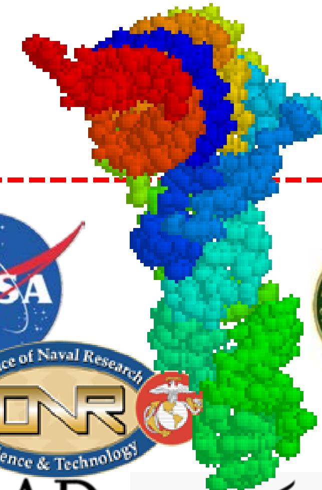
Human Germline Modification

1-Dec-2015 2 PM NAS



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National Cancer Institute
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National Heart Lung and Blood Institute
NSF
NASA
NIGMS
NHGRI
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Office of Naval Research
Boston University

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Harvard Stem Cell Institute
MIT
mit media lab
The Center for Bits and Atoms
Broad Institute
Wyss Institute

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Complete genomics

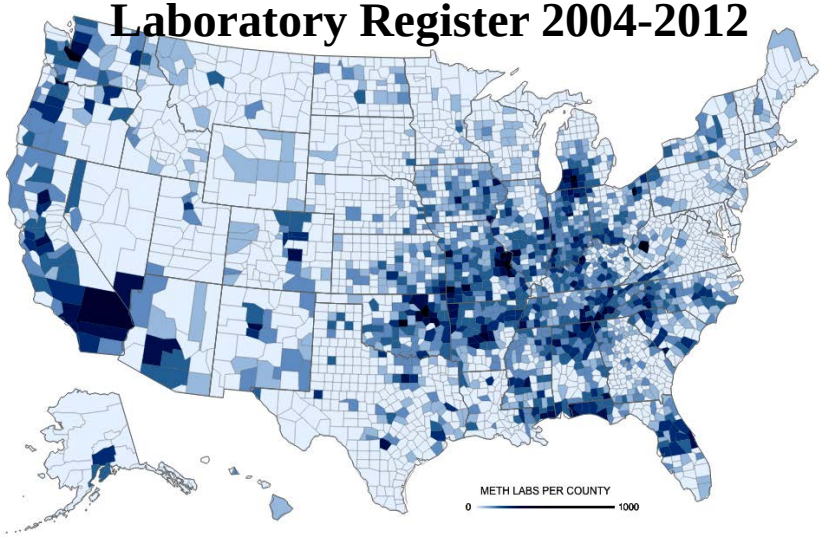
------Reading----------------Writing-------------Arithmetic-------

