Human Embryology: Naturally Occurring Stem Cells
Lab made Stem Cells: iPS - induced Pluripotent Stem Cells

Stem Cells and Somatic Cells occurring NATURALLY

SOMATIC Cells - normal functioning unique cells all over the human body
  Each Highly Specialized for a particular function.....~220 somatic cell types
  Examples: muscle, blood, nerve, skin, intestinal, bone, heart, liver etc.
  As they go through specialization - lose the ability to go through Mitosis

STEM Cells - word borrowed from the plants - ‘Stem’ gives rise to branches
  Non-specialized - BUT with multiple Potentials to produce 220 Somatic Cells
  Very active Mitosis

*Any newly formed human cell.....comes from a pre-existing Stem Cell*

STEM CELLS categorized two ways:
1. Stem Cells: based on Somatic Potentials - three groups:
   a. Pluripotent Stems - can produce ANY / ALL of the 220 CELL TYPES
   b. Multipotent Stems - can produce MANY, but not all, of the 220 cells
   c. Totipotent Stems - can produce fetus, umbilical cord, placenta, membranes
      .....Zygote - through the eight cell embryo cells

2. Stem Cells: based on Embryo Function - three types:
   a. Embryonic Stem Cells (ESC).....~10 day life span - Natural
   b. Adult Stem Cells (ACS).........4 types - Natural
   c. Cancer Stem Cells (CSC).........ASC Mutate => cancer - Natural

Embryology.....it takes eight weeks - formation of a human fetus - 2/7 Rule
When does this all begin? Egg from the Ovary + Sperm from the Testis

Female: Ovum = Oocyte = Egg / Male: Spermatozoa = sperm
Ovulation - release of oocyte from ovary
Fertilization - sperm enters and activates the oocyte
Oocyte nucleus - Female Pronucleus (23); Sperm nucleus - Male Pronucleus (23)

Zygote Nucleus - fusion of female and male pronuclei - contains the Fetal Genome

Genome = sum total of all the genetic information for any organism;
>3,200,000,000 nucleotide pairs - >6 meters of DNA packed in 46 chromosomes;
~24,500 coding genes - code for proteins;
~0.000055% of genome - not in nucleus…..16,569nts in mitochondrial DNA

Zygote = fertilized egg, largest human cell, point of straight pin, visible with naked eye

Zona Pelucida (ZP) = gelatin surrounding zygote; sperm penetrate ZP for fertilization
Two Cell Embryo at about 36 hours = two blastomeres; 6 cleavage divisions
Cleavage Divisions: 1 >2 >4 >8 >16 >32 >64; 64 cell embryo = morula
Human Hatching: ZP turns into a hard case = shell, embryo ‘Hatches’ on day 4

Blastocyst - embryo turns into a hollow sphere with ESCs by days 5 - 6
hollow sphere, point of a pin, implants in uterine wall ~6 days

Inside the blastocyst: fetus form….produces 220 new cell types…..HOW?
DIFFERENTIATION of SOMATIC CELLS:

Amazing process ‘Specialization’ of somatic cells.....220 Different Ways
220 One Way Streets - 220 Sets of Signals - bring into existence 220 somatic cells
Terminal Differentiation - end point of 220 Streets - 220 Specialized Cells
All Natural Embryology - ‘One Way Street’ - Basic Tenet Human Embryology
Programming - another word for Differentiation.....220 programs!

EMBRYONIC STEM CELLS.....day 9, 10, 11 - morph into four major branches:

ADULT STEM CELLS:
1. HSCs - Hematopoietic SCs > forms all blood cells - RBCs, WBCs. Bs, Ts, Macs etc.
2. MSCs - Mesenchymal SCs > forms muscle, bone, cartilage, heart etc.
3. ESCs - Endodermal SCs > forms liver, stomach, lungs, pancreas etc.
4. NSCs - Neural SCs > brain, spinal cord, astrocytes, glia, epidermis

PROGRAMMING: an unspecialized, pluripotent nucleus, in a zygote, follows a specific set of signal instructions, to become a highly specialized nucleus, in one of 220 specific somatic cells

Where does the fetus form?
in the Amniotic Cavity – forms within the embryonic stem cell mass
implantation day 6-10; embryo covered with sprouts = chorionic villi
Day 28 - the size of a pea; 6 weeks - chorion covered with villi
Day 34, 35, 36 - face forms - Day 49 - fontanel (soft spot) present; neural tube closes
Where does the water in the amnion come from? Fetal kidneys / Recycles
Where does the umbilical cord go? the placenta - develops from the villi
What are the signals:  
1. Transcription Factors  
2. Epigenetic Chromatin Regulators

Signals - Turn Genes ON and OFF
They can Activate or Repress dozens to hundreds of genes at one time
Embryonic Stems => Adult Stems => Progenitor Stem Cells => Somatic

Progenitor Stem Cells…..Stem fully committed to that Somatic Cell Line

What do Adult Stem Cells do?
1. Bring into existence all 220 somatic cells….in first 8 weeks!
2. Replace all the worn out somatic cells…..for the rest of your life!

