Combination Chemotherapy in Chronic Lymphocytic Leukemia (CLL)

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There are two types of tumors: Malignant and Benign.

- **Benign tumors** stay in one place.
- **Malignant tumors** spread to other areas in the body.
  - Invade neighboring tissues.
  - Enter bloodstream and metastasize to different sites.

These are the dangerous ones.
Cancer- “The silent killer”

- Cancer is a genetic disease
- Cancer is clonal in nature
- Cancer results from mutations
  - Oncogenes
  - Tumor Suppressor Genes
  - DNA Repair Genes
  - Genome Instability Genes

“Tumor Formation Arises as a Consequence of Alterations in the Control of Cell Proliferation and Disorders in the Interactions between Cells and their Surroundings that Result in Invasion and Metastasis”
ACQUIRED CAPABILITIES OF CANCER CELLS

- Self-sufficiency in growth signals
- Evading apoptosis
- Insensitivity to anti-growth signals
- Sustained angiogenesis
- Tissue invasion & metastasis
- Limitless replicative potential
Cancer is a life-style disease

Cancer Does Not Discriminate

Nobody is Safe
## US Mortality

<table>
<thead>
<tr>
<th>Rank</th>
<th>Cause of Death</th>
<th>No. of deaths</th>
<th>% of all deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Heart Diseases</td>
<td>710,760</td>
<td>29.6</td>
</tr>
<tr>
<td>2.</td>
<td>Cancer</td>
<td>553,091</td>
<td>23.0</td>
</tr>
<tr>
<td>3.</td>
<td>Cerebrovascular diseases</td>
<td>167,661</td>
<td>7.0</td>
</tr>
<tr>
<td>4.</td>
<td>Chronic lower respiratory diseases</td>
<td>122,009</td>
<td>5.1</td>
</tr>
<tr>
<td>5.</td>
<td>Accidents (Unintentional injuries)</td>
<td>97,900</td>
<td>4.1</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetes mellitus</td>
<td>69,301</td>
<td>2.9</td>
</tr>
<tr>
<td>7.</td>
<td>Influenza and Pneumonia</td>
<td>65,313</td>
<td>2.7</td>
</tr>
<tr>
<td>8.</td>
<td>Alzheimer’s disease</td>
<td>49,558</td>
<td>2.1</td>
</tr>
<tr>
<td>9.</td>
<td>Nephritis</td>
<td>37,251</td>
<td>1.5</td>
</tr>
<tr>
<td>10.</td>
<td>Septicemia</td>
<td>31,224</td>
<td>1.3</td>
</tr>
</tbody>
</table>
5% of all cancers are cancers of the blood

One in 25 of the population will develop leukemia, lymphoma or myeloma in their lifetime

One in 45 of the population will die of leukemia, lymphoma or myeloma
Haemopoiesis – the process of blood cell production

The body produces 3,000,000,000,000 blood cells every day.

This rate of production goes on throughout ones life.

One damaged cell is enough to give rise to a leukemia
The Stem Cell

Oxygen & breathing
To stop bruising & bleeding
To fight infections

Immune cells
Antibodies
Fight viruses
e.g. chicken pox

leukaemia
# Classification of Leukemias

<table>
<thead>
<tr>
<th>Myeloid origin</th>
<th>Acute</th>
<th>Chronic</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Acute Myeloid Leukemia (AML)</td>
<td>Chronic Myeloid Leukemia (CML)</td>
</tr>
<tr>
<td></td>
<td>Acute Lymphoblastic Leukemia (ALL)</td>
<td>Chronic Lymphocytic Leukemia (CLL)</td>
</tr>
</tbody>
</table>
Leukemia