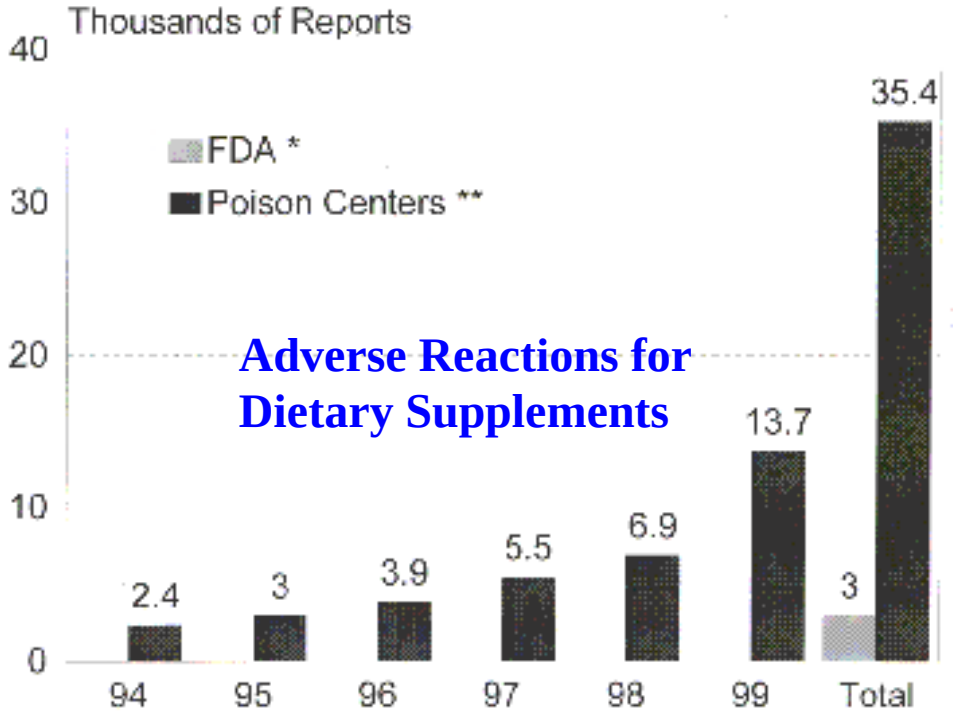
Ineffective & unsafe, yet widespread

Medicine's Wild West — Unlicensed Stem-Cell Clinics in the United States

Hermes Taylor-Weiner, Ph.D., and Joshua Graff Zivin, Ph.D.

Meth lab seizures per county
DEA's National Clandestine Laboratory Register 2004-2012





Safe & effective
relative to alternatives,
yet concerns persist

Vaccination vs smallpox, polio ...

GMO Golden rice vs 1M deaths/yr

Test-tube babies vs infertility

Random vs engineered

Compelling medical applications of germline therapies

1) Mitochondrial diseases

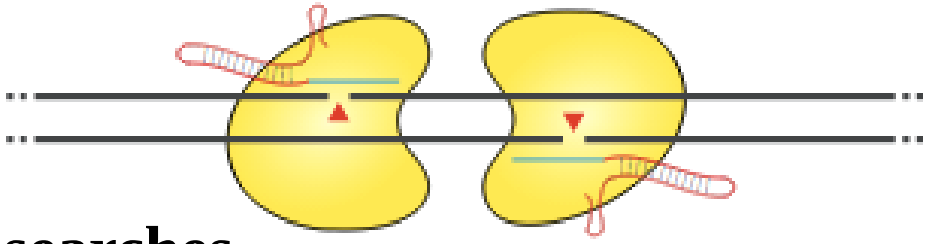
2) Families in which post-natal remedies are not optimal and both parents are fully afflicted (20% of marriages involve close relatives)

3) Treating & screening single germ cells is safer than treating millions of somatic cells, since each cell adds to the collective risk of cancer.

4) PGD-IVF is not acceptable for medical or personal reasons.

5) Complex diseases with single-target solutions, which work better when applied earlier.

Nuclease Specificity



100-fold: **Theoretical & empirical searches**
Mali et al., Cong et al. (Church & Zhang labs) Science 3-Jan-2013

1000-fold: **Paired nickases**. Mali, et al, Ran et al (Church, Zhang labs). Nat Biotechnol. 1-Aug-2013. Cell 12-Sep-2013

<5,000-fold: **Truncated guide RNAs**. Fu, et al. (Joung lab). Nat Biotechnol. 26-Jan-2014

>100-fold: **RNA-guided FokI nucleases** (Joung & Liu labs) Nat Biotechnol. 25-Apr-2014

>25-fold: **Cas9 charge** Slaymaker (Zhang lab) Science 1-Dec-2015

Efficiency & specificity goals?

Off-target: 10,000 to 10 events per million cells

Spontaneous error rate = 0.1 to 10 per cell.

Clonal cells, e.g. spermatogonial stem cells (SSC)
Increase efficiency from 5-50% to 100%. (& Specificity.)

Analysis: BLESS, Guide-Seq, Digenome-seq, HTGTS, IDLV, whole genome, targeted seq.

Haploinsufficient = ~3000 journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1003484
bioinfo.mc.vanderbilt.edu/TSGene #cells: pubmed/23829164

We are not limited by natural variants or complexity:

GH therapies for non-GH alleles

- Turner syndrome
- Chronic renal failure
- Prader–Willi syndrome
- Intrauterine growth retardation
- Idiopathic short stature
- AIDS Muscle wasting

Also, Not limited to gene therapies:
genetic counseling, gene drives, xenotransplantation

Enhancement via somatic gene therapies

Somatic protocols can spread more rapidly than germline modifications, since only 1% of the population per year (that is births) are of the correct age for germline impact, while somatic therapy is applicable at any age.

Pathogen resistance CCR5, FUT2
Aging GFD11-MSTN, TERT-CDKN2A-TP53
Cognitive (ASD/AD) NGF, NEU1, GRIN2B, PDE4B

Equitable access to technology?
Super-exponential technologies.
Encouraging innovation.

Not
6 decades
but
6 years

arep.med.harvard.edu/gmc/genome_services.html