Example - Skin: 
Replaced every ~30 days!

HUMAN EMBRYOME PROJECT: find all 220 Sets of Signals
STEM CELLS and SOMATIC CELLS
produced in a LABORATORY
the DREAM…..

Millions of people around the world are suffering and dying.....
because one cell, out of 220, is slowly disappearing!
Millions of people around the world are suffering and dying.....
because one cell out of 220 has disappeared - they have 219!

DREAM.....‘CELL REPLACEMENT THERAPY’

1. IF we could find all 220 sets of signals.....and
2. IF we had access to Pluripotent Stem Cells in the laboratory
We could potentially produce any human cell in the laboratory!

Dilemma: Need Pluripotent Stem Cells
We only had access to Pluripotent Stems in an embryo
Difficult to study; Rejection problems; Ethical concerns

.....and then there was a MIRACLE:

Dr. Shinya Yamanaka - Kyoto University in Japan
Discovered how to convert SKIN cells into STEM CELLS:
Help from a very special female from Scotland.....who was dead!
Dolly - the famous cloned sheep Nature, Cover article, June 30, 2016
To clone Dolly: Researchers had to take a mammary gland somatic cell
nucleus and reprogram it backwards to a pluripotent state.

Yamanaka and his team found the signals in the oocyte of the sheep that
‘Reprogrammed’ the somatic nucleus in reverse back to pluripotency.
They converted the one-way street into a two-way street!
Found the four ‘Stemness Signals’ could reprogram a somatic nucleus
CELL REPLACEMENT THERAPY came true thanks to Dolly!!

July 7, 2006: Yamanaka announces he has taken somatic cells from the skin
of a MOUSE, inserted four ‘Stemness Signals’ - Oct3/4, Sox2, c-Myc &
Klf4, also known as (OSMK), and reprogrammed the skin cells into
pluripotent stem cells = mouse iPSC.
The 4 signals also called the ‘Yamanaka Factors’.

November 20, 2007: Yamanaka announces he repeated the same procedure
with HUMAN skin fibroblast cells and created:
Human induced Pluripotent Stem cells = iPSCs or iPS.
‘Lab-Made’ human pluripotent stem cells.

REPROGRAMMING: a highly specialized nucleus, in one of 220 specific somatic
cells, follows a set of four ‘stemness signal’ instructions, to reverse the
specialization process and revert to a zygotic, unspecialized, pluripotent nucleus

December 10, 2012: Yamanaka received Nobel Prize for Medicine
That paved the way for the “Miraculous Swap”:
TRADE: Pluripotent Embryonic Stem Cells in a blastocyst - 10 days
FOR: Pluripotent iPSCs in a petri dish - can grow forever
POTENTIAL APPLICATIONS of iPSCs:

1. **CELL REPLACEMENT THERAPY**:

   Autologous = no rejection because you use your own skin.
   
   Skin Regeneration - produce new skin for Epidermolysis Bullosa
   
   In Utero Amniotic Fluid Stem Cell Therapy for Myelomeningocele
   
   Heart Cell Regeneration for Hypoplastic Left Heart Syndrome
   
   Dopamine producing neurons
   Parkinson Disease: Skin > iPSC > human DPCs > Dopamine Neurons
   
   Retinal Cells for Age Related Macular Degeneration
   Future: *National iPSC Repositories*
   …..use iPSC cells of someone who matches your
   By matching transplantation HLA antigen types
   
   Blood: Skin > iPSC > human HSCs > platelets & erythrocytes
   Blood Cell Factories in the future
   
   Multiple Sclerosis: Skin > iPSC > human OPCs > Oligodendrocytes
   
   Diabetes Type I:
   Alginate Capsules protect the iPSC Beta Cells from immunity
   
   VIDEO: [https://www.youtube.com/watch?v=Q6](https://www.youtube.com/watch?v=Q6)
2. DISEASE MODELING:

