What Is Cancer? Is it a Single Disease?

“Tumors can best be defined as diseases in which a single cell acquires the ability to proliferate abnormally, resulting in an accumulation of progeny. Cancers are those tumors which have acquired the ability to invade through surrounding normal tissues. The most advanced form of this invasive process is metastasis, a state in which cancer cells escape from their original location, travel through hematogenous (blood stream) or lymphogenous (lymphatic system) channels, and take up residence in distant sites. The only difference between a malignant tumor (a.k.a. cancer) and a benign tumor is the capacity of the former to invade. Both benign and malignant tumors can achieve large size, but benign tumors are circumscribed and therefore generally can be removed by surgery. Malignant tumors often have invaded surrounding or distant tissues prior to detection, precluding surgical excision of the entire tumor cell population. It is the ability of cancers to destroy other tissues through invasion that makes them lethal.

There are as many tumor types as there are cell types in the human body. Cancers thus represent NOT a single disease but a group of heterogeneous diseases that share certain biological properties (in particular, clonal cell growth, and invasive ability). Cancers can be classified in various ways. Most cancers in adults are carcinomas, representing cancers derived from epithelial cells. Leukemias and lymphomas are derived from blood-forming cells and lymphoid cells respectively. Sarcomas are derived from mesenchymal tissues. Melanomas are derived from melanocytes, and retinoblastomas, neuroblastomas and glioblastomas are derived from stem cells in the retina, neurons and glia, respectively.”


I. Genes and Cancer…..

A. North America Statistics - 2017-2018

<table>
<thead>
<tr>
<th>Estimated New Cases</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>161,360</td>
<td>18%</td>
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<tr>
<td>Lung &amp; bronchus</td>
<td>116,990</td>
<td>12%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>71,420</td>
<td>9%</td>
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<td>Urinary bladder</td>
<td>60,490</td>
<td>7%</td>
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<td>Melanoma of the skin</td>
<td>52,170</td>
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<td>Kidney &amp; renal pelvis</td>
<td>40,610</td>
<td>5%</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>40,080</td>
<td>5%</td>
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<tr>
<td>Leukemia</td>
<td>36,560</td>
<td>4%</td>
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<tr>
<td>Oral cavity &amp; pharynx</td>
<td>58,720</td>
<td>4%</td>
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<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>29,200</td>
<td>3%</td>
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<tr>
<td>All Sites</td>
<td>836,150</td>
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<table>
<thead>
<tr>
<th>Estimated Deaths</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>84,590</td>
<td>27%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>27,150</td>
<td>9%</td>
</tr>
<tr>
<td>Prostate</td>
<td>26,720</td>
<td>8%</td>
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<tr>
<td>Pancreas</td>
<td>22,300</td>
<td>7%</td>
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<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>19,610</td>
<td>6%</td>
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<tr>
<td>Leukemia</td>
<td>14,360</td>
<td>4%</td>
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<tr>
<td>Esophagus</td>
<td>12,720</td>
<td>4%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>12,200</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>11,460</td>
<td>4%</td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>9,620</td>
<td>3%</td>
</tr>
<tr>
<td>All Sites</td>
<td>318,420</td>
<td>100%</td>
</tr>
</tbody>
</table>
B. 10 Basic Principles of Oncology:

1. Central Dogma of Molecular Genetics - Terminology

Genome - sum total of all human DNA information > 3,000,000,000 nts
Exome - sum total of all Exon DNA = Coding information
Transcriptome - sum total of all the mRNA transcript information
Proteome - sum total of all the Proteins

Genome of.....a person, an organ, a tissue, a cell, a tumor.....etc.

2. Mitosis and Apoptosis

a. Mitosis - Cell Birth - programmed cell birth
   one cell divides to become two cells
   prophase / metaphase / anaphase / telophase
   “In the old days.....we thought all cancer was the result of too much mitosis”

b. Apoptosis - Cell Death - programmed cell death
   eliminates cells that are dying, worn out or no longer needed
   i. shrinkage / blebbing / fragmentation (apoptotic bodies) / engulfment
      via macrophage
   ii. ‘Programmed Cell Death’ / ‘Cellular Suicide’ / ‘Blebbing to Oblivion’
      neat, clean, tidy.....way to get rid of an old cell.....no muss, no fuss

c. Mitosis and Apoptosis -
   i. cellular equilibrium.....cellular homeostasis mechanism
      maintains the normal number of cells in the body
   ii. if equilibrium is upset.....tumor = inappropriate accumulation of cells
      Too Much Mitosis = tumor.....Mitosis program out of control
      Not Enough Apoptosis = tumor.....Apoptosis program out of control

d. Chemotherapy kills cancer most cells..... via Apoptosis
e. Zygote becomes a Fetus in 8 weeks - 220 new cells must form
Differentiation - production of 220 new somatic cells

