A Vision of the Future of MPNs

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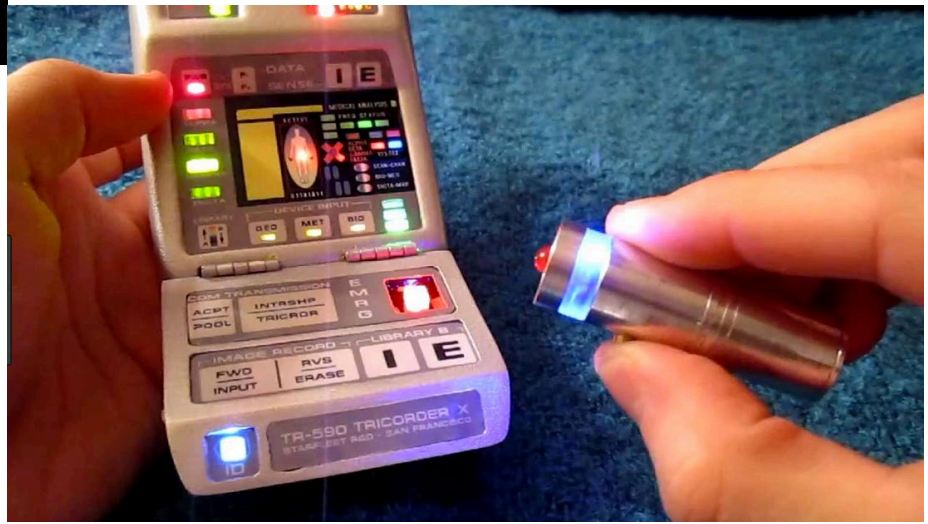
Stanford Cancer Institute

Mayo Patient Conference
March 3, 2019

Leonard “Bones” McCoy and his tricorder

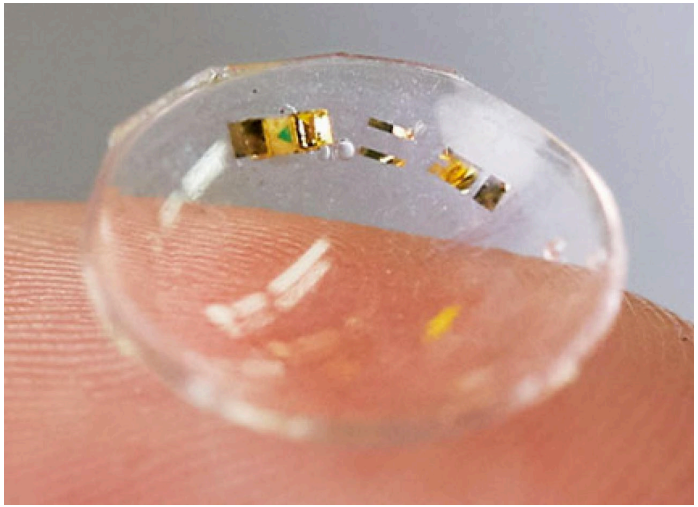
Star Trek has always reminded us of the future possibilities of medicine



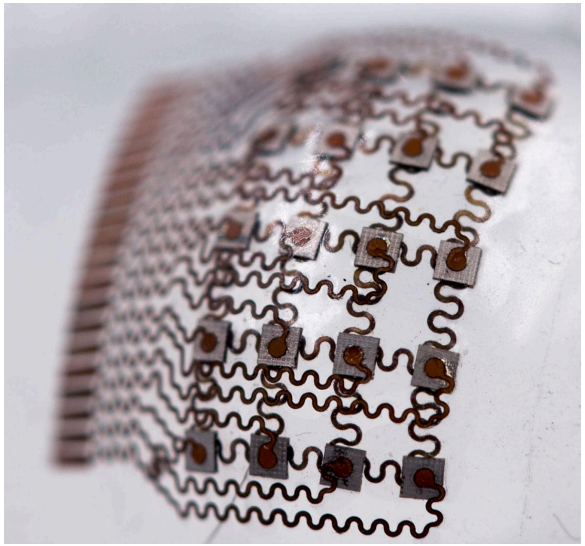
Elements of the Future of Medicine

- Personalized technology
- High speed and wireless
- Miniaturization
- Wearables and internal sensors for monitoring of health & disease
- Ever-present, analytics-enabled, real-time, individualized attention to prevent and treat disease

The Revolution in Sensors (1)



Lens with glucose sensor to track sugar level in tears



Wearable postage-stamp size patch that measures blood pressure

Kraft, Nat Geo, 2019

The Revolution in Sensors (2)

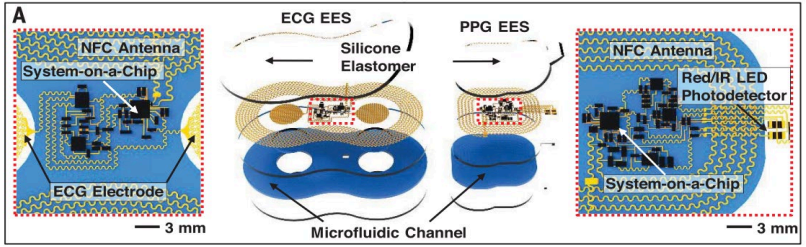
RESEARCH ARTICLE

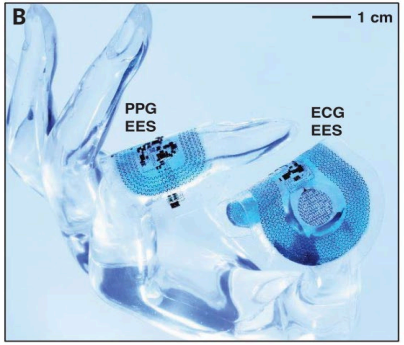
BIOMEDICINE

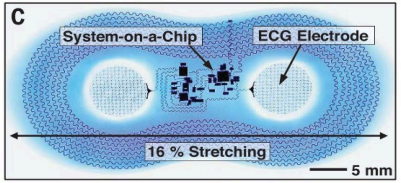
Binodal, wireless epidermal electronic systems with in-sensor analytics for neonatal intensive care

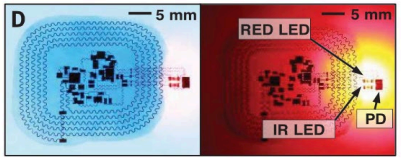
Ha Uk Chung[1,2*], Bong Hoon Kim[1,3,4,5*], Jong Yoon Lee[4,6*], Jungyup Lee[4*], Zhaoqian Xie[3,7,8*], Erin M. Ibler[9,10], KunHyuck Lee[1,3], Anthony Banks[1,4,5,11], Ji Yoon Jeong[4], Jongwon Kim[3,12], Christopher Ogle[1,5], Dominic Grande[4,6], Yongjoon Yu[4], Hokyung Jang[4], Pourya Assem[6], Dennis Ryu[1,5], Jean Won Kwak[1,8], Myeong Namkoong[1,13], Jun Bin Park[4], Yechan Lee[4], Do Hoon Kim[4], Arin Ryu[4], Jaeseok Jeong[4], Kevin You[4], Bowen Ji[3,7,8,14], Zhuangjian Liu[15], Qingze Huo[3,7,8], Xue Feng[16], Yujun Deng[7,17], Yeshou Xu[7,18], Kyung-In Jang[19], Jeonghyun Kim[20], Yihui Zhang[16], Roozbeh Ghaffari[1,5,13], Casey M. Rand[10,21], Molly Schau[22], Aaron Hamvas[21,22,23], Debra E. Weese-Mayer[10,21,23], Yonggang Huang[3,5,7,8], Seung Min Lee[24], Chi Hwan Lee[25], Naresh R. Shanbhag[6], Amy S. Paller[5,9,23†], Shuai Xu[1,5,9†], John A. Rogers[1,3,4,5,8,13,26,27†]

