressor are needed to see this picture.
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Other Drugs: DNA Topoisomerase II Inhibitors
DNA Topoisomerase Inhibitors

- Topo I inhibitors
- Camptothecins
- Chinese tree *Camptotheca acuminata*
- S-phase most sensitive
- Topotecan
  - Ovarian and lung cancer
  - Toxicity - myelosuppression
- CPT-11
  - Colon, lung and ovarian
  - Myelosuppression and diarrhea

- Topo II inhibitors
- Doxorubicin
- Etoposide
  - Binds tubulin
  - Inhibits Topo II
  - SCLC, testicular, lymphomas, pediatric tumors
  - Synergy with cis-platin
  - Toxicity - myelosuppression, alopecia, mucositis at high doses
Mechanisms of Resistance
Drug Resistance

Depends on:

- Proliferative/intrinsic status of cells
- Status of the immune system
- Tumor microenvironment
  - Limited vascular access
  - Penetration of drug into tissue
General Mechanisms of Anticancer Drug Resistance

- Decreased uptake
- Increased efflux (P-glycoprotein)
- Decrease in drug activation
- Increase in drug catabolism
- Change in target enzyme levels
- Activation of alternate growth pathways
- Inactivation by binding to sulphydryls
- Increased DNA repair
- Decreased ability to undergo apoptosis
- Epigenetic mechanisms
Drug Resistance Due to Impaired Uptake or Increased Efflux

Defects in:

- Passive diffusion
- Facilitated diffusion
  - Cyclophosphamide
- Active transport
  - Melphalan

Expression of Multiple Drug Resistance (MDR) proteins:

- P-glycoprotein
  - ATP-binding cassette (ABC) molecules
- Multi-drug resistance protein (MRP)
- Lung resistance protein (LRP)
P-glycoprotein

- Encoded by mdr1
- Elevated in many chemo-resistant tumors

- Anthracyclines
  - Doxorubicin
  - Daunorubicin
  - Epirubicin
- Mitoxantrone
- Vinca alkaloids
  - Vinblastine (low level resistance)
  - Vincristine
- Etoposide
- Taxanes
  - Paclitaxel
  - Docetaxel
- Actinomycin D
Multidrug Resistance Protein (MRP)

- ABC molecule
- 15% homology with P-glycoprotein
- Expressed in leukemia and lung cancer

- Anthracyclines
  - Doxorubicin
  - Daunorubicin
  - Epirubicin
- Vinca alkaloids
  - Vinblastine (low level resistance)
  - Vincristine
- Etoposide
Lung-Resistance Protein (LRP)

- Protein expressed on intracellular vaults
- Pumps drugs into vaults and exports them
- Anthracyclines
  - Doxorubicin
  - Daunorubicin
- Mitoxantrone
- Alkylating agents?
- Cis-platinum?
Monoclonal Antibody Therapy
Differences Between Chemotherapy and Monoclonal Antibody Therapy

**Chemotherapy**
- Injury to cancer cells and normal cells
- Side effects/toxicity can be cumulative and may lead to long-term sequelae
- Multi-drug resistance

**Monoclonal Antibodies**
- Specifically target tumor cells
- Fewer side effects to normal cells
- Less chance of drug resistance
- Fewer cumulative side effects
- Few dose-limiting side effects
Monoclonal Antibodies: Unconjugated
Monoclonal Antibodies: Conjugated
Types of Monoclonal Antibodies

- Human: -umab
- Murine: -momab
- Chimeric: -ximab
- Humanized: -zumab
Therapeutic Effects of Monoclonal Antibodies

• Direct Effects
  – Induction of apoptosis
  – Block growth factor receptors
  – Anti-idiotype Ab formation

• Indirect Effects
  – Ab-dependent cellular cytotoxicity (ADCC)
  – Complement-mediated cellular cytotoxicity

Green MC, Cancer Treatment Rev 2001, 26:269
Mechanisms of Action: ADCC

ADCC: Recruitment of natural killer (NK) cells, macrophages and monocytes by MoAB through its binding to their Fcγ receptors

Complement Dependent Cytotoxicity (CDC)

CDC: Induced by MoAB binding to C1q, resulting in activation of the complement cascade and generation of the membrane attack complex.