3 types of Naturally Occurring Stem Cells
1. Embryonic Stem Cells - 10 day life span
2. Adult Stem Cells
3. Cancer Stem Cells

Embryonic Stem Cells - gone in 10 days - morph into Adult Stem Cells
Adult Stem Cells: in Embryo and Fetus - Produce all the Original 22 Cells
in you and me today - Produce 220 Replacement Cells

f. Two Ways to do Mitosis: Symmetric and Asymmetric

Any human cell brought into existence for the first time…..
Comes from a Stem Cell via Asymmetric Mitosis

3. All tumors (all cancers) originate from one single somatic cell

a. one normal body cell mutates to become one abnormal body cell
b. the mutation is due to some type of DNA Damage in the original normal cell
d. that first single mutated cell is the ‘Founder Cell’ - the Founder of a Tumor
e. the conversion of a normal cell into Founder Cell is ‘Transformation’
f. from the Founder Cell - the entire tumor > cancer develops - may take decades
g. Founder Cell = 1 cell out of 80,000,000,000,000 – can lead to the tumor!

FIND IT…..GET RID OF IT…..F I G R O I !
4. **Genome Stability** - Human Genome >3,000,000,000 nucleotide pairs
   
   we want our genomes to be Stable - No Changes!
   
   Changes = Mutations = DNA Damage > Tumor > Cancer

   a. **GENE MUTATIONS**: ~20,000 code for proteins = ~20,000 proteins - Proteome
      
      ~600 normal genes can mutate to cause cancer

   i. **ONCOGENES (ONC)** – genes normally inactive, turned OFF
      cause Cancer when they are mistakenly activated, turned ON
      the gene mutation mistakenly turns them ON
      ‘Activation Mutation’ - Activates a gene by mistake
      acts as Dominant Genes - need only one of the two genes mutated
      ~80% of the 600 cancer causing genes

      SRC  SaRComa  first ONC discovered
      RAS  RAAt Sarcoma  most frequently mutated
      SIS  SImian Sarcoma
      ERB  ERythroBlastosis
      MYC  MYeloCytosis
      ABL  ABelson Leukemia virus
      BCL2  B-Cell Lymphoma-2

   ii. **TUMOR SUPPRESSOR GENES (TSG)** - gene normally turned ON
      cause Cancer when they are mistakenly inactivated, turned OFF
      the gene mutation mistakenly turns them OFF
      ‘Inactivation Mutation’ - Inactivates gene by mistake
      acts as Recessive Genes - need both of the two genes mutated
      ~20% of the 600 cancer causing mutations = 120 - nicknames

      BRCA1  BReast CAncer #1
      BRCA2  BReast CAncer #2
      RB1    RetinoBlastoma
      APC    Adenomatous Polyposis Coli
      DCC    Deleted in Colon Cancer
      P53    Protein 53

   iii. **ONCOMirs** - microRNAs from Non-Coding Genes
      mistakenly turn genes ‘ON’ - used for early cancer diagnosis
iv. All somatic cells accumulate cellular mutations over time

**Driver Mutations** - Drive Cancer development - ONCs, TSGs & ONCOMirs drives cell growth / invasiveness / metastasis

**Passenger Mutations** - No Cancer affect / ‘along for the ride’

v. Cancer Causing Mutations may be **EXOGENOUS** or **ENDOGENOUS**

**EXOGENOUS MUTATIONS** - Caused by Factors **OUTSIDE** the Body
Carcinogen Exposure.....Carcinogens = Cancer Causing Agents
Most Carcinogens are Mutagens.....they cause DNA mutations!

Environmental or Life Style Exposures Examples:
- Cigarette Smoke - over 55 Carcinogens > Lung Cancer
  - Benzo[a]pyrene - main lung cancer mutagen
  - PAH - Polycyclic Aromatic Hydrocarbons
  - Nicotine-Derived Nitrosoamines
- Primary - Secondary - Tertiary Smoke Exposure
- Ultra Violet Light > Skin Cancer
- Ionizing Radiation - X-rays & Gamma Rays
- Asbestos > Mesothelioma
- Virus > Liver Cancer - Hepatitis B, Cervical Cancer - Human Papilloma Virus
- Bacteria > Stomach Cancer - Helicobater pylori
- Lead > Lung Cancer
  - * Over 220 Exogenous Carcinogens Known!*

**ENDOGENOUS MUTATIONS** - Caused by factors **INSIDE** the body
Random DNA Mutations inside our cells
S-Phase DNA replication errors - replication mistakes before mitosis
MisMatch Repair - most corrected by proof-reading enzymes:

DNA Replication during S Phase - Replication enzyme is DNA Polymerase
DNA Polymerase always makes a few mistakes: 1 / 1,000,000 nucleotides

- Human Genome contains > 3,000,000,000 nucleotides (x2 both sides)
- ~6,000 replications errors / per cell / per mitosis
- Average stem cell acquires 2-3 mutations per mitosis!