Stages of Maturation/Differentiation

- ALL
- CLL

- AML
- CML

- Lymphoid Stem Cell
- Myeloid Stem Cell
- Trilineage Stem Cell

- Lymphoid
- Myeloid

- T-cell
- B-cell
- Erythrocyte
- Platelets
- Neutrophil
- Macrophage
- Monocyte
- Eosinophil
<table>
<thead>
<tr>
<th></th>
<th>ADULTS</th>
<th>CHILDREN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Lymphocytic</td>
<td>11%</td>
<td>75-80%</td>
</tr>
<tr>
<td>Acute Myeloid</td>
<td>32%</td>
<td>20%</td>
</tr>
<tr>
<td>Chronic Myeloid</td>
<td>15%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Chronic Lymphocytic</td>
<td>26%</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Leukaemia Therapy

18th century
- Arsenic

1950s
- Mustard Gas
- Conventional chemotherapy

21st century
- Bone Marrow Transplant
- Targeted Therapy
Mechanisms / sites of action

- **Pentostatin**: Inhibits adenosine deaminase
- **6-MP & Thioguanine**: Inhibits purine ring synth
- **Methotrexate**: Inhibits dihydrofolate reduction and purine synth
- **Bleomycin Etoposide**: Damage DNA & prevent repair
- **Daunorubicin Doxorubicin Mitoxantrone**: Intercalates with DNA, Inhibits RNA synthesis
- **Hydroxyurea**: Inhibits ribonucleotidase reductase
- **Fludarabine Cytarabine 2-CDA**: Inhibits DNA Synthesis
- **Alkylating agents Procarbazine**: Form adducts with DNA
- **L-Asparaginase**: Deaminates asparagine and inhibits protein synthesis
- **Vinca Alkaloids**: Inhibits microtubule function
Chronic Lymphocytic Leukemia

- Most frequent type of adult leukemia in Western countries
- Usually age > 50 yrs; Men:Women approximately 2:1
- Progressive accumulation of neoplastic, long-lived, immunologically incompetent, clonal lymphocytes
- Median survival ~ 9 years
- Advanced disease has increased morbidity & mortality, often from infection
Clinical Course of CLL

- Asymptomatic at diagnosis and for prolonged periods
- Diagnosis often incidental
- Initial symptoms: lymph node ↑/anemia
- Progression: bone marrow impairment, ↑ susceptibility to infection
- Hypogammaglobulinemia ↑ with advanced disease
- Long-term complications: autoimmune phenomena, Richter’s transformation
Major Clinical Features of CLL

- B cell origin: > 98%; T cell: 1-2%
- Approximately 50% enlarged liver and/or spleen
- CD19, CD20, CD23, co-express CD5
- low level surface Ig (IgM, IgD)
- >30% lymphocytes in bone marrow
- >5,000/mL monoclonal lymphocytes (Lymphocytosis)
CLL PROGNOSTIC FACTORS

- Lymphocyte count
- Short lymphocyte doubling time
- Atypical lymphocyte morphology
- 80% Lymphadenopathy
- Trisomy 12 or complex CG
- Elevated LDH
- Elevated $\beta_2$ (beta) microglobulin
- Autoimmune anemia, thrombocytopenia
- Infections
- Immunoglobulin rearrangement
- Others
Atypical Cell Morphology in CLL

(a) BM smear  (b) Blood smear
Common Chromosome Abnormalities in CLL

- **+12** - Progressive disease, poor survival, atypical morphology
- **del(13)(q14.3)** - Good prognosis (D13S319)
- **(14)(q32)** - Hallmark change in B-cell lymphoproliferative disorders IgH gene
- **del(6)(q)** - Progressive disease, associated with prolymphocytic transformation
- **del(17)(p13.1)** - Shorter survival (p53)
- **Del(11)(q22)** - Rapid disease progression (ATM)
Partial Trisomy of 12

- Advanced clinical stage
- Atypical morphology
- Increased proliferative activity
- Less favorable prognosis

GENES ON CHROMOSOME 12

- TEL
- KIP1
- K-ras
- MDM2
- IGF-1
- h-PRL-1
Vysis CLL Panel-- *R Slide (tube)
ATM (11q22.3)/p53(17p13.1)
spectrum green: 11q22.3    spectrum red: 17p13.1

normal cells
monosomy 17/del 17p
monosomy 11/del 11q

Vysis CLL Panel-- *B Slide (tube)
D13s319/13q34/CEP 12    spectrum green: 12p11.1-q11
spectrum red: 13q14.3    spectrum aqua: 13q34
del 13q14.3
normal cells
monosomy 13
trisomy 12
Chronic Lymphocytic Leukemia (CLL)

- At present there is no curative treatment.