“Organoids” - primitive organ-like 3-D clusters of **differentiated cells** grown in a laboratory

**ORGANOIDS** - Cover article, *Science, June 7, 2019 - 5 articles*

Inner Ear Organoid: sensory epithelia via 3-D Culture - IUMC  
*Nature, August 8, 2013*

Retinal Rod Cell Organoids: from iPSCs for Retinitis Pigmentosa Therapy

Eye Organoids: Optic Cup Retina

Liver Organoids: Vascularized and Functional Human Liver from iPSCs-liver bud transplant into mesentery

Heart Organoids: Cardiac Repair after MI (Myocardial Infarction)  
Cardiac Fibroblasts > Cardiomyocytes - 3 signals

Large Intestine Organoid: Study the development stages of colon cancer  
*Sequential Cancer Mutations, Nature, May 7, 2015*

Living Colon Cancer Patient Organoid Biobank - *Cell, 161, pp. 933-945, May 7, 2015*

Kidney in a Dish.....First Man-Made Mini-Kidney  
Human kidney organoid with a full set of renal cell types!  
*Cover Article - Nature, vol 526, pp. 512 and 564, October 22, 2015*

Vocal Cords in a Dish.....First Cells for Potential Vocal Cord Replacement  
*Science Translational Medicine*

Human Brain Organoids - Mini-brains - size of an apple seed  
Resembles the Fetal Brain at ~9 Weeks / Microcephaly Brain Organoid - smaller  
‘Cerebral organoids model human brain development and microcephaly’  
Model for study of ASD - autism using the skin from a child with ASD diagnosis  
Model for developing drugs for various brain related conditions

Human 5 Week Fetal Brain in a petri plate made from iPSCs - size of a pea  
99% of normal genes working - contains part of spinal cord and retina  
Takes ~12 weeks in vitro to reach the 5 week in vivo stage  
May be a model to study experimental drugs and causes of brain disease  
May be as a model to study: ASD, Parkinson, Alzheimer - using their skin  
Attach to nerves and muscles in vitro - on their own - Intrinsic  
Primitive brain waves discovered in lab made mini-brains!!  
Could those Mini-Brains become sentient - develop the ability to feel and sense?  

“Organoid on a Chip” - Multichannel, 3-D, microfluid, cell culture on a plastic, chip.....  
with living vascular cells to mimic the body’s circulatory system  
Simulates the basic anatomy and function of entire organs.  
can be used to test chemical toxicity, normal cells v. disease

Lung Organoid on a Chip  
Heart Organoid on a Chip  
Nephron Organoid on a Chip  
Artery Organoid on a Chip  
Lymph Vessels on a Chip  
Person on a Chip  
*Microfluidic organs-on-chips - Nature Biotechnology, Vol 32, August 2014, pp. 760-770*

2. DRUG THERAPY SCREENING:

Screen thousands of small molecules, all at one time, in a petri dish, find **one** out of 1000s, with desired beneficial therapeutic effect

Lou Gehrig's Disease (Amyotrophic Lateral Sclerosis)  
screened over 5,000 molecules to find one: Kenpallone
3. REGENERATIVE MEDICINE:

Produce human tissues and organs in the laboratory

Cell Replacement Examples: *Cell Stem Cell* - July 2, 2015

Scaffold = Framework - basic shape of the organ
.....spherical, tubular, sheet etc.

Blood Vessels.....artery and vein replacement

HSE - Human Skin Equivalent - scaffold: sheet of bovine collagen
Dermal fibroblasts + Epidermal Keratinocytes

Nose - biodegradable nose scaffold

Ear - ear shaped scaffold, grows under the skin of forearm

Bladder - therapy for bladder cancer

Liver - liver cell jet printer

What about making.....Heart, Lung, Liver or Kidney in a lab

originally.....“cannot do it”.....organ scaffolds too complex
today.....Bio-Artificial Organs

Video: [http://www.nature.com/news/tissue-engineering-how-to-build-a-heart-1.13327](http://www.nature.com/news/tissue-engineering-how-to-build-a-heart-1.13327)

Future Heart Transplant: Recipient skin > iPS > cardiomyocyte cells
Donor does not have to be compatible - Decellularize, and Recellularization - , re-start > transplant.....2020!