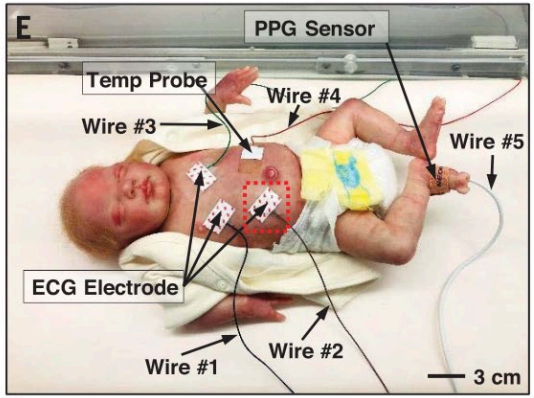
Chung *et al.*
Science
March 1, 2019

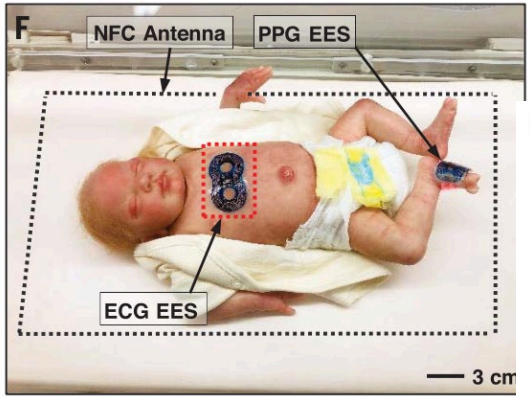


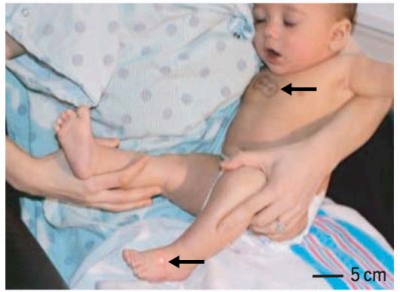


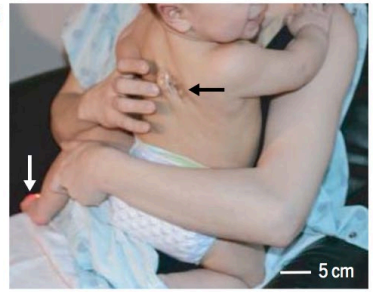








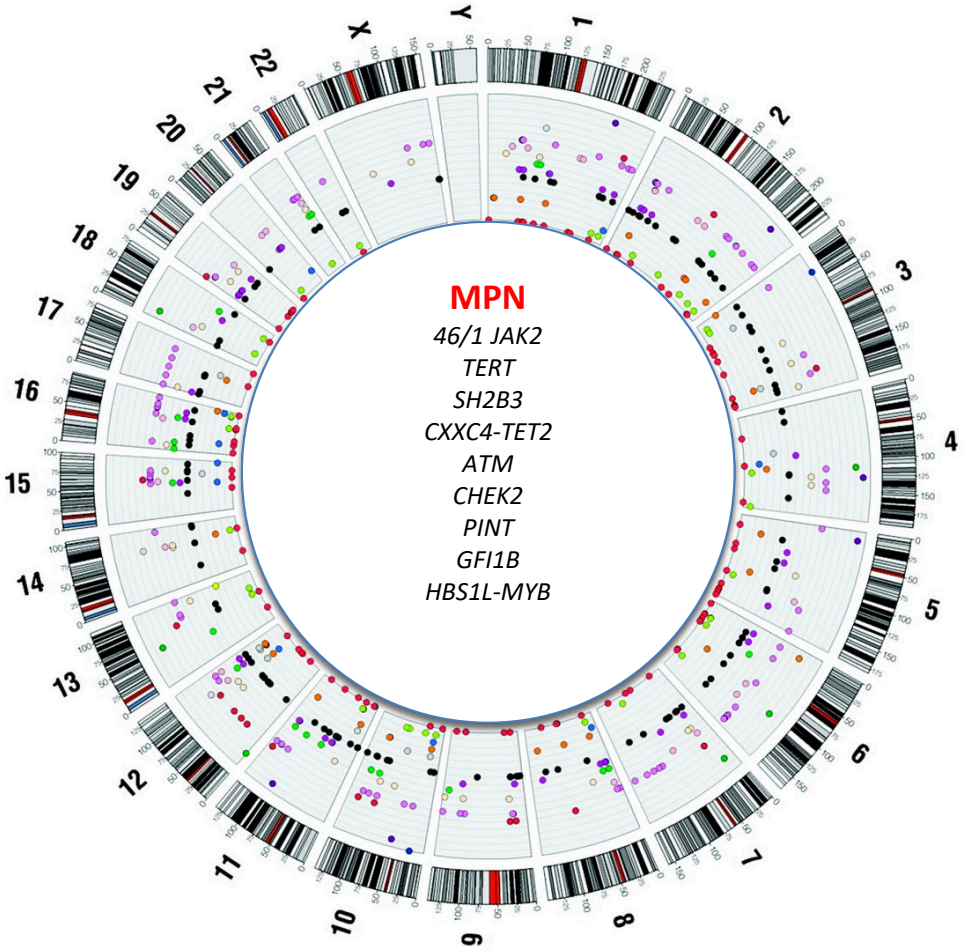




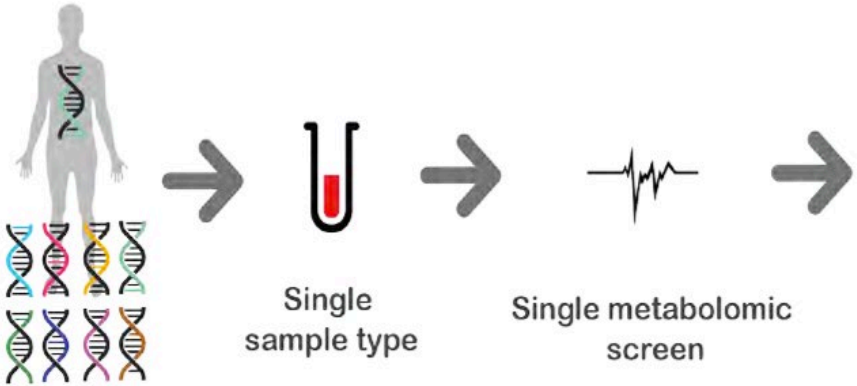
Predisposition to MPN

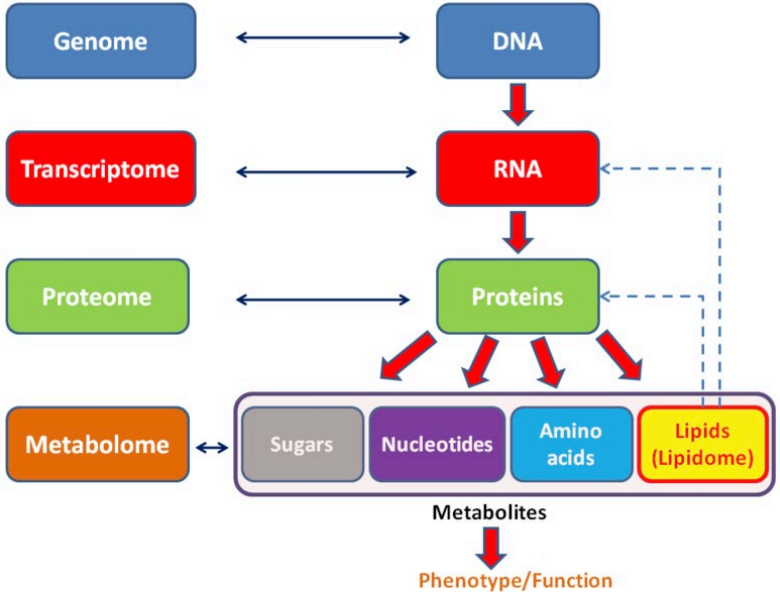
In 2033, Alex Saven is born and his parents wish to understand what are his future risks of disease

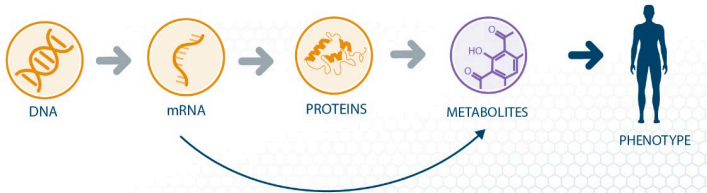
A fingerstick prick of blood or internal biosensor will be used to conduct an integrated scan of current health and a probability assessment of future heath risks, including MPN