- About 25% of all CLL patients and 50% of patients with advanced disease are resistant to cytotoxic drugs.

- Drug resistance is a major obstacle in therapy.
Treatment of CLL

- No strong evidence that therapy prolongs survival
- Asymptomatic: watch and wait
- Symptomatic:
  - Radiation for local complications
  - Chemotherapy
  - Monoclonal antibodies
  - Stem cell transplantation
Traditional approaches to therapy of CLL

- Chlorambucil ± Steroids
- Follicular lymphoma therapy (CVP, CHOP)
- Transplant as last resort

NOW

- New Chemotherapeutic drugs
  Fludarabine, Campath, Rituximab, alkylators, combinations
- Better transplantation
- Improved understanding of CLL
Purposes of Chemotherapy

- **Curative**
  - elimination of all known tumor mass (i.e., complete response)

- **Palliation**
  - treatment aimed at improving symptoms and controlling tumor growth

- **Adjuvant**
  - chemotherapy which attempts to eliminate micrometastatic disease following primary treatment

- **Neoadjuvant**
  - chemotherapy offered prior to other primary potentially curable therapy
Why Combination Therapy?

- Clinical success
- Tumor cell heterogeneity
  - genomic instability
    - emerging resistance
  - cytokinetics
    - cycling vs quiescent populations
  - multiple approaches to a single target or pathway
  - sanctuary sites
  - biochemical modulation
Design of Combination Therapies

Conventional Considerations

- Two active drugs (A + B)
- Dose intensity
- Overlapping toxicities
- Cross-resistance
Oxaliplatin (Eloxatin)

- Used in treatment of advanced colorectal cancer
- DNA is the critical target for its anti-tumor activity
Mechanism of action of Oxaliplatin

Oxaliplatin-Induced DNA damage:

- Inhibit replication and transcription
- Block cell cycle
- Induce apoptosis
DNA damage, repair mechanisms and consequences

**Damaging agents**
- X-rays
- Oxygen radicals
- UV light
- Alkylating agents
- X-rays
- cisplatin

**Consequences**
- Replication errors
- Uracil
- Single-strand break
- Bulky adducts
- Interstrand crosslink
- Double-strand break
- A-G Mismatch
- T-C Mismatch
- Insertion/Deletion

**Repair mechanisms**
- Inhibition of:
  - Transcription
  - Replication
  - Chromosome segregation
- base excision repair (BER)
- nucleotide excision repair (NER)
- recombinational repair (HR, EJ)
- mismatch repair (MMR)

**Cell-cycle arrest**
- Apoptosis
- Cancer
- Ageing
Fludarabine is a Functional DNA Chain Terminator

- Poor substrate for extension
- Resists Exonucleases
- Inactivates Polymerase
- Not a substrate for Ligases
- Inhibits Ribonucleotide Reductase

Fludarabine; F-ara-A
Mechanism of Fludarabine Action

F-ara-A

F-ara-AMP

dCK

F-ara-ADP

F-ara-ATP

Masked chain Termination
Nucleoside Analog-induced S Phase Arrest in Human ML-1 Myeloid Leukemia Cells

- Control
  - 33% S phase

- 10 nM Gemcitabine
  - 65% S phase

- 50 nM ara-C
  - 71% S phase

- 1000 nM F-ara-A
  - 63% S phase
Cleavage of death substrates
Nuclear and cytoplasmic damage
Cell death / Apoptosis
Synergistic Killing of CLL by Oxaliplatin and Fludarabine
Combination leads to enhancement of apoptosis