Obstacles that Limit the Effectiveness of Monoclonal Antibodies

- Heterogeneity of ag distribution on malignant cells
- Non-homogenous blood flow to tumors
- High interstitial pressure in tumors
- Unbound ag-binding Ab
- Human anti-mouse Ab (HAMA)
- Human anti-human Ab (HAHA)
- Cross reactivity with normal tissue ag

Green MC, Cancer Treatment Rev 2001, 26:269
Monoclonal Antibodies for Treatment of B-cell Malignancies

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Type</th>
<th>Investigational Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>Rituximab (Rituxan)</td>
<td>Chimeric</td>
<td>FDA approved</td>
</tr>
<tr>
<td></td>
<td>Tositumomab (Bexxar)</td>
<td>$^{131}$I-Murine</td>
<td>Submitted</td>
</tr>
<tr>
<td></td>
<td>Ibritumomab (Zevalin)</td>
<td>$^{90}$Y-Murine</td>
<td>Submitted</td>
</tr>
<tr>
<td>CD52</td>
<td>Alemtuzumab (Campath)</td>
<td>Humanized</td>
<td>FDA approved</td>
</tr>
<tr>
<td>CD22</td>
<td>Epratuzumab (Lymphocide)</td>
<td>Humanized</td>
<td>Phase II/III</td>
</tr>
</tbody>
</table>
Rituximab: An Anti-CD20 Monoclonal Antibody

- Genetically engineered chimeric murine/human monoclonal antibody
  - Variable light- and heavy-chain regions from murine anti-CD20 antibody IDEC-2B8
  - Human IgGκ constant regions
CD20 Expression in B-Cell Tumors

- Hairy Cell
- Burkitt's Lymphoma
- Large Cell
- Mantle Cell
- Marginal Zone
- Follicular Small Cell
- Small-Cleaved
- CLL/PLL
- CLL

Mean Channel Fluorescence:

0 100 200 300 400 500 600
Rituximab for Initial Treatment of LGNHL: Duration of Response

Phase II Trial Rituximab Plus CHOP: Overall Survival

TTF after initiation of cytoxan, doxorubicin, vincristine, and prednisone (CHOP) vs. rituximab and CHOP (R-CHOP)

Example of Antibody Mechanism of Action: Trastuzumab (Herceptin)

- Binds to HER2 receptor
- Potential mechanisms of action
  - Inhibits proliferation of tumor cells
  - Sensitizes cells to chemotherapy
  - Kills cells by recruiting other immune cells
Herceptin® (Trastuzumab): Humanized Anti-HER2 Antibody

- Targets HER2
- High affinity ($K_d = 5\text{ nM}$) and specificity
- Humanized
  - 95% human
  - 5% murine
Herceptin: Potential Mechanism of Action

- Down-regulates HER2 receptor expression
- Inhibits proliferation of human tumor cells that overexpress HER2 protein
- Enhances immune recruitment and antibody-dependent cellular cytotoxicity (ADCC) against HER2 protein overexpressing cancer cells
- Down-regulates angiogenesis factors?
- Induces T cell immune response against HER2?
# Herceptin® Combination Pivotal Trial: Objective Response Rate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Herceptin + CT (n = 235)</th>
<th>CT (n = 234)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>PR</td>
<td>43</td>
<td>28</td>
</tr>
<tr>
<td>ORR (%)</td>
<td>50</td>
<td>32</td>
</tr>
<tr>
<td>95% CI</td>
<td>44–57</td>
<td>26–38</td>
</tr>
</tbody>
</table>

*p value* < 0.001

Herceptin® Combination Pivotal Trial: Time to Progression

---

**Survival**

- **Herceptin + CT (n = 235)**
- **CT alone (n = 234)**

*p < 0.001*

Median follow-up: 30 mo

---

Herceptin® Combination
Pivotal Trial: Overall Survival*

**Herceptin + CT (n = 235)**

CT (n = 234)†

RR = 0.80

\( p = 0.046 \)

% of patients crossing over to Herceptin at progression*

<table>
<thead>
<tr>
<th>Month</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>15</td>
<td>62</td>
</tr>
<tr>
<td>25</td>
<td>65</td>
</tr>
<tr>
<td>40</td>
<td>72</td>
</tr>
</tbody>
</table>

*Median follow-up: 35 mo (range: 30–51).
†These patients are still reported in the CT arm.