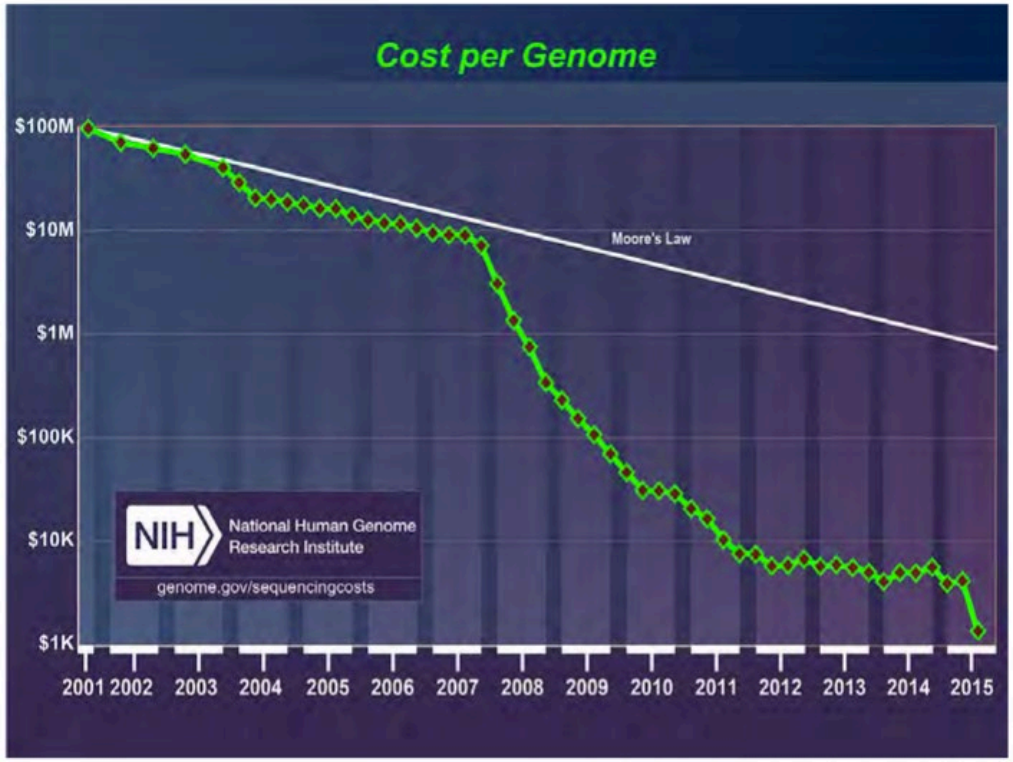
Assessing Inherited Predisposition: More than Just Gene Variants





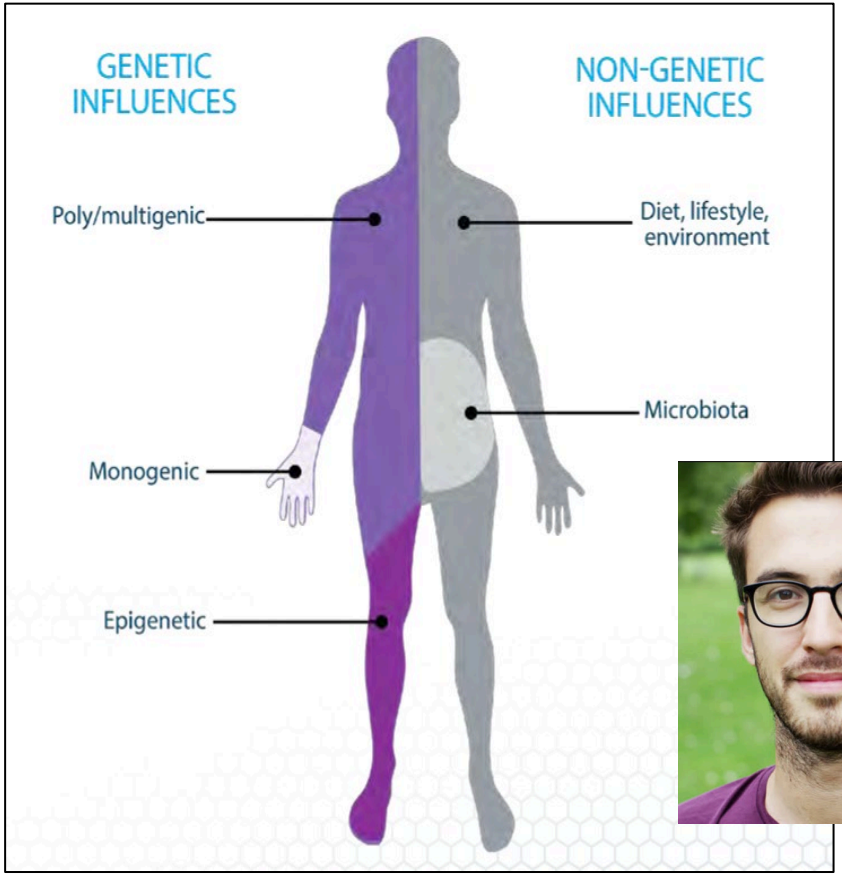


Cost is Decreasing Dramatically

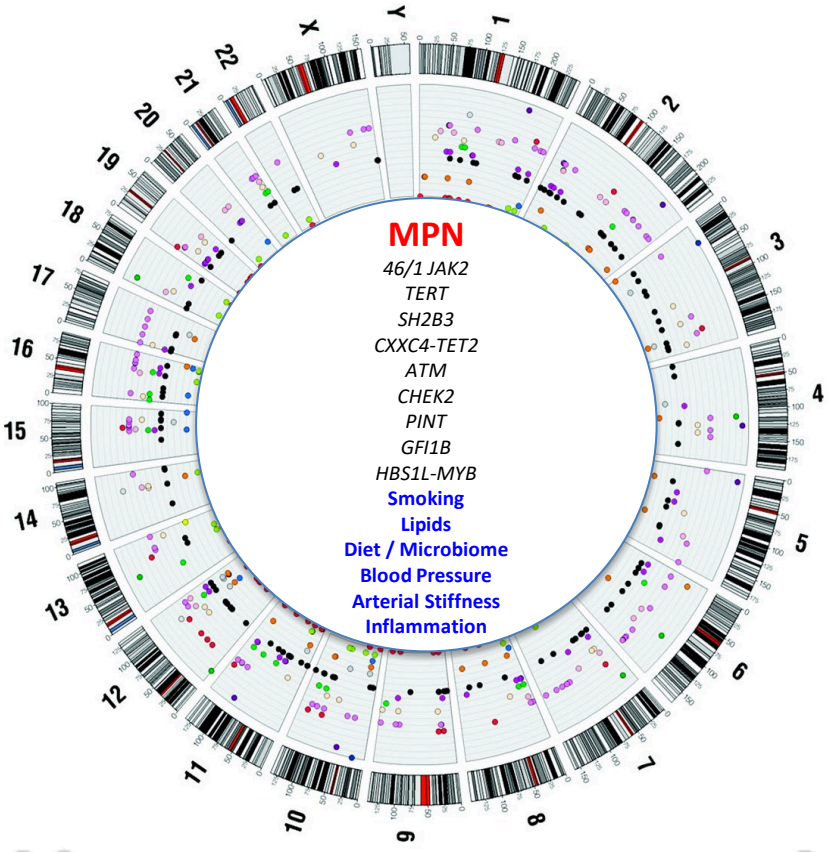


Source: Wetterstrand KA - DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: genome.gov/sequencingcosts.