Replication Errors can result in Endogenous Cancer Causing Mutations
Random Mutations.....'Bad Luck' Cancer Mutations

**Human Cancer:** **Endogenous** - Random Mutations = ~65%
**Exogenous** - Carcinogen Mutations = ~35%

*Science, vol. 355, no. 6331, pp.1330-1334, March 24, 2017*

#1 factor connected to acquiring cancer in the world?
**AGING!**
b. CHROMOSOME ABNORMALITIES

i. Translocations - two chromosomes break and rejoin - exchange parts

- exchange parts of between two chromosome arms
- often equal exchanges = reciprocal translocation
- an oncogene can be activated at the break point = ONC
- leading cause of leukemias and lymphomas

ii. Aneuploidy - due to mitotic nondisjunction - add or lose chromosomes

- extra chromosomes - extra copies - activate ONCs
- missing chromosome - lose copies - inactivate TSGs

ii. INsertions or DELetions - INDELs

- INsertions - can ramp up an ONC = turn it ON
- Amplifications - ramp up an ONC
- DELetions - can cause loss of a TSG = turn it OFF

c. CHROMOTHRYPSIS - Chromosomes Pulverized into Small Fragments

i. multiple chromosomes break and rejoin at multiple different positions
ii. some chromosome fragments are lost in the process
iii. rejoining can lead to ONC Activation and / or TSG Inactivation

d. EPIGENETIC CHROMATIN ERRORS - Opens and / or Closes Chromatin

i. HyperMethylation - Closes - genes OFF - can Inactivate a TSG

ii. HypoMethylation - Opens - genes ON - can Activate an ONC
5. **All Cancer is Genetic…..but Most Cancer is not Inherited**

Remember: the words **Genetic** and **Inherited** do not mean the same thing

**a. SPORADIC CANCER - 90 to 95% of Cancer**

Inherited means: a trait that runs through the family, 
passes from parent to child through the egg and sperm
most cancer NOT inherited - just ‘crops up’…..~90 to 95%
90 to 95% of cancer is **SPORADIC** –
Sporadic Cancer usually occurs later in life

**SPORADIC:** We inherit, from our parents perfectly NORMAL DNA
We inherit NORMAL genes and chromosomes
Then: some time during our life time…..

i. **a NORMAL GENE MUTATES > DRIVER MUTATION**
an ONC or TSG > somatic cell mutation > ‘FOUNDER’

ii. **CHROMOSOME TRANSLOCATION occurs, activates**
an ONC > somatic cell translocation > ‘FOUNDER’

iii. **CHROMOTHRIPSIS occurs - activates ONCs or inactivates**
TSGs > chromothriptic somatic cell > ‘FOUNDER’

iv. **EPIGENETIC CHROMATIN ERROR occurs - ONC or TSG**
leads to somatic cell with epigenetic error = ‘FOUNDER’

Most DNA Damage that leads to cancer - occurs during our lifetimes!
It is **GENETIC** - caused by DNA damage…..BUT NOT INHERITED

**b. INHERITED PREDISPOSITION - 5 to 10% of Cancer**

Predisposition = increased chance / tendency
occurs with the TSGs.....BRCA1 / RB1 / APC / P53
usually Early Onset - show up earlier in life
About 5 to 10% of Cancer.....an inherited factor.....TSG gene

Mendelian Inheritance:

\[ \begin{array}{c}
B B - Normal \\
B b - Predisposition - INHERIT HETEROZYGOSITY \\
b b - cancer
\end{array} \]

Inherit one (of the two copies) with a germ line driver mutation
mutation is passed through the egg or sperm
passing through the family = passed to half the offspring
d. Breast Cancer - women in US - 1 in 8 will develop breast cancer = 12%

   i. 90 - 95% - Sporadic (usually late age of onset)
   ii. 5 - 10%  - Inherited Predisposition (usually early age of onset)

   Inherit Heterozygosity…..B  b

   If a WOMAN inherits one (of the two) BRCA1 genes with an inactivating TSG driver mutation…..B  b

   Breast Cancer  - risk goes from ~12% to ~80%
   Ovarian Cancer - risk goes from ~1.5% to ~50%

   If a MAN inherits one (of the two) BRCA genes with an inactivating TSG driver mutation…..

   increased chance pancreatic, prostate and male breast cancer

6. How do Most Tumors Develop?

   Process of Clonal Expansion - may take decades

   Solid Tumors - Individual tumor cells packed together in solid mass
   Liquid Tumor - Individual tumor cells dispersed in liquid - blood

   a. Founder Cell gives rise to the Primary Tumor - 8 to 12 years or more
   Few cancer deaths are caused by the primary tumor....~8%.
   Exception: GBM - GlioBlastoma Multiforme brain cancer = primary tumor
   Most cancer deaths due to Benign Tumors that Metastasize = Spread
   Spreading through the body to form Lethal Secondary tumors = Malignant

   b. Metastasis - migration of tumor cells from the Primary Tumor into other remote body tissues.....where they can ‘seed’ and establish Secondary Tumors:
Most cancer deaths, ~92%, from secondary tumors!