Decellularization - remove all heart cells and endothelial cells with detergent, under pressure, left with an acellular human heart scaffold

Recellularization - in a Bioreactor: add new heart cells and endothelial cells made ahead of time in the lab, from iPSCs of the recipient

*Biomaterials* - vol. 52, pp. 103-112, June, 2015

Lungs - Decellularization and Recellularization

Pig lung formed in a Bioreactor in lab / successfully transplanted back


Liver - Decellularization and Recellularization

*Bioengineered transplantable porcine livers – Biomaterials, February, 2015*

Kidneys - Decellularization and Recellularization
Limbs - Bio-Limbs - Decellularization and Recellularization

Detergent removal of all cells, leaving cell free scaffold of blood vessels, tendons and muscle from a rat.....took several weeks.....then in a bioreactor, supplied the progenitor stem cells for replacement cells, even outer skin covering - and the new limbs responded normally to electric stimuli and the fingers contracted.


Biotech company produces human heart with 3-D printer

Step toward ‘INK’ development for 3-D Printing of bio-prosthetic Ovary

MAKING MEAT FROM STEM CELLS

Producing ground beef, sausage or chicken from muscle stem cells
STEMburgers - available by 2020!
Cultured Meat / Clean Meat / Synthetic Meat / in vitro Meat
‘Impossible Burger’ - different - it has all plant components
Stem Cells make steak in space!

STEM CELLS AND CANCER:

Example - Skin:

Squamous Carcinoma
Melanoma
Basal Bell Carcinoma
CRISPR DNA and RNA Editing Systems
Applications and Implications.....the CRISPR Toolbox

REVIEW: DNA Double Helix.....A - T / G - C .....Complementary Pairs

1 Gene transcribed > Millions of mRNAs translate > Millions of Protein molecules
Genes - sit in a chromosome! Proteins do all the work!
Proteins are how the genes manifest themselves!
Genome: 3,200,000,000 nucleotides.....
Coding DNA = Exons: ~2% of human genome.....Non-Coding DNA = Introns: ~98%

DNA is DNA is DNA.....Virus, Bacteria, Plant, Animal, Human
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 >50,000 Monogenic Diseases

1. IF we could Surgically modify the Human Genome

2. IF we could control the Precision of the Surgical Incision
   We may be able to Surgically Correct any genetic single gene
disease in the laboratory!

3. Human Genome DNA is less than 1/40,000th the diameter of a human hair
   Surgically remove a... 'Bad Gene'....'Knock Out'
   Surgically implant a....'Good Gene'....'Knock In'
   Precision Genome Surgery - 3.2 billion incision sites!

DREAM.....’GENOME EDITING’ of the human genome
Definition.....to modify a gene or gene product by inserting,
   deleting or replacing a DNA or RNA sequence

‘GENE REPLACEMENT THERAPY’

Previous methods of Genome Editing:
   1. ZFNs   - Zinc Finger Nucleases
   2. TALENs - Transcription Activator Like Effector Nucleases
      very slow, tedious and lacked locus accuracy

DREAM: GENE Replacement Therapy: fast and easy
      99% faster than before.....Precision Genome Editing

   Precision: cut at over 3,200,000,000 human nucleotide pairs

Initial discovery - 2005.....mechanism in Bacteria for ‘Adaptive’ Immunity
   primitive bacteria / archaea immune systems

Human Immune System   - trillions of white blood cells

Bacterial Immune System - three molecules:
   1. Helicase
   2. CRISPR
   3. Nuclease

   Immune System - ‘ADAPTS’ to fight Repeated Viral Infections
      - ‘Remembers’ previous infections
      - Basis of our Immunizations / Vaccinations
Bacterial Immune System…..

Bacteriophage viruses can attack and destroy bacterial cells:

**Bacterial Immune System:**

1. Interrogates Foreign DNA
2. Eliminates Non-Self DNA

Interrogates and Identifies foreign DNA entering the bacterial cell
Eliminates harmful viral, destroys non-self DNA
Remembers previous viral infections = Adaptive Immunity
If virus re-enters bacterium - destroyed by surgically cutting virus DNA

Discovery came from a ‘cup of yogurt’ - studying Streptococcus thermophiles
by dairy scientists trying to better understand how certain bacteria help yogurt develop its distinctive taste or ‘Tang’

Bacterial genome DNA generates multiple random repeated RNA sequences
which turned out to be the mechanism for virus DNA recognition

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

**CRISPR:**
the random repeated RNA Sequences
Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR/Cas9 System makes up the natural bacterial immune system

CRISPR RNA - IDENTIFIES virus DNA entering bacterial cell - DNA/RNA
in order for it to be destroyed by cutting into small pieces