Dynamic Re-Assessment of Predisposition

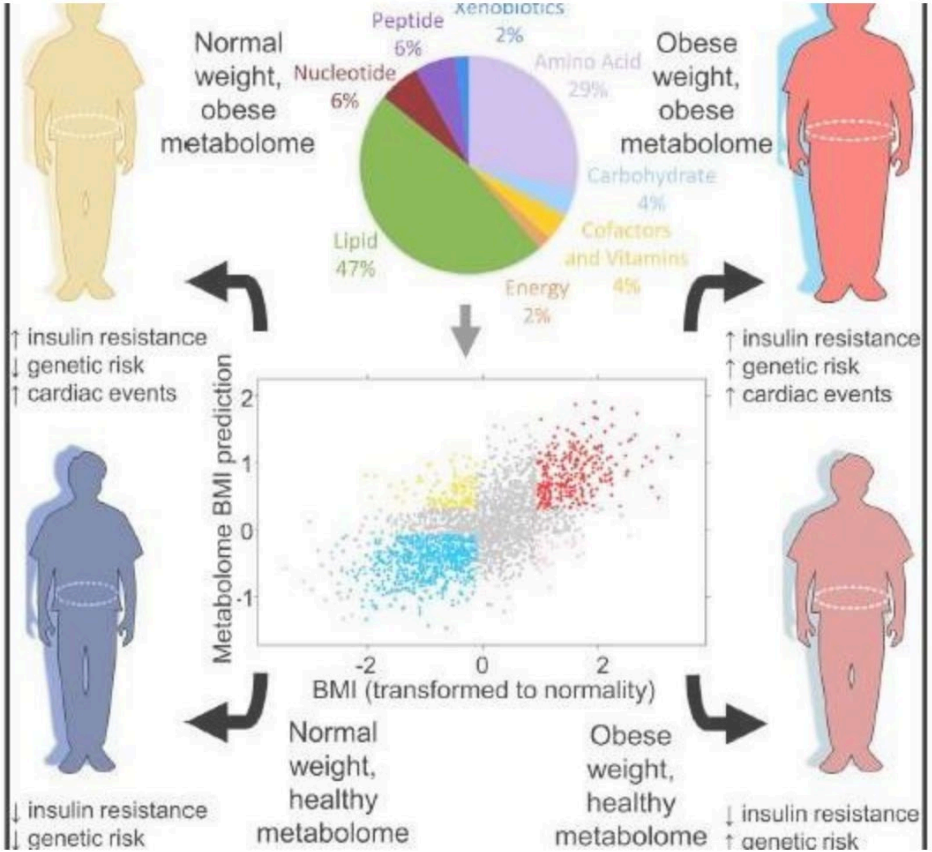


Alex is now 35



Predisposition

Low MPN Risk
Low risk of thrombosis
Low risk of AML



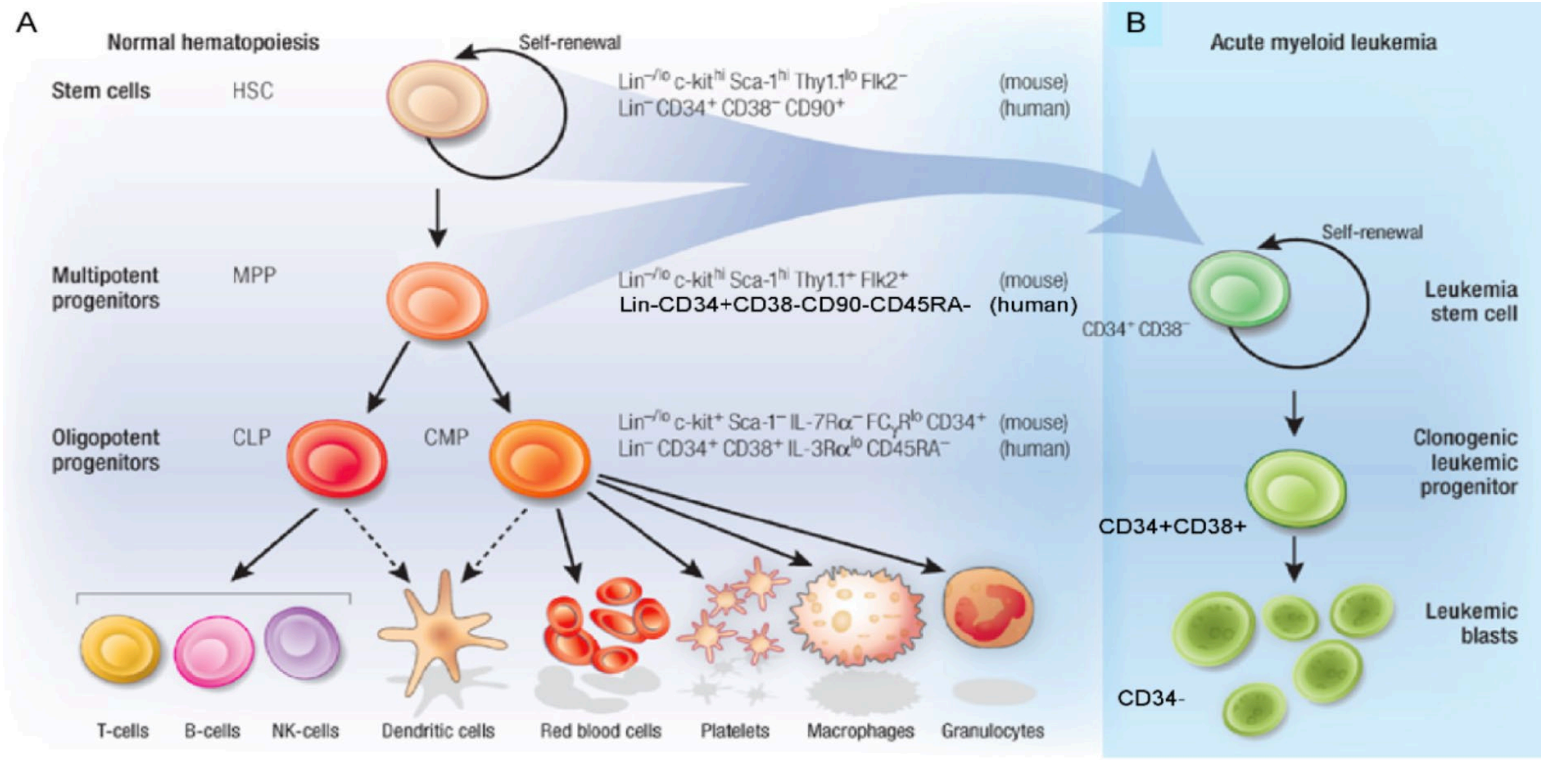
High MPN Risk
High risk of thrombosis
High risk of AML

High MPN Risk
Low risk of thrombosis
High risk of AML

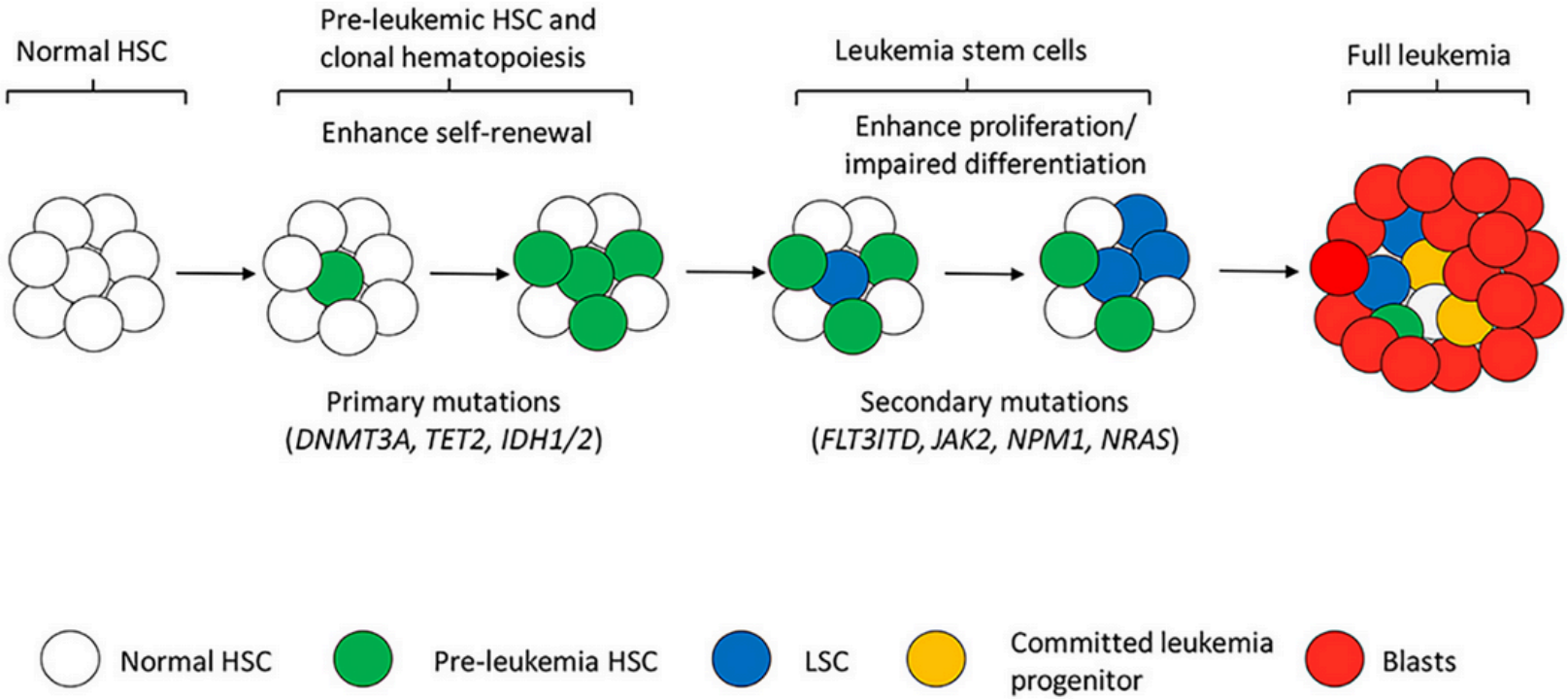
Low MPN Risk
High risk of thrombosis
Low risk of AML