Control
Fludarabine
Oxaliplatin
combo

Caspase-3 expression

Moufarij et al., 2005
Fludarabine enhances DNA damage

Moufarij et al., 2006
Typical comet images from CLL cells treated with cytotoxic drugs
Action of Fludarabine on Repair of Oxaliplatin-induced DNA damage

control

(-) fludarabine

(+) Oxaliplatin treatment

4-hr repair
Oxaliplatin in combination with fludarabine

Mitochondrial envelope

mtDNA

Nuclear envelope

Repair pathways

Intrastrand adducts
Monoadducts
Interstrand crosslinks
Deoxyribose Base Phosphate
Proposed model for oxaliplatin/Fludarabine combination

- Damage to DNA
- Excision of damaged DNA
- DNA re-synthesis in repair patch
- F-ara-ATP incorporation to inhibit repair synthesis
- Signals for cell death
Clinical Investigation (OFAR)

A Phase I-II Study of Oxaliplatin, Fludarabine, Cytarabine and Rituximab in Patients with Richter’s Transformation, Prolymphocytic Leukemia or Refractory/Relapsed B-CLL

- Determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT)
- Assess the complete response (CR) and partial response (PR) rate
- Determine the safety and toxicity profile of combination therapy

- Characterize the pharmacodynamics of oxaliplatin in leukemia cells with respect to total adduct formation, crosslink formation and excision DNA responses. Compare these parameters in cells from the same patient after treatment with oxaliplatin in combination with fludarabine and cytarabine.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage</th>
<th>Day(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxaliplatin</td>
<td>17.5 mg/m²</td>
<td>1-4</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>30 mg/m²</td>
<td>2, 3</td>
</tr>
<tr>
<td>Ara-C</td>
<td>1 g/m²</td>
<td>2, 3</td>
</tr>
<tr>
<td>Rituximab</td>
<td>375 mg/m²</td>
<td>3</td>
</tr>
</tbody>
</table>

Moufarij, Plunkett & Keating, 2005
Oxaliplatin in Therapy of Aggressive CLL

Actions of Fludarabine/ara-C

Annexin Positivity

Hours

Moufarij, 2005
Combination action on γH2AX activation is at least additive.
γH2AX Confocal Studies

Control

DAPI

FITC

Overlay

combination

DAPI

FITC

Overlay
Use of Microarrays to identify genes responsible for clinical resistance
The repertoire of gene products produced by a cancer cell might differ in two ways from its normal counterpart.

**Quantitatively**, as shown for gene B, which is expressed at an abnormally high level, and gene A, which is not expressed at all.

**Qualitatively**, as shown for gene C* which is mutated such that it produces an altered gene product.
A microarray scan comparing a cisplatin resistant ovarian cell line, C13*5.25 (labelled red), with its parental cisplatin sensitive cell line, 2008 (labelled green). Probes were hybridised against a 10,500 human gene cDNA glass array, washed and scanned on a Packar Scanarray 5000. Differential gene expression is seen as deviation of an elements colour towards either the red (Cy3) or the green (Cy5) channel. Red spots represent genes overexpressed in C13*5.25. Green spots represent genes overexpressed in 2008. Yellow spots represent genes that are equally expressed. Black spots represent genes that are not expressed in either cell line. A 600 element gene matrix has been enlarged for clarity.
Clustering of gene expression profiles
Hierarchical clustering for 275 genes was performed using Pearson correlation as a measure of similarity, after average linkage and median centering of values using GeneSpring v6.1 program. Each column corresponds to a gene, with the row corresponding to expression levels in different pairs of cells. These relationships are summarised in a dendrogram, in which the pattern and length of the branches reflects the relatedness of the samples. Experiments were performed in triplicate. (Red represents up-regulation, green represents down-regulation, and black represents no change.)
Benefits of Characterizing Genetic Alterations in Cancer

**Clinical Utility**
- May provide *objective markers for primary diagnosis* and for the detection of minimum residual disease.
- May identify *disease subtypes and relationships between them*.
- May provide *targets for screening of drugs*.

**Basic Biological Value**
- Provides insights into *oncogenetic mechanisms underlying tumor development*.
- Provides insight into *basic biology of the normal cells or tissues in which the tumors arise*.
Acknowledgments

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