Primary Tumor develops in the breast where it is localized at the site where it originated. But as the tumor cells continue to proliferate they accumulate new mutations and becomes ‘Invasive’..... invading areas of the tissue beyond the limits of the original tumor.

The invasive cells may gain entry into a blood vessel and then travel all through the body via the circulatory system.....hematogenous or it may gain entry into lymph node..... lymphogenous, and then travel all through the body via the lymphatic system.

The cancer cells can then exit from the blood vessel or lymphatic vessel and some distant sight and ‘seed’ secondary tumors in the bone, lung, liver, colon etc. Most cancer deaths from secondary tumors!

d. Epithelium and EMT

i. Epithelial Cells form a covering or act as a lining in the body
ii. sits upon a basement membrane - laminar matrix
iii. below the basement membrane is the connective tissue with:
   blood vessels, lymphatic vessels, nerves, muscle etc.
iv. Epithelial cells have cell to cell adhesion - like a rigid box
   BUT.....epithelial cells can lose their rigidity and convert into migratory stem cells - Mesenchymal Stem Cells - a pliable blob like an amoeba tp migrate throughout the body. EMT: Epithelial to Mesenchymal Transition cells transition into a cell that can be Invasive and Metastasize
e. Step by Step of Tumor Formation

i. One epithelial cell acquires a mutation - ONC or TSG
   that single cell (orange cell) is the ‘Founder Cell’
   it can grow faster than the other normal cells
   Leads to a ‘Clone of the Founder Cell’ = ‘Clonal Expansion’ = One clone in Tumor
   Check back at the same site.....four years later.

ii. Four years since the origin.....
   Observe an accumulation of orange clone cells = ‘Hyperplasia’
   One orange cell acquires a second mutation (pink)
   Pink cells can grow faster than the orange cells
   Second clonal expansion of pink cells begins = Two clones in the tumor
   Check back at the same site.....four years later.

iii. Eight years since the origin.....
    Observe an accumulation of pink and orange clones = Dysplasia
    One of the pink cells acquires a third mutation (lavender)
    Lavender cells grow faster than the pink cells
    Third clonal expansion of lavender cells begins = Three clones in the tumor
    Check back at the same site.....four years later.

iv. Twelve years since the origin.....
    Observe Primary tumor - still localized - ‘cancer in situ’.....three clones
    One of the lavender cells acquires a fourth mutation (blue)
    Blue cells go through the EMT conversion - becomes invasive
       penetrates through the basement membrane and invades the
       underlying connective tissue - ‘Invasive Tumor’ = four clones in tumor
    Check back at the same site.....four years later.

v. 16 years since the origin.....
    One of the blue cells acquires a fifth mutation which
    Permits the cell to break away from the tumor mass and enter
    a blood vessel - beginning of Metastasis - five clones in the tumor
    Check back at the same site.....four years later.

vi. 20 years since the origin.....
    5 mutations - 5 clonal expansions
    Invasive, Metastasizing, Life Threatening Carcinoma
    Carcinoma is a tumor that originates from an epithelial cell
    Tremendous Tumor Heterogeneity
6. **Tumor Heterogeneity - Within one Tumor**
   Between and Among Multiple Tumors
   
   a. All the combinations of over 400 mutated ONCs and TSGs
      
      **Drivers and Passengers**
   
   b. Plus chromosomes: Translocations / Aneuploidies / INDELs
   
   c. Plus Chromothripsis: chromosome shattering
   
   d. Plus Epigenetic Chromatin Errors: open and close chromatin
   
   e. Plus ONCOmirs transmitted to normal cells via Exosomes
   
   f. Complexity of Diagnosis and Therapy - Mix of Expanded Clones
      some with MET and others with EMT cell reversion
   
   g. **TCGA - The Cancer Genome Atlas**: [https://cancergenome.nih.gov/](https://cancergenome.nih.gov/)

Lung Adenocarcinoma - leading cause of cancer death in the world....>1,000,000 per year

Cancer Cell Mutations: Drivers + Passengers = 8.9 / 1,000,000

Normal Cell Mutations: Passengers = 1 / 30,000,000

Cancer Cells: 265 fold increase!