Early Disease Detection

Early disease detection: Biosensors to detect abnormal expansion blood progenitor cells

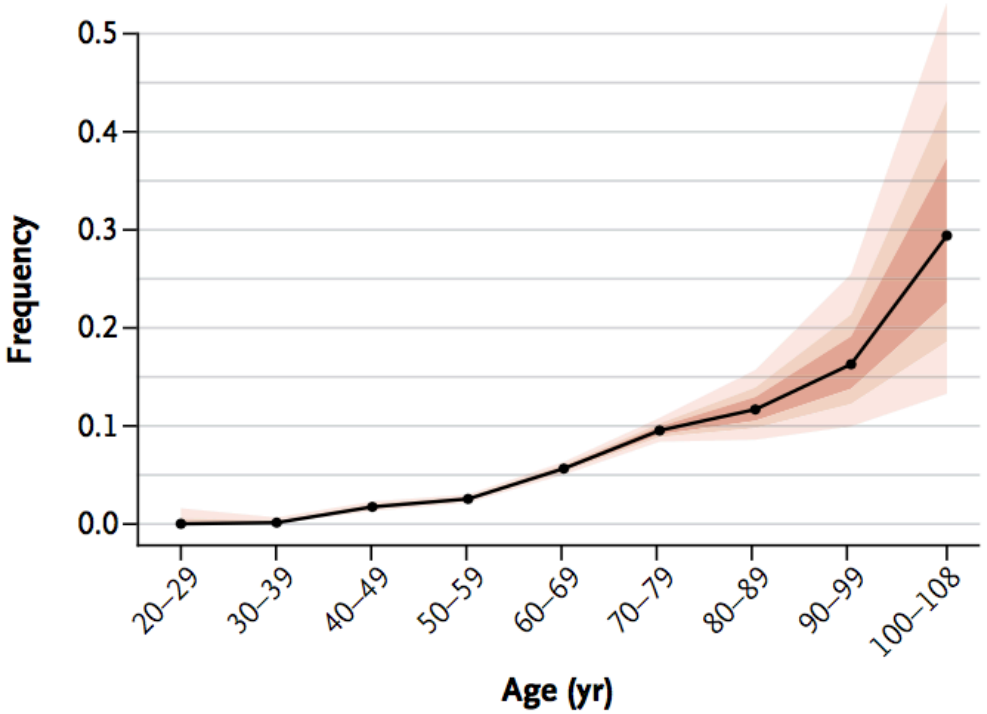


Biosensors for early detection of mutated pre-leukemic cells



Clonal hematopoiesis of indeterminate potential (CHIP)

a.k.a Age-related clonal hematopoiesis (ARCH)

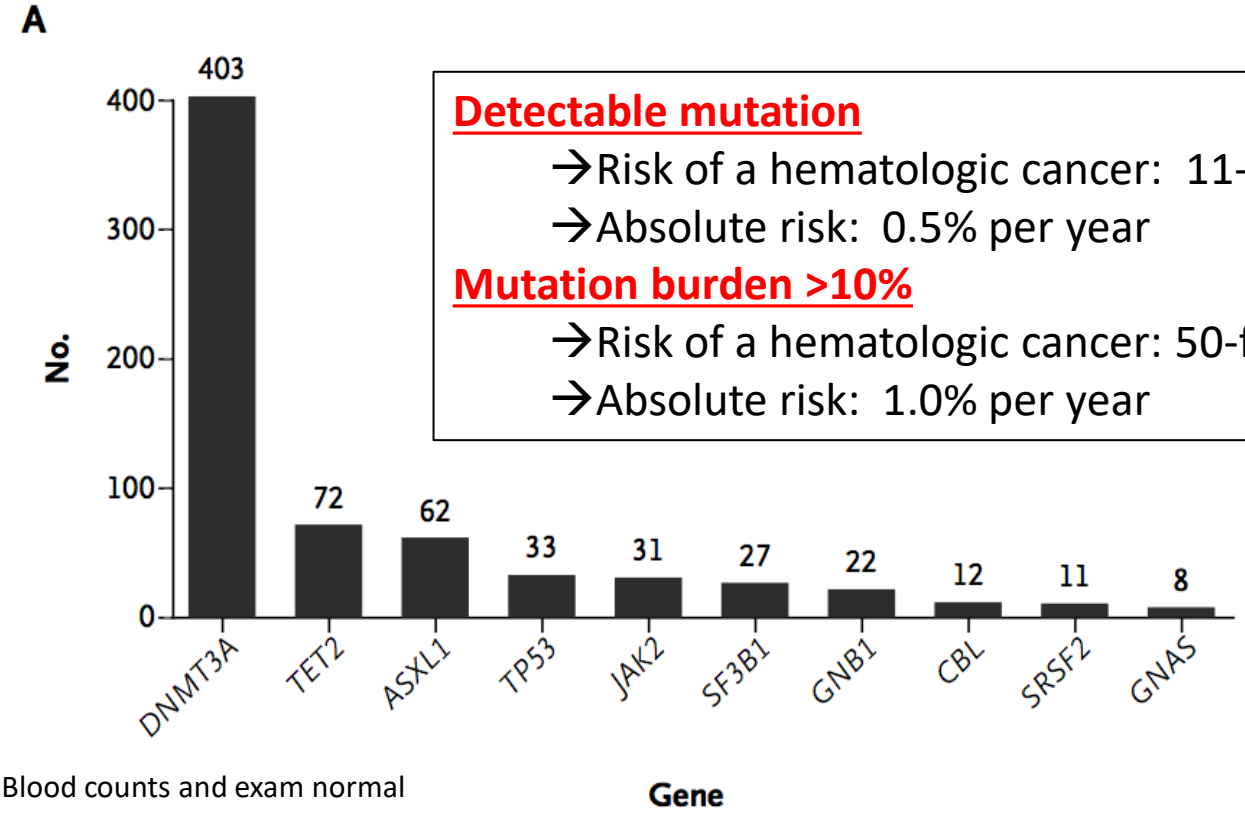


No. with Mutation	0	1	50	138	282	219	37	14	5
Total	240	855	2894	5441	5002	2300	317	86	17

Jaiswal *et al*, *NEJM*, 2014

Clonal hematopoiesis of indeterminate potential (CHIP)

The most common genes identified



Detectable mutation

→Risk of a hematologic cancer: 11-fold increase

→Absolute risk: 0.5% per year

Mutation burden >10%

→Risk of a hematologic cancer: 50-fold increase

→Absolute risk: 1.0% per year

Alex, now 50, feels great. Blood counts and exam normal
Biosensor reading picks up a new *JAK2* V617F mutation.
Burden of 2% circulating in the blood.