Cas PROTEINS - CRISPR associated system - DESTROYS viral DNA

1. Helicase - unwinds virus DNA helix - separates two DNA strands
2. Nuclease - creates Double Strand Break - (DSB) = Target GAP

GAP closure creates mutations in virus DNA when
filling the GAP > leading to inactivated virus
Nuclease is an RNA-Guided DNA Endonuclease enzyme
CRISPR/Cas9 - 2 Components:

   Nucleases - cut virus DNA: 2 pairs of scissors > GAP

2. CRISPR: natural molecule of bacterial immune system
   identifies foreign (virus = non-self) DNA
   then Guides it to Nuclease for precise incision and Gap

What is gRNA = guide RNA:
Modified CRISPR RNA made in a laboratory
biochemically linked with tracrRNA

Example: CRISPR/Cas9 with Viral DNA
PAM - Protospacer-Adjacent Motif - NGG sequence on the virus DNA where gRNA initiates attachment for target recognition
PAM from SpCas: NGG = any nucleotide/guanine/guanine
NGG = 9.9%.....of human genome
PAM from ScCas: NNG = any/any/guanine = >50% of HG

CRISPR/Cas9 is a bacterial cell Organelle

What if we could learn to control that organelle?
How can we take control.....WE PROVIDE the GUIDE.....
Lab-made gRNA combined with Cas protein enzymes

Guide RNA is 20 RNA nucleotide sequence made in a laboratory
Lab made gRNA recognizes ANY Target we Specify in ANY cell
Precision DNA incision in the Genome = DSB = creates a GAP

How do we fill the nuclease incision GAP?

LEFT SIDE of the diagram - NHEJ: Non-Homologous End Joining.....
Leads to INDELs - Inserions / DEletions = Frame Shift Mutations
Fill DNA gap via natural mechanism - with RANDOM nucleotides
creates DNA Frame Shift mutations
‘KNOCK OUT’ Inactivates a gene - mutates the DNA

RIGHT SIDE of the diagram - HDR: Homology Directed Recombination
Fill DNA gap with TEMPLATE directed Nucleotides
‘KNOCK IN’ Corrects the genetic mutation - a normal gene
‘Cut Out a Bad Gene / Paste in a Good Gene’ - in your own DNA
CRISPR - where does this term come from? / what does it mean?

P - Palindromic / Palindrome - extended sequence of letters or numbers; sequence the same starting from either end - mirror image from the middle

**PALINDROMEEMORDNILAP**

POP
ABBA
RADAR
RACECAR
A TOYOTA
AIBOHPHOBIA

DNA Palindrome: GGAATCGATCTTAAGATCTCCGATTCC
CCTTAGCTAGAA

CRISPR Locus…..on a circular Bacterial Chromosome

Spacer DNA (black) is a short segment of viral DNA – captured at previous infection
When it is transcribed from DNA into RNA => CRISPR RNA

How is CRISPR delivered to the target cell?

1. Delivered to therapeutic cell inside an AAV - Adeno-Associated Virus infects cell
2. Electroporation - electric shock, 1,100 volts, creates pores in target cell
3. Lipid Nanoparticles - synthetic lipid layer which will break down when it enters a cell - they carry mRNA versions of all the CRISPR machinery which will be translated into proteins in the cell…..~90% efficient

G. The Miracle in Genome Editing Technology - August 17, 2012

Dr. Jennifer Doudna (DO U DNA) / Dr. Emmanuelle Carpentier:
CRISPR/Cas9 Programming to seek out and cut any target
*Science, vol. 337, pp.816-821, August 17, 2012*

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

Cal Berkley Appealed…..MIT Broad won appeal
Cal Berkley - Re-appealed and WON – August 20, 2019!
References and Videos on CRISPR

1. CRISPR 101 - FREE 2019 text book:  
   [https://app.hubspot.com/documents/2418554/view/38573039?accessId=08e364](https://app.hubspot.com/documents/2418554/view/38573039?accessId=08e364)

2. Review Article: ‘CRISPR/Cas guides the future of genetic engineering’  
   *Science* - Vol 631, pp.866-869, August 31, 2018

3. God in a kit: the perils and possibilities of a toolbox called CRISPR  


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

6. ‘What is CRISPR?’ - [https://www.youtube.com/watch?v=MnYppmstxJs](https://www.youtube.com/watch?v=MnYppmstxJs)

7. ‘Editing Humanity’ - *The Economist* - August 22, 2015

8. ‘Genome Editing with CRISPR’ - [https://www.youtube.com/watch?v=2pp17E4EO8](https://www.youtube.com/watch?v=2pp17E4EO8)