Herceptin® Combination
Pivotal Trial: Overall Survival

FISH+

Herceptin + CT (n = 176)
CT (n = 169)

RR = 0.71
p = 0.007
26.2 mo

FISH−

Herceptin + CT (n = 50)
CT (n = 56)

RR = 1.11
p = NS
24.0 mo

Inhibition of VEGF Pathway

Somatic mutation → Small avascular tumor → Tumor secretion of VEGF stimulates angiogenesis → Rapid tumor growth and metastasis → Angiogenic inhibitors may reverse this process
Targeting VEGF: Antibody

- Bevacizumab = humanized monoclonal antibody against VEGF (93% human, 7% murine)
- Binds to VEGF with high affinity
- Prevents VEGF from binding to its receptors and inhibits angiogenesis
- Proven clinical activity in colorectal, lung, breast, kidney cancers
Anti-angiogenic therapy: Toxicity

• While specific agents may have unique toxicities (such as rash), there are several toxicities which seem to be a class effect:
  – Hypertension
  – Proteinuria
  – Bleeding / Thrombosis

? Impaired wound healing
? Spontaneous bowel perforation
Tumor Immunotherapy
Immunosurveillance: The Immune System Prevents Tumors

- T-cells
- Interferon-γ
The immune system can induce regression in some human cancers

MART-1 (green)
Caspase 3 (red)

CD3 (blue)
NK 1.1 (red)
Nuclei (green)
Tumor regression is mediated by T cells

Zweifach 2005
Tumor infiltrating T cells is correlated with cancer survival

Zhang et al. NEJM 2003
Pages et al. NEJM 2005
Hiraoka et al. Br J Cancer 2006
Ladanyi et al. Clin Cancer Res 2004
Melanoma Tumor Antigens

- **Differentiation**
  - Tyrosinase
  - gp100
  - MART-1/Melan-A
  - Tyrosinase-related proteins 1 & 2

- **Cancer-Testis**
  - MAGE
  - NY-ESO-1

- **Mutational**
  - $\beta$-catenin

- **Viral**
  - HERV
Types of Immunotherapy

• PASSIVE
  – Monoclonal Antibody Therapy
  – Cytokine Therapy
  – Adoptive T cell Therapy

• ACTIVE
  – Vaccination
Adjuvant Therapy for Melanoma: Interferon-alpha2b

- Direct anti-proliferative effect
- Cytotoxic effect
- Potentiation of B- and T-cell responses
- Enhances dendritic cell antigen presentation
- Anti-angiogenesis activity
E1684: Relapse-Free Survival

Median RFS

Arm | Median RFS
---|---
IFN-α2b | 1.72 yr
Obs | 0.98 yr

E1684: Estimated Overall Survival

<table>
<thead>
<tr>
<th>Arm</th>
<th>Median OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNα2b</td>
<td>3.82 yr</td>
</tr>
<tr>
<td>Obs</td>
<td>2.78 yr</td>
</tr>
</tbody>
</table>

**Graph**

- **Y-axis:** Probability of survival
- **X-axis:** Time (range: 0-8 years)
- **Lines:**
  - Blue: IFN-α2b (n=143)
  - Yellow: Observation (n=137)

**Statistical Significance:**

\[ P = 0.0237 \]

*Citation:

J Clin Oncol. 1996;14:7-17.*
Rates of Recurrent Melanoma: High-Dose and Low-Dose IFN

- HDI reduced risk of disease recurrence by 26%, $P_2 = 0.00009$
- Trend for increased benefit with high dose, $P = 0.02$

**High Dose Trials**
- ECOG 1684
- Intergroup E1690 (H)
- NCCTG 83-7052
- ECOG 2696
- Subtotal:

**Low Dose Trials**
- WHO 16
- Intergroup E1690 (L)
- UKCCCR AIM-High
- French CGM
- Austrian MMCG
- Scottish MG

GM-CSF for Melanoma

Stage III

Stage IV

Interleukin-2

- T cell growth factor
- 4 helical bundle cytokine
- Produced by T cells
- Induces T and NK cell proliferation
- Exhibits strong anti-tumor properties in mice
- FDA approved for renal cell carcinoma in 1992
- FDA approved for melanoma in 1998
Clonal T cell expansion

Signal 1
CD28
TCR
Naive T cell

Signal 2
CD40L

Signal 3
IL-2

In vitro
IL-2

In vivo
IL-2, IL-4, IL-6, IL-12, TNF, other γc family cytokines?
4-1BB, OX40, CD27, others?