Jaiswal *et al*, *NEJM*, 2014

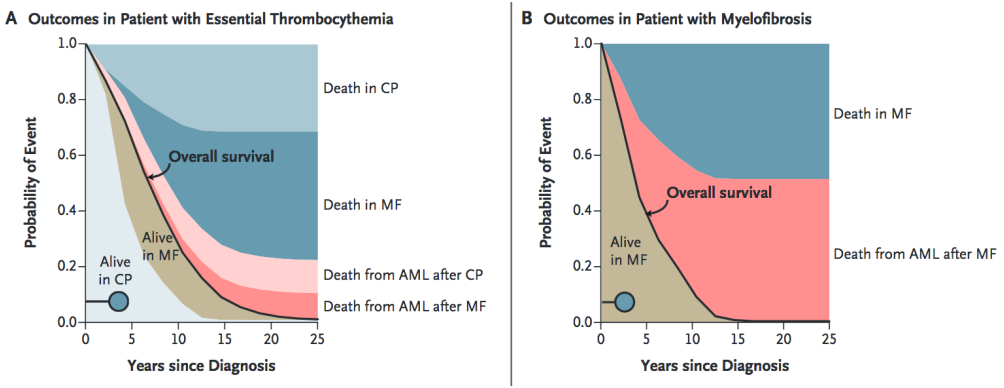
Disease Monitoring

2019 Prognostic Scoring Systems

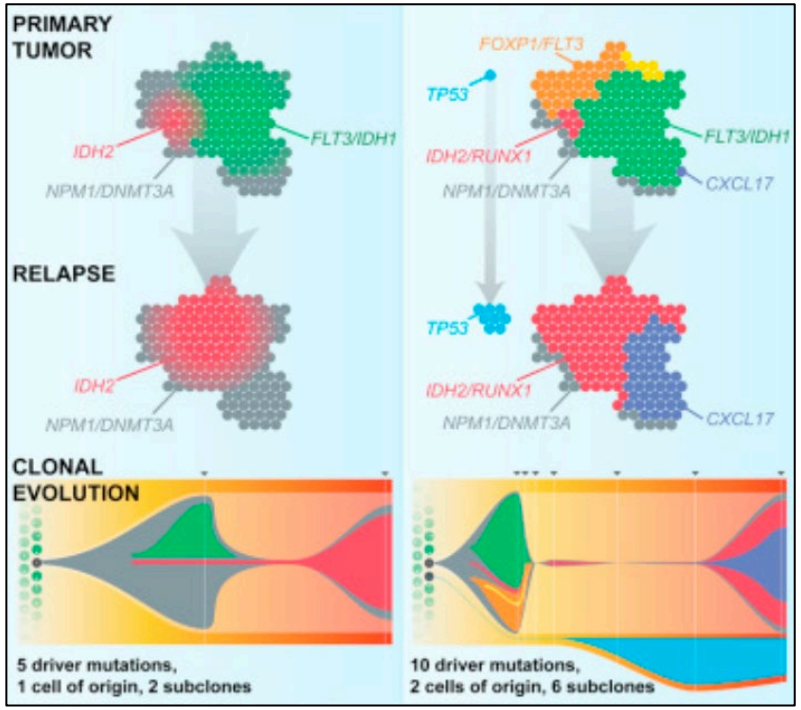
Alex is now 62 and has a new diagnosis of primary myelofibrosis.

Myelofibrosis/MPN Scoring Schemes

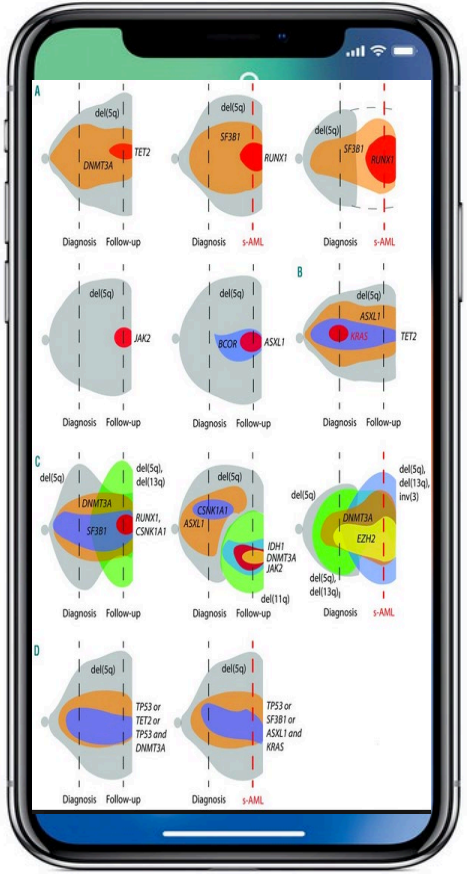
- IPSS
- DIPSS
- DIPSS-Plus
- MIPSS70(Plus)
- GIPSS
- MYSEC (secondary MF)
- Sanger MPN risk calculator

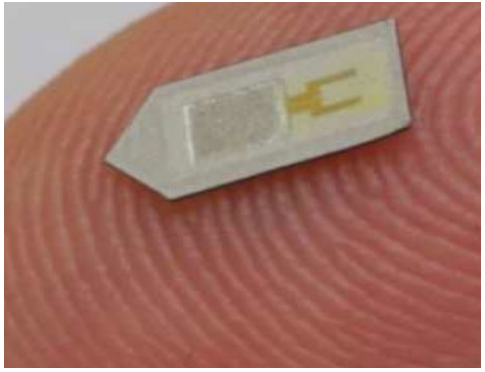


2019:
A snapshot in time of ‘mutation landscape’

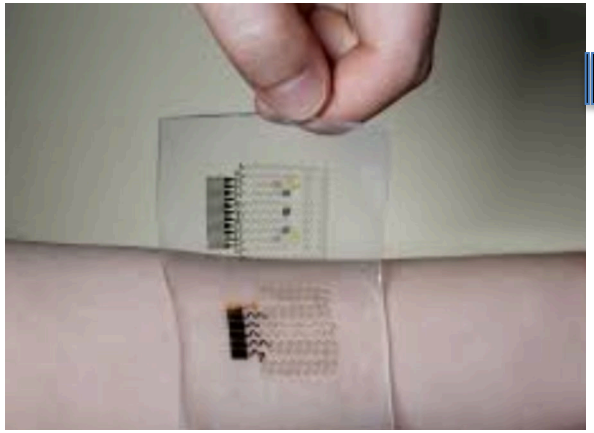


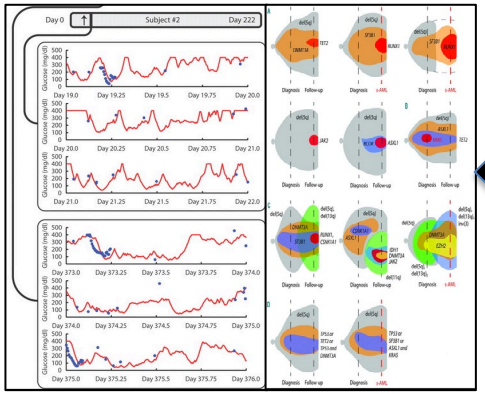
2030 ?
Continuous monitoring of mutation dynamics





Refined, Continuous Risk Re-Stratification in MPNs





- **Thrombosis & Bleeding**
- **Progression to:**
  - **MF**
  - **AML**
- **Inherited Risk**
- **Co-Morbidity Status & Infection Risk**