New System for Cancer Classification:
Molecular vs. Histopathologic (microscopic)


Multiplatform Analysis of 12 Cancer Types Reveals Molecular Classification within and across Tissues of Origin

Cancers are more likely to be genetically similar based upon their cell type of origin rather than their organ of origin

“MOLECULAR ONCOLOGY” - diagnose with ONC / TSG

About 10% of cancers today, would be improperly classified with the new system.....diagnosis.....therapy.....prognosis

e. **Example of Tumor Formation - Colorectal Cancer**

![Colorectal Cancer Diagram](image)

**Colorectal Cancer.....**

May take 30 years to develop

- Normal epithelium
- Hyperproliferative epithelium
- Aberrant cryptic foci
- Small adenoma
- Large adenoma
- Colon carcinoma

↑ APC (TSG)
↑ COX-2 (ONC)
↑ K-RAS (ONC)
↑ P53 (TSG)
↑ DDC (TSG)
Cancer Prevention by Early Cancer Screening Tests:
- Colonoscopy - beginning age 50 - earlier if there is a Family History if discovered in the first 27 of those 30 years - probably curable!
- Cologuard - Rx... send in a stool sample for evaluation
- Mammogram - beginning age 40 - earlier if FH or BRCA1 / BRCA2
- Cervical Cell Samplet - begin age 25 - earlier if FH or HPV virus
- Prostate - begin age 40 - earlier if FH or elevated PSA Uncomfortable? Yes... Increase Life Expectancy / Save Your Life!
- Skin - Moles: A - Asymmetric shape / B - Border irregular / C - Color varied / D - Diameter > 6mm / E - Evolving
- Lung Cancer Screening - to be covered by Medicare / Breath Test

7. How do Oncogenes and Tumor Suppressor Genes Cause Cancer?

a. What is a tumor - inappropriate accumulation of cells!
What causes - inappropriate accumulation of cells?
- inappropriate mitosis - mitosis at wrong time

b. Cell Cycle - Life Cycle of a Cell

i. G1 - Gap in time between the last mitosis before S
S - Synthesis of DNA - DNA Replication go from 46 to 92 chromosomes
G2 - Gap in time between S and Mitosis
M - Mitosis: Prophase / Metaphase / Anaphase / Telophase

j. Cell Cycle Check Points - check before progressing on
ii. Cell Cycle usually turned off - only turns on for mitosis

iii. What keeps the cycle turned off? Protein Padlock

c. What turns the cycle ON? What unlocks the Padlock? a KEY!

d. CELL SIGNAL PATHWAY - TURNS CYCLE ‘ON’
e. Normal Genes, ~400, can mutate to cause cancer
   i. Oncogenes - ONC:
      Dominant Trait - One Allele Mutates
   ii. Tumor Suppressor Genes – TSG;
      Recessive Trait - Both Alleles Mutated
      TSG mutation = ‘HIT’

f. TSG - Tumor Suppressor Genes…..
   INHERITED PREDISPOSITION
   i. Retinoblastoma (RB) - cancer of the retina of the eye
      RB Gene at 13q14.2 - produces the RB Protein

```
TSG Paradigm: RB
Sporadic 2 - HITS 55%
RR → Rr → rr
Inherited Predisposition 45%
Rr → rr
Inherited Heterozygosity
```

1. What does the RB Protein do?
   Cell Cycle Repressor…..RB Protein is the PADLOCK

```
RR RR
R r r
rr rr

NO Padlock! Cycle Wide Open
```

14
8. Cancer Stem Cells

a. ‘STEMNESS’ - the Unique Characteristics of Stem Cells

i. Self Renewal - Asymmetric mitosis - makes copies of itself
   1x, 10x, 1,000,000x

ii. Biologic Immortality - Telomerase to maintain Telomeres

iii. Differentiate - Produce any of 220 human somatic cell types

iv. Migrate - throughout the developing embryo

v. DNA Damage Repair - pump / purge harmful chemicals from cell - repair potential ‘lethal’ DNA damage

b. What if the Founder Cell.....is a Stem Cell?

Founder Cell “STEMNESS” = Cancer Stem Cell “CANCERNESS”

i. Self Renewal - 1x, 10x, 1,000,000x

ii. Biologic Immortality - Cancer Cells grow forever
   Telomerase is upregulated in 85 - 90% of advanced cancers

iii. Differentiate - Produce any of >200 human cancer cell types

iv. Migrate - Invade and Metastasize throughout the body

v. DNA Damage Repair = Resistant to Chemo & Radiation therapy

c. perhaps All Founder Cells.....All Tumors.....All Cancers
   are produced from mutated Adult Stem Cells!
d. Cancer Stem Cells - small subpopulation of cells in most human tumors

Small subpopulation of cells in most human tumors
1. Drive Tumor Growth - asymmetric mitosis
2. Drive Tumor Development - ~200 cancer cell types
3. Drive Tumor Metastasis - migratory cells
4. Drive Tumor Recurrence - resistant to Chemo and Radiation

e. Often times chemo therapy will destroy all the mass of pink cells
Cancer disappears - cannot find it on X-ray or CT scan
Cancer appears to be gone!.....”Cancer Free”!
But Sometimes - 5 or 6 years later - it returns.....Cancer Recurrence!
WHY? Chemotherapy killed the bulk of the pink differentiated Cancer Clones - but ultimately failed because it did not eliminate the Cancer Stem Cells - Resistant to chemo - they survive and slowly continue asymmetric mitosis until tumor returns!

f. Future - Combinational Therapy
Chemo or radiation to eliminate the bulk of the clonal differentiated tumor cells
Followed by a different therapy to selectively get rid of the Cancer Stem Cells

g. Hematopoietic Stem Cells Mutate > Leukemias and Lymphomas
Intestinal Crypt Stem Cells > ColoRectal Cance

Skin - Epidermal Stem Cells > Squamous Cell Carcinoma
Melanocytes > Melanoma
Dermal Stem Cells > Basal Cell Carcinoma
9. **Cancer Therapy** - the ‘DREAM’….Magic Bullet!