Activation

Activated T cell

Limited clonal expansion

Effector T cell

Extensive clonal expansion and effector development
High-dose Bolus rIL-2 Regimen

- IL-2 600,000 IU/kg every 8 hours by 15-minute IV infusion for a maximum of 15 doses
- 9-16 day rest period
- Repeat schedule for another 15 doses
- Maximum 30 doses per course of therapy

- Excessive toxicity treated by withholding dose or discontinuing treatment for that cycle
## Pivotal High Dose IL-2 Trials

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>No. of Patients (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CR</td>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>182</td>
<td>12(6.6)</td>
<td>15(8.2)</td>
<td></td>
</tr>
<tr>
<td>Renal Cell</td>
<td>227</td>
<td>21(9.3)</td>
<td>22(9.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>409</td>
<td>33(8.1)</td>
<td>37(9.0)</td>
<td></td>
</tr>
</tbody>
</table>

High-Dose IV Bolus rIL-2

Duration of Response

High Dose IL-2 promotes durable disease free survival in responders

- CR is durable
- 27/33 CR (81%) without recurrence at 39-148 months

IL-2 Side Effects are related to Capillary Leak Syndrome

- Constitutional (flu-like)
- Cardiovascular
- Gastrointestinal
- Pulmonary
- Metabolic
- Neurologic

- Hepatic
- Renal
- Dermatologic
- Capillary leak
- Hematologic
## Key Biochemotherapy Trials

<table>
<thead>
<tr>
<th>Regimen</th>
<th>N</th>
<th>RR (%)</th>
<th>Survival (months)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD/BCG vs. DTIC + BCG</td>
<td>386</td>
<td>29/18</td>
<td>6.7/6.9</td>
<td>Costanzi, et al.</td>
</tr>
<tr>
<td>DTIC + IFNα vs. DTIC</td>
<td>60</td>
<td>53/18</td>
<td>17.6*</td>
<td>Falkson, et al.</td>
</tr>
<tr>
<td>DTIC + IFNα ± Tam vs. DTIC ± Tam</td>
<td>255</td>
<td>16/21</td>
<td>9.5/8</td>
<td>Falkson, et al.</td>
</tr>
<tr>
<td>IL-2 + IFNα vs. Cisplatin + IL-2/IFN</td>
<td>126</td>
<td>18/35</td>
<td>9/9</td>
<td>Keilholz, et al., 1997</td>
</tr>
<tr>
<td>mDartmouth vs. mDartmout/IL-2/IFN</td>
<td>102</td>
<td>27/44</td>
<td>15.8/10.7</td>
<td>Rosenberg, et al., 1999</td>
</tr>
<tr>
<td>CVD vs. CVD/IL-2/IFN</td>
<td>183</td>
<td>25/48</td>
<td>9.5/11.8</td>
<td>Eton, et al., 2002</td>
</tr>
<tr>
<td>CD + IFN vs. CD + IFN + IL-2</td>
<td>363</td>
<td>23/21</td>
<td>9/9</td>
<td>Keilholz, et al., 2003</td>
</tr>
<tr>
<td>CVD vs. CVD + IL-2 + IFN</td>
<td>405</td>
<td>11/17</td>
<td>8.7/8.4</td>
<td>Atkins, et al., 2003</td>
</tr>
</tbody>
</table>
Biochemotherapy: NCI Randomized Trial

E3695: Preliminary Survival Data

OS - Eligible Cases

Log-rank p-value = 0.439

<table>
<thead>
<tr>
<th>Group</th>
<th>Time Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10</td>
</tr>
<tr>
<td>CVD</td>
<td>85/192</td>
</tr>
<tr>
<td>CVD-BIO</td>
<td>89/194</td>
</tr>
</tbody>
</table>

(# events/# at risk)
Why doesn’t immunotherapy work better? Tumor Escape

- Tumor Factors
  - Genetic instability
  - Antigen loss
  - Defects in antigen processing and presentation
  - Lack of co-stimulation
- Host Factors
  - T cells
  - Dendritic cells
  - Local microenvironment

Nature Immunol 2002
Why doesn’t immunotherapy work better? Immune Suppression

• Mice deficient in IL-2 or IL-2R demonstrate lethal autoimmunity
• IL-2 also induces Tregs that suppress immunity
• IL-2 as a regulatory cytokine mediating tolerance?
Immune Suppression in the Tumor Microenvironment

T cells are tolerized in the tumor microenvironment

IFN-γ

Perforin

Granzyme B

Zippelius et al. Cancer Res 2004
Regulation of T cell activation is a complex process

Antigen-specific T cell Activation
- TCR : Antigen MHC
- CD28 : B7 Co-stimulation

Activated T cell
- IL-2 secretion
- Proliferation
- Effector function
- Induction of CTLA-4

CTLA-4 : B7 suppression
Termination of response
Anti-CTLA induces clinical responses in melanoma patients