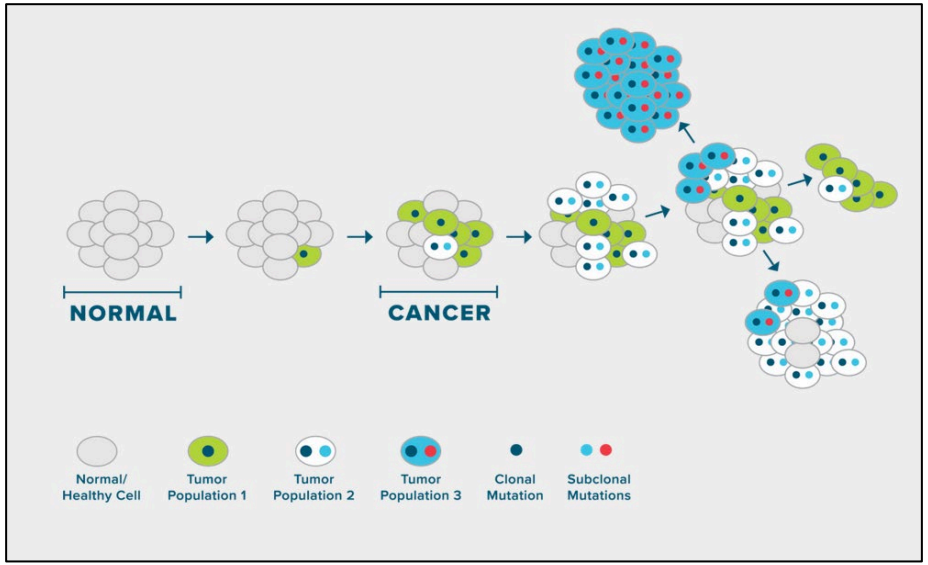
RBC, WBC, platelets
Coagulation genomics
Platelet RNA expression
Endothelial cell biome
VWD Factor Activity
Bioactivity Profile

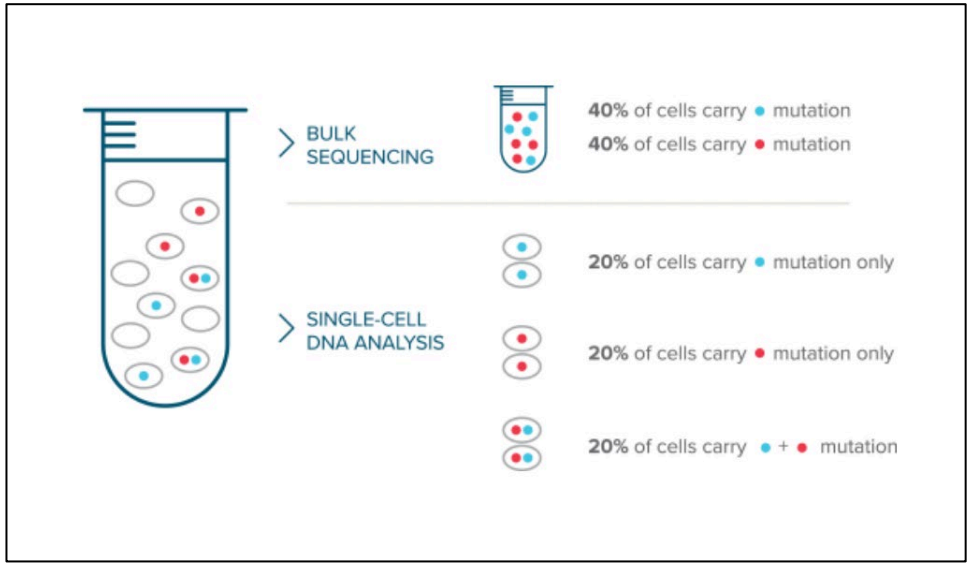
Mutation profile
Mutation allele burden
High risk molecular mutations
Marrow fibrosis
Flow markers of early blood progenitors
Single cell analysis

Inherited DNA variants and metabolome

Lipid / glucose profile
Metalobome
Microbiome
T/B-celll and other immune cell markers

Now: single cell rather than bulk analysis of mutations





In the future:

Real time, monitoring of single cell mutation landscape and early detection of treatment effects and resistance/relapse

Mission Bio

Treatment

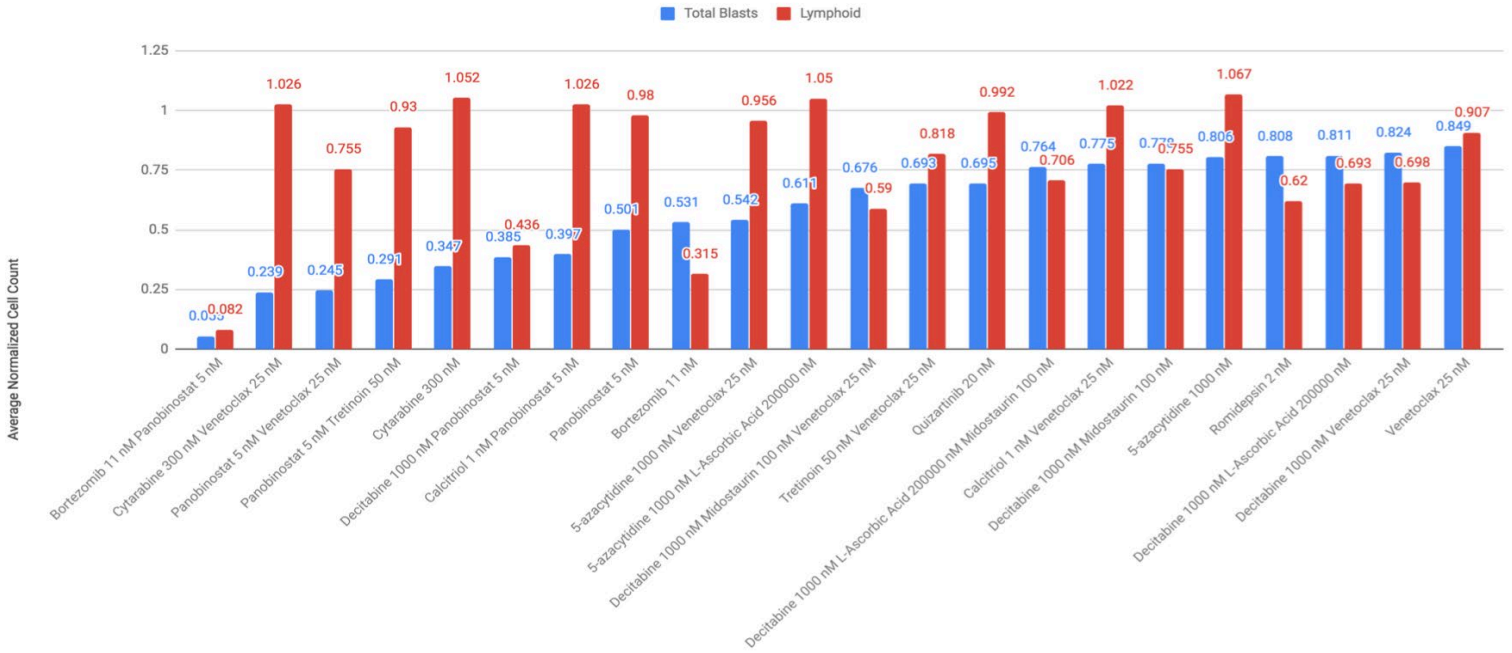
Notable Labs

Stanford Hospital
Patient 689AML Results
Bone marrow (689AML1), Peripheral Blood (689AML2

Ex vivo drug sensitivity testing

Intrapatient drug sensitivity

Bone marrow (689AML1)



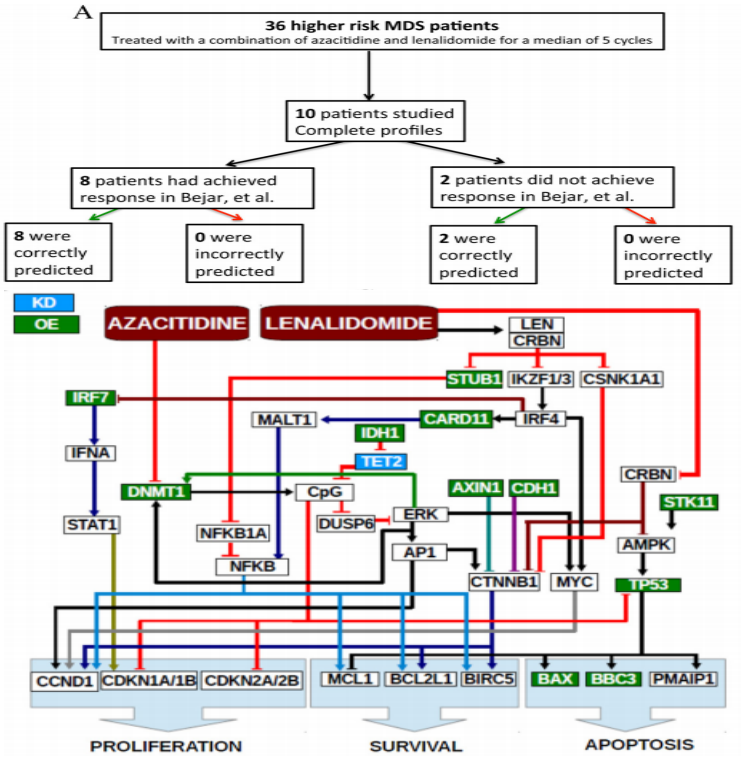
Limitations of ‘Biospecific’ Therapies

- Tumor heterogeneity
  - Polyclonality
  - Numerous potential targets
  - Co-mutations
  - Clonal evolution
  - Driver vs. passenger mutations
  - Variable gene expression
  - Innate drug sensitivity

In silico and *ex vivo* Assays Guiding Personalized Treatment Selection in Myeloid Malignancies

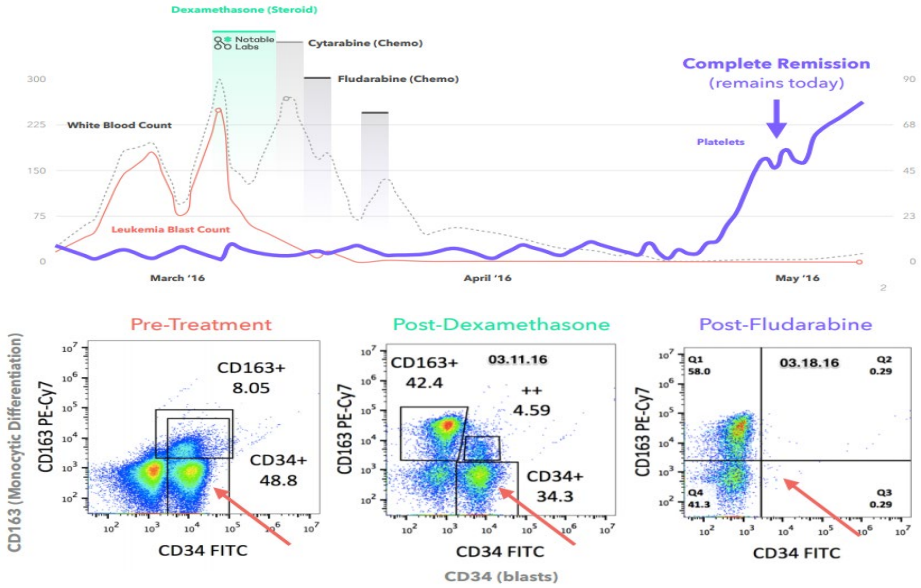
In silico Assay

CELLWORKS™



Ex vivo Assay

Notable Labs



1. McKeown et al Cancer Discov, 2017,
2. Drusbosky et al, Leukemia Research 2017

Genomic Signatures Predict Venetoclax Response in AML

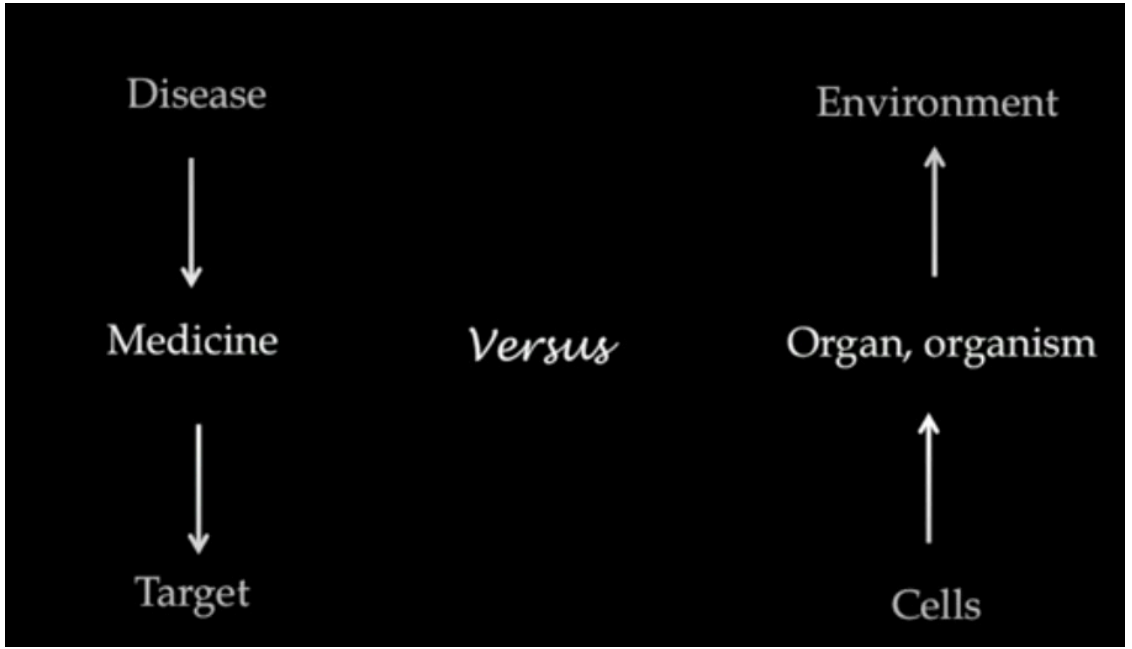
Computational protein network mapping/ex vivo drug sensitivity

- 74 samples from patient with refractory/relapsed AML
  - *86% ex vivo* venetoclax responses matched computer simulation prediction
  - Correctly predicted responses of 2 treated patients

- *Computer derived genomic signatures identified resistance/sensitivity to venetoclax*

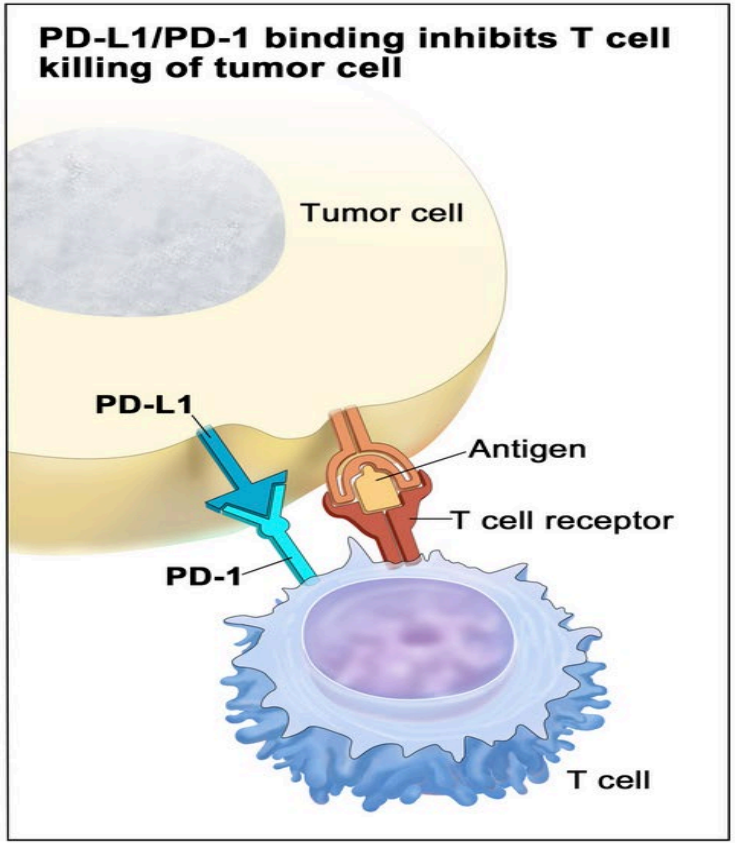
Drusbosky et al, ASH 2017, #2707 (Cellworks)