9. TED Talk: ‘How CRISPR lets us edit our DNA’ - Jennifer Doudna  
   [https://www.youtube.com/watch?v=TdBAHexVYZc](https://www.youtube.com/watch?v=TdBAHexVYZc)

10. Genetic engineering will change everything forever - CRISPR  
    [https://www.youtube.com/watch?v=jAhjPd4uNFY](https://www.youtube.com/watch?v=jAhjPd4uNFY)

11. MIT Technology Review - ‘We Can Now Engineer the Human Race’  
    *Vol. 118, May/June, 2015*

CRISPR/Cas9 Applications…..

1. Baby gets In Utero Stem Cell Transplant from Mother with Thalassemia

2. Gene Editing therapy for Hunter Syndrome using TALENs

3. CRISPR/Cas9 corrects mutation in Duchenne Muscular Dystrophy

4. Fragile X Syndrome neurons restored by CRISPR/Cas9 Reactivation  
   Epigenetic Demethylation to Reactivate an inactive gene

5. CRISPR/Cas9 generates over 4,000 mutations in BRCA1 cancer gene

6. Yeast - reprogramming genes to convert sugars into Biofuels

7. Wheat - knock out genes to gain resistance to Powdery Mildew

8. Mushrooms – knock out gene to prevent browning and early decay

9. Tomato – knock out PL gene for better fruit softening, shelf life and flavor

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

11. Mosquitos - knock out gene to cause sterilization…..Malaria, Zika, Dengue

12. Bacteria - enhance plastic degradation genes…..‘plastic eating bacteria’

* *CRISPR in high school lab: the-odin.com - Genetic Engineering Kits $159
New California Law - not for use with humans!*

16
13. ‘Can CRISPR Feed the World?’ - Discover Magazine - April 2018, pp 43-51
   a surprising number of common foods are in peril from infections!

14. How GMO Corn Produces up to 10% more than similar types.

15. Golden Rice – add Beta Carotene to produce Vitamin A…..for prevention of blindness
   Letter to Greenpeace signed by 107 Nobel Prize winners:

16 ‘Impact of Genetically Engineered Maize of Agronomic, Environmental and Toxicological traits: a meta-analysis of 21 years of field data’
   “GMOs are not only SAFE…..but are beneficial”
   https://www.nature.com/articles/s41598-018-21284-2

17. Viewpoint: Why CRISPR-edited crops should be allowed in organic agriculture
   a. Genetic Engineering - insertion of a gene at random into host genome
   b. CRISPR - precise locus insertion into the host genome
   c. Mutation breeding - induce mutations in plant seeds with chemicals or radiation and the resulting plants are screened for beneficial mutations

18. Genetically edited microbes produce their own fertilizer
   increase crop production for world’s poorest populations

19. Save elephant species by inserting genes from extinct woolly mammoth
   so elephants can survive in the frozen tundra habitat

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

21. Delete myostatin gene to create ‘Double Muscling’ in cattle and beagles

22. Macaque - Knock out three zygote genes show ease of procedures in primates

23. Duchene Muscular Dystrophy - knock out exon #41 that harbors the mutation

24. ‘CURE’ for Sickle Cell Anemia - edit and replace SCA mutation in the Beta Hemoglobin gene in HSCs - ‘Phenotypic Cure’
25. ‘CURE’ for Sickle Cell Anemia - Reactivate Fetal Hemoglobin
Knock Out the BCL11A inhibition of the gamma hemoglobin gene

26. Huntington Disease- ‘correct’ abnormal mRNA FOCI in Huntington Disease

27. Cancer research - Knock Out PD-1 receptor - for Immunotherapy

28. Leber Congenital Amaurosis (blindness) - inject CRISPR into the retina of the patient = therapy in a person.....not a cell in vitro

29. CRISPR Diagnostic Platform to ID specific viruses, pathologic bacteria, genotype human DNA and diagnose cancer  
Science, Apr 2017, pp.438  
From RNA Virus that attacks bacteria

Dr. Feng Zhang - CRISPR/13a - RNA Guided to an RNA Target
Can locate active viral genes.....RNA Genomes

Can locate virus RNA at attomolar concentrations - 1 part per quintillion!
Cas13a RNA enzyme - cuts target then makes collateral RNA cuts
Then.....it, cuts ANY RNA indiscriminately
Add ‘fluorescent reporter RNA’ to test system > fluorescent glow when cut
 Fluorescent Glow is signal for positive test
Diagnose viral infections with no high tech lab equipment - in the field
Diagnose Cancer early with Liquid Biopsy (blood) - destroy Onco-mRNA
Oncoprotein cannot be produced – Cas-13a STOPS CANCER!