Magic Bullet Therapy: attacks and effects only the cancer cells and leaves normal cells unharmed!

a. **Tumor removal before invasiveness and metastasis:**
   - polyp snare / skin cancer resection / surgery to remove tumor

b. **High Risk Organ removal before any cancer occurs:**
   - mastectomy and or oophorectomy with a BRCA1 mutation carrier

c. **Chemotherapy:** harsh cytotoxic chemicals - damage DNA
   - Systemic Therapy - causes death of rapidly dividing cancer cells via apoptosis

d. **Radiation:** radiation directed at rapidly growing cancer cells
   - Directed Therapy leading to DNA and chromosome damage and cell death

e. **Bone Marrow Transplant:** destroy leukemic WBCs…..
   - and replace them with new normal WBCs - from donor or self

f. **RAS = Targeted Therapies:** Gleevec - to correct the BCR/ABL abnormal cancer protein in Chronic Myelocytic Leukemia

g. **Hormonal Therapy** - some breast and prostate tumors can be affected by adding or removing certain hormones like estrogen or testosterone

h. **Angiogenesis Inhibitors** - all tumors need a blood supply
   - these therapies stop the formation of new blood vessels in a developing tumor

i. **Antibody Blocking:** block signal pathway receptor - Herceptin
   - blocks the HER/NEW receptor – blocks abnormal breast cancer mitosis

j. **micro RNA:** man-made microRNA molecule can selectively
   - turn off one specific oncogene - prostate cancer

k. **Vaccines** - for cancer caused by Oncoviruses like Hepatitis
   - for liver cancer and HPV for cervical cancer

l. **Therapeutic Cancer Vaccines** - use cancer cell proteins to
   - stimulate the immune system to attack the cancer

m. **Epigenetic Therapy** - Demethylation or Hypermethylation
   - of Chromatin for ONC inactivation or TSG activation

n. **Liquid Biopsy** - nearly every tumor sheds DNA into blood
   - new tests coming to diagnosis cancer from a simple, annual, blood evaluation.
10. Immunotherapy for Cancer

A. Human Immune System - White Blood Cells

Our body’s natural defense system against: viruses, bacteria, fungi, protozoa and CANCER

INNATE IMMUNE SYSTEM - first line of defense
Immediate and Short Term Protection - Non-specific Generic Protection
WBCs: Neutrophils, Macrophages and Natural Killer Cells

ADAPTIVE IMMUNE SYSTEM - Adapts to a Specific Infection
Recognition is Retained.....Remembered - ‘Immunologic Memory’
Basis for Immunizations and Vaccinations
Recognizes Non-Self Molecular antigens
WBCs: B Lymphocytes (CD8) and T Lymphocytes (CD19)

SELF v NON-SELF

SELF - molecular components of YOU - your body
e.g. HLA Type Human Leukocyte Antigens - transplantation markers

NON-SELF - Foreign molecular components of viruses, bacteria, protozoa etc. and CANCER CELLS

Foreign molecules are often ANTIGENS = ANTIbody GENERator

LYMPHOCYTES: B Lymphocytes - produce AntiBodYs - Y shaped molecules immunoglobulins

T Lymphocytes - aTTack cells

IMMUNE SYSTEM MISTAKES - mistakenly attack SELF
Autoimmune Diseases: Diabetes Type 1, Crohn’s Disease, Multiple Sclerosis

B. IMMUNOTHERAPY.....CAR T / PD-1 / CD47

T Cells: 1. Surveillance, 2. Interrogation,

CAR T Cells - Chimeric Antigen Receptor
Genetic Manipulation of Normal T Cells and the Immune System
Ignores Self (CD19) and Kills CLL Leukemia Cells

“Living Medicine” - Therapy from WITHIN rather than WITHOUT
**PD-1 and PD-L1**

**PD-1 is an Immune Checkpoint Inhibitor**

**T Cell Surveillance Identification**

Keytruda and Opdivo - both are Anti-PD1

Tecentriq and Bavincio - both Anti-PD-L1

**CD47 Immunotherapy with MACROPHAGES**

Dr. Irv Weismann - Stanford University

1. Surveillance, 2. Interrogation, 3. Identification and 4. Elimination by Phagocytosis = Eats the Non-Self Cell - like amoeba

Gets rid of: old cells / damaged cells / CANCER cells

CD47 - cell surface molecule.....“Don’t Eat Me”