Phan et al. PNAS 2003
Anti-CTLA4 induces autoimmunity

Phan et al. PNAS 2003
Strategies for Adoptive T cell Transfer in Melanoma

Adoptive transfer of MART-1-specific T cells persist and mediate tumor regression

Morgan et al. Science 2006
Homeostatic Repopulation

Mackall et al. JI 1996
Adoptive transfer of tumor-specific T cells in lymphodepleted patients

- Cytoxan/Fludarabine
- TIL + HD IL-2
- 18/35 (51%) had objective clinical response
  - 3 CR
  - 15 PR

Dudley et al. JCO 2005
Clinical response to adoptive T cell transfer
Alpha Interferon Induced Autoimmunity Correlates with Increased DFS

Gogas, NEJM 354(7):2006
TLR Signaling Pathways

Imiquimod is a TLR 7/8 agonist

- Synthesized in 1980’s
- No direct anti-viral action
- Effective against:
  - HSV related warts
  - Basal cell carcinoma

Miller et al. Antiviral res 1999
Topical imiquimod for lentigo maligna melanoma

Mechanisms of Resistance

• Tumor Cell Factors
  – Loss of tumor antigen expression
  – Loss of MHC Class I expression

• Tumor Microenvironment
  – Suppressive soluble factors (IL-10, TGF-β)
  – Membrane bound factors (FasL)

• Host Factors
  – Poor performance status
  – Genetic polymorphisms
Targeted Molecular Therapy
Targeted Therapy

• Agents that target selected cells or pathways important to tumors, optimally sparing normal cells
• May or may not lead to direct cell death
• Examples of currently relevant targets (meaning currently available drugs):
  – PML-RARα, BCR-ABL, c-kit, EGFR, VEGFR, CD20, CD33, CD52, PDGFRα/β, HER2/neu
Targeted therapy: Reagents

- Target pathway by disrupting protein-protein interactions: technically challenging, but small molecule antagonists in development
- Antibodies
- Tyrosine Kinase inhibition
- Proteasome inhibition
Example of TKI: Imatinib Mesylate (STI-571)

- Protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase (TK), the abnormal TK created by the Philadelphia chromosome abnormality in chronic myelogenous leukemia (CML)

- Inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukemic cells

- Inhibits proliferation and induces apoptosis in gastrointestinal stromal tumor (GIST) cells that express activating c-kit mutant.
Chromosomal Translocations May be Initiating Events Hematopoietic Malignancies

Normal

Clonal Selection
Chromosomal Translocations Result in Fusion Proteins
BCR-ABL

Oligomerization  Tyrosine Kinase Domain
Imatinib Mechanism

Adapted from Imatinib / CML Prescribing Guidelines, Novartis
Brief review of Imatinib

- CML = chronic myelogenous leukemia
- t(9;22) = Philadelphia chromosome
- Bcr-Abl fusion
- Constitutive phosphorylation (activation) leads to proliferation and suppression of apoptosis
- Gleevec curative
- Resistance can develop
- Also helpful in GIST and possibly mucosal melanoma
Imatinib Resistance

- Gene Amplification
- Tyrosine Kinase Mutation
- Leukemic Stem Cells
Ras-raf-MEK signaling in melanoma

The organisation and function of the Ras-Raf-MEK-ERK pathway

Expert Reviews in Molecular Medicine © 2002 Cambridge University Press
Targeting EGFR: TKI’s

- Orally-delivered small molecules inhibiting the tyrosine kinase of EGFR
- Block downstream signaling
- Currently approved for non small cell lung cancer

[Chemical structure of erlotinib]
Targeting VEGFR: TKI’s

- Orally-delivered tyrosine kinase inhibitors of VEGFR (plus others)
- Block signaling after receptor activation
- Sunitinib
- Proven clinically in kidney cancer, GIST and hepatocellular carcinoma
Stem Cell Therapy
The sequential dysplasia to malignancy hypothesis

Benign Nevi

Melanocytes

Melanomas
The stem cell hypothesis

Melanocytes

Benign Nevi

Melanocytic "stem cell"

Melanomas
A Model For “Cancer Stem Cells” In Treatment Resistance and Disease Recurrence

A. Standard Rx

B. CaSC Rx

C. Combination Complete Response

- Cancer Stem cell (CaSC)
- Daughter cells
Figure 1. The hoped-for pathway of development of personalized medicine for the treatment of leukemia

Davies, S. M. Hematology 2006;2006:111-117
Questions?

QuickTime™ and a decompressor are needed to see this picture.