30. ‘Boys Only’ Cattle Herds - Male ‘Terminator Cattle’
Use CRISPR to move SRY (Testes Determining) gene from Y chromosome to
the X chromosome of a bull and converts all XX, females to infertile XX
males! Steer meat production about 10% more efficient than heifers

31. CRISPR editing of human sperm with .....Germ Line Editing
For treating male infertility
Preventing genetic disease when the father carries the harmful gene
May be impossible – due to highly condensed DNA in sperm

32. CRISPR/Cas12k
Targeted Gene Insertion - new CRISPR function beyond bacterial immunity
CAST - CRISPR Associated Transposase mediates highly efficient, RNA guided
insertion of cargo DNA into the bacterial E. coli genome.

33. Human / Animal Chimeras = Human / Animal Hybrids - Xenotransplantation
Produce human organs in Organogenesis Deficient mammalian embryos
Use CRISPR to Knock Out organ specific stem cell organ receptors
Can be used between any two mammals - for future human organ transplantation
34. Transplant CRISPR Pig organs into humans…..
   Pigs - must delete all 62 PERV virus genes and 20 pig antigen genes
   Knock out PERV and ANTIGEN genes in somatic cells
   Nuclear Transfer PERV Knock Outs to create cloned pigs with No PERVs
   Cloned pigs are bred to create herds of pigs with no PERVs or AGs
   For ‘Human Safe’ organ transplants!

35. CRISPR editing of human sperm with…..Germ Line Editing
   For treating male infertility
   Preventing genetic disease when the father carries the harmful gene
   May be impossible – due to highly condensed DNA in sperm

36. SATI - Single homology Arm donor mediated intron-Targeting Integration
   Inserts normal minigene into intron beside the mutated gene = ‘Knock In’
   Corrected Progeria (premature aging syndrome) in mice

37. “Prime Editing” – could surpass CRISPR…..more efficient / fewer errors
   Uses Cas9 Nuclease that only Nicks one strand on target DNA
   Plus Reverse Transcriptase (RT)….converts RNA into DNA
   pegRNA (prime editing guide) to locate and ‘home in on’ target DNA
   carries correct RNA sequence on ‘edited sequence’, RT corrects RNA to DNA

New “Prime Editing” could surpass CRISPR
Nature - October 21, 2019

Cas9 Enzymes
‘Nicks’ One Strand - SSB
Reverse Transcriptase
RNA => DNA
pegRNA (prime editing guide)

A new way to modify DNA, “prime editing”, couples two enzymes, Cas9 (blue)
and reverse transcriptase (red), to a pegRNA, prime editing guide, (green) that
takes the complex to a specific place on DNA’s double helix (yellow and purple)
and also holds the code for an insertion of new DNA at that spot.
crispr
crispr
EDITING HUMAN EMBRYOS:

GERM LINE EDITING.....the Germ Line: Zygote, Embryo, Eggs or Sperm:
Affects all future Generations!!
Affects every cell of the body of the offspring!
Alters the individual forever! Can lead to Genotypic Genetic Cures!!
Humans are tinkering with animal and human evolution!

(Non-Viable)
GENOME Editing Human embryos - China: 2. HIV receptor - April 6, 2016
(Non-Viable)
GENOME Editing Human embryos - London: study early embryo cell divisions
(Non-Viable)
GENOME Editing Human embryos - Stockholm: study early embryos
(Non
GENOME Editing Human Embryos – Cal Berkley (Non-Viable)
72% Success Rate! Correcting Hypertrophic Cardiomyopathy!!

BASE EDITING: CRISPR/dCas9 - VIABLE human embryos - China - Aug 13, 2018

CRISPR/dCas9 = deactivated Cas9 or ‘dead’ Cas9.....
No Nuclease enzyme activity! Does not cut the target DNA - No Gap
Corrected Marfan Syndrome - corrects point mutations: C-to-T or G-to-A
With NO double strand GAPs and NO normal donor TEMPLATES needed

First Gene Edited Human Babies reported born in China

November 28, 2018
CLAIM: First Genome-Edited babies born in China ..... Non-Identical Twin Girls:
Lulu and Nana
CRISPR/Cas9 Knockout of CCR-5 - HIV Protection
Never Documented
MORATORIUM??
1. Academy of Science National ‘Gene Editing Summit’
   Moratorium…..How will it be controlled?…..by Whom?

2. Human Gene Editing Receives science panel’s support
   for NON-VIABLE Embryos ONLY!

THERAPY v ENHANCEMENT:

1. **THERAPY** - CRISPR procedure Correct a Genetic Condition
   GENOTYPIC CURE - correct disease and egg & Sperm

2. **ENHANCEMENT** - procedure to improve above and beyond the norm.
   DNA Editing for Stronger Bones
   DNA Editing for Cardiovascular System
   DNA Editing for Perfect 20/20 Vision
   DNA Editing to be HIV free
   DNA Editing for 6’3” Adonis
   DNA Editing for higher IQ….CCR-5??

BUT is any of this really Necessary?? We have PGD!

**PGD** - Pre-Implantation Genetic Diagnosis can give same results
Remove one cell from 8 cell embryo - check for the harmful gene
Only normal embryos with no mutation implanted into the uterus

The remaining 7 cells form a perfectly normal embryo
Additional References:

   ....a World of Genetic Perfection!
4. ‘Could / Should Dilemma’:
   “.....just because we Could.....does that mean we Should?”
5. Jennifer Doudna Book - ‘A Crack in Creation’ - the history of CRISPR’s discovery and ethical issues
6. The Future of Babies’ - Time Magazine - January 14, 2019
7. ‘Editing Humanity’ - The Economist, August 22-28, 2015
8. "We can now Engineer .....the Human Race!"

[Image of baby with a family tree]
Sam Rhine - College and Career Information and Suggestions:

Check Out:  [http://www.kumc.edu/gec/prof/career.html](http://www.kumc.edu/gec/prof/career.html)

1. **Undergraduate** go to your favorite college and obtain your 4-year Bachelor's degree. Major in biology, biochemistry, molecular biology, bio-engineering etc. Make sure you satisfy the Pre-Med requirements so you can apply to medical school if you decide that is the best route for you.

2. **Medical School** is four years and the curriculum is very similar at all medical schools in the US. The reason for that is that everyone must pass the same national exam after finishing medical school - therefore the schools must cover the basic subject matter. If you pass that exam the summer after finishing medical school, you can then put M.D. behind your name.

3. **Residency** is then 4 - 8 years of ‘Specialty Training’ to become a pediatrician, obstetrician, orthopedic surgeon, oncologist, neurosurgeon or whichever specialty you choose. If you want to pursue a career in Tissue Engineering then you might want to get a residency with Dr. Anthony Atalla at Wake Forest University. If you want to use CD-47 antibodies to functionally disable cancer stem cells you might want to do your residency in oncology at Stanford University with Dr. Irv Weissman or Dr. Michael Clarke.

   Keep your ‘antennae out’ during your four years of medical school – to determine who is doing the research you want to pursue for a career - and go do your residency with that person - he or she.

_________________________________________________________________________

2. For those who are not interested in medical school - they might want to pursue a career in research and they will go on after their undergrad work and get their Masters and Ph.D. which may be 4 to 6 more years.

3. The Ph.D. is usually followed by Post Doctoral studies, Post Doc, for 2 - 4 years to gain more special expertise for the research career you want to follow. Then you will be ready to join the faculty at a university to do research and teach. Others will opt to get a job doing research in industry for biotech companies. Also, some of these people are getting their Ph.D. in biostatistics or computer sciences where they will help with the planning and evaluation of research data.

4. M.D. / Ph.D. Most major Medical Schools offer a combined M.D. / Ph.D. for a person who may one day be the chairperson of the Department of Molecular Medicine at some medical college - check that out for each individual medical school.

5. **Masters Degree in Genetic Counseling** - is another option for some. There are almost 30 places in the US where those programs are available. For more information - check out this web site:


6. Teaching - Also Remember…..many people who will make a major contribution to all these careers in the future will do so by majoring in Education in college and will be preparing young people in the future…..as your Teachers have been preparing you!

   "Teachers Make All Other Professions Happen!"

   Also Consider:  Physician Assistant (PA):  [http://www.aapa.org/](http://www.aapa.org/)

   Student Academy:  [http://www.aapa.org/ssapa/](http://www.aapa.org/ssapa/)

   MD/MS Genomic Medicine:  [http://admissions.med.miami.edu/md-programs/md-ms-in-genomic-medicine](http://admissions.med.miami.edu/md-programs/md-ms-in-genomic-medicine)

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