CD47 Disappears - old red blood cells / apoptotic bodies

CD47 covers surface of Hematopoietic Stem Cells: HSC, MPC, LPC

Protects normal stem cells from Mac Attack immune destruction

BUT also:

Protects Cancer Stem Cells from Mac Attack immune destruction

CD47 Monoclonal Antibody - blocks CD47 “Don’t Eat Me” molecule

Investigation under way for: Ovarian, Breast, Colon, Brain, Pancreatic

Dr. Michael Clarke – Stanford; Indiana University; Greenfield HS

Pediatric brain Tumor Success - reported March 15, 2017

11. LIQUID BIOPSY:

nearly every tumor sheds cancer cell DNA into the blood

A. Tests being developed for simple, annual, blood screening evaluations

B. MasSpec Pen - rapidly and accurately detects cancer cells during surgery

C. Future annual CSC Screening - ‘Stop Cancer before it Begins’

D. That, of course, is called: FIGROI
CRISPR-Cas Editing Systems - Applications and Implications

.....the DREAM

A. Millions of people around the world suffering and dying.....

Because one of their two genes is not working - AD (Autosomal Dominant)
Because both of their two genes are not working - AR (Autosomal Recessive)

OMIM Database - details on >24,000 Monogenic Diseases

1. IF we could find a way to Dissect the Human Genomic DNA
2. IF we could accurately control the precision of the cut

We may be able to correct any genetic disease in the laboratory
Dissect out a... ‘Bad Gene’..... ‘Knock Out’
Replace with a... ‘Good Gene’..... ‘Knock In’

Precision Genome Surgery!

B. DREAM..... ’GENOME EDITING’ of the human genome

Cut and Paste our own DNA
Cut out a bad gene - Paste in a good gene..... ‘Knock Out / Knock In’

DREAM: GENE REPLACEMENT THERAPY

Previous methods of Genome Editing:

1. ZFNs - Zinc Finger Nucleases
2. TALEN - Transcription Activator Like Effector Nucleases
   very slow and tedious and lacked accuracy

Gene Replacement Therapy: fast and easy - 99% faster than before
.....Precision Genome Editing

Precision: cut any one of over 3,000,000,000 human nucleotides

Initial discovery - 2005.....mechanism of bacterial ‘Adaptive’ immunity

primitive bacterial / archaea immune system

‘Adaptive Immunity’

Immune System - ‘ADAPTS’ to fight Repeated Infections
- ‘Remembers’ previous infections
- Basis of Immunizations / Vaccinations
Review: Bacteriophage viruses can attack and destroy bacterial cells

Bacterial Immune System.....

Interrogates foreign viral DNA
Eliminates harmful viral DNA
Remembers previous viral infections = Adaptive Immunity

If virus enters bacterium again - it is destroyed by chopping up virus DNA

Discovery came from a ‘cup of yogurt’ - studying Streptococcus thermophiles

By dairy scientists trying to better understand how certain bacteria helps yogurt develop its distinctive ‘Tang’

Bacterial genome DNA generates multiple random repeated RNA sequences which turned out to be the mechanism for virus DNA recognition by the bacterium and leads to viral DNA destruction - kills virus

The Random Repeated RNA Sequences referred to as:

Clustered Regularly Interspersed Short Palindromic Repeats - ‘CRISPR’

C. CRISPR/Cas9 System for natural bacterial immunity

CRISPR Recognizes virus DNA when it enters the bacterial cell > destroys it

Destroys virus DNA with CRISPR associated proteins = Cas proteins

1. Helicase - unwinds virus DNA helix - separates two DNA strands
2. Nuclease - destroys the virus DNA with a double strand cut

CRISPR/Cas9 - two components

1. Cas9: Enzyme component - Nuclease and Helicase
   Nuclease - cuts virus DNA: double scissors/cut both strands

2. CRISPR Guide RNA = gRNA - interrogates foreign (virus) DNA then Guides it to the Nuclease for precise cutting
gRNA recognizes foreign virus DNA in bacterial cell and destroys it..... by guiding it to the Cas9 Nuclease enzyme for DNA double strand cut

gRNA ‘Homing Mechanism’- homes to specific viral DNA sequence

Example – CRISPR/Cas9 with Viral DNA

D. CRISPR/Cas9 System Controlled in the Lab for Human Gene Editing

CRISPR/Cas9 is a bacterial cell Organelle - How can we take control? WE PROVIDE the GUIDE.....lab-made gRNA combined with CRISPR Guide sequence is usually ~20 nucleotide sequence made in a laboratory Lab made gRNA recognizes any Target we Specify in any cell Precision DNA cut

NHEJ: Non-Homologous End Joining
Fill DNA gap with RANDOM nucleotides - creates DNA errors Inactivates DNA - ‘Knock Out’

HDR: Homology Directed Recombination ’Knock In’ add normal gene Fill DNA gap with TEMPLATE directed Nucleotides - corrects DNA ‘Knock In’ a normal gene - corrects a genetic mutation

‘Cut Out a Bad Gene and Paste in a Good Gene’ - in your own DNA Edit DNA to Correct: Sickle Cell, Cystic Fibrosis, Parkinson Dx, MS
E. The Miracle in Genome Editing Technology - 2012

Dr. Jennifer Doudna and Dr. Emmanuelle Carpentier

*Science, vol. 337, pp.816-821, August 17, 2012*

Dr. Feng Zhang and Dr. George Church - CRISPR/Cas9 in human cells

*Patent Battle: Cal Berkeley v. Broad Institute - MIT* - MIT Won! Cal Berkley is Appealing

F. References and Videos on CRISPR/Cas9

1. ‘Applications of CRISPR technologies in research and beyond’
   *Nature Biotechnology, September 2016, pp. 933-940*

2. ‘The Gene Machine’
   - *Time Magazine, Cover Article, July 4, 2016*

3. ‘Dawn of the Age of Genome Editing’
   - *Nature - March 10, 2016, pp.155-167*

4. ‘The DNA Revolution’
   - *National Geographic - Cover Article - August 2016*

5. ‘What is CRISPR?’
   - *https://www.youtube.com/watch?v=MnYppmsxtxIs*

6. ‘Genome Editing with CRISPR’
   - *https://www.youtube.com/watch?v=2pp17E4E-O*

7. TED Talk: ‘How CRISPR lets us edit out DNA’
   - Jennifer Doudna
   - *https://www.youtube.com/watch?v=TdBAHexVYzc*

8. Genetic engineering will change everything forever - CRISPR
   - *https://www.youtube.com/watch?v=jAhjPd4uNFy*

9. ‘The CRISPR Pioneers’
   - *Time Magazine, pp. 116-122, December 19, 2016*

G. CRISPR/Cas9 Applications…..

1. Yeast - reprogramming to convert sugars into Biofuels

2. Wheat - delete genes to gain resistance to Powdery Mildew

3. Mushrooms - delete gene to prevent browning and early decay

4. Tomato - delete PL gene for better fruit softening, shelf life and flavor

5. Cabbage - knock out Psbs gene for better flavor and shelf life
   first GM fried cabbage and first cabbage pasta salad

6. Mosquitos - delete gene to cause sterilization…..Malaria, Zika, Dengue

7. Bacteria - enhance plastic degradation genes…..‘plastic eating bacteria’
   CRISPR in high school lab: the-odin.com $150

8. Golden Rice - altered to produce Vitamin A…..prevention of blindness
   *See letter on page 7 regarding safety of GMOs*

9. Save elephant species by inserting genes from extinct wooly mammoth
   so they can survive in the frozen tundra habitat for elephants

10. Prevent AIDS - delete CCR-5 Gene and CCR-5 T-cell receptor for HIV

11. Delete myostatin gene to create Double Muscleing in beagles
12. Macaque - Delete three zygote genes show ease of procedures in primates
13. Duchene Muscular Dystrophy - delete exon 51 that harbors the mutation
14. CURE for Sickle Cell Anemia - edit and replace SCA mutation in HSCs
15. iPS + CRISPR - ‘correct’ medium spiny neurons iPS in Huntington Dx
16. Cancer research - modify PD-1 receptor the mechanisms for Immunotherapy
17. CRISPR Diagnostic Platform to ID specific viruses, pathologic bacteria, genotype human DNA and diagnose cancer  
   *Science, April 32, 2017, pp.438-442*
18. Pigs - deleted all 62 PREV virus genes and 20 pig antigen genes 
   transplant CRISPR Pig organs into humans.....~12 months away!
   EDITING.....the Germ Line, zygote, embryo, eggs or sperm:
   Affects every cell of the body! Alters that individual forever!
   Humans are tinkering with human evolution!
21. Edit Human embryos in London: study early embryologic cell divisions
22. Edit Human embryos in Stockholm: study early embryologic development
   PROBLEMS: Insertion timing; Embryo Mosaicism; Editing success; Off target edit

H. Is Genome Editing really necessary??

   PGD - Preimplantation Genetic Diagnosis.....most editing cases would not be necessary

I. Moratorium?? CRISPR Summit:

   1. National Academy of Science ‘Gene Editing Summit’ - December 2015
   2. Scientists Seek Ban on Method of Editing the Human Genome
   3. Human Gene Editing Receives Science Panel’s Support

J. THERAPY v ENHANCEMENT:

   1. Therapy - a procedure to help a person reach normality
   2. Book - Brave New World by Aldous Huxley - 1932
   3. Movie - GATTACA - with Ethan Hawk and Uma Thurman - 1997
   3. ‘Editing Humanity’ - The Economist, August 22-28, 2015
   4. The Could / Should Dilemma
   5. Jennifer Doudna - “A Crack in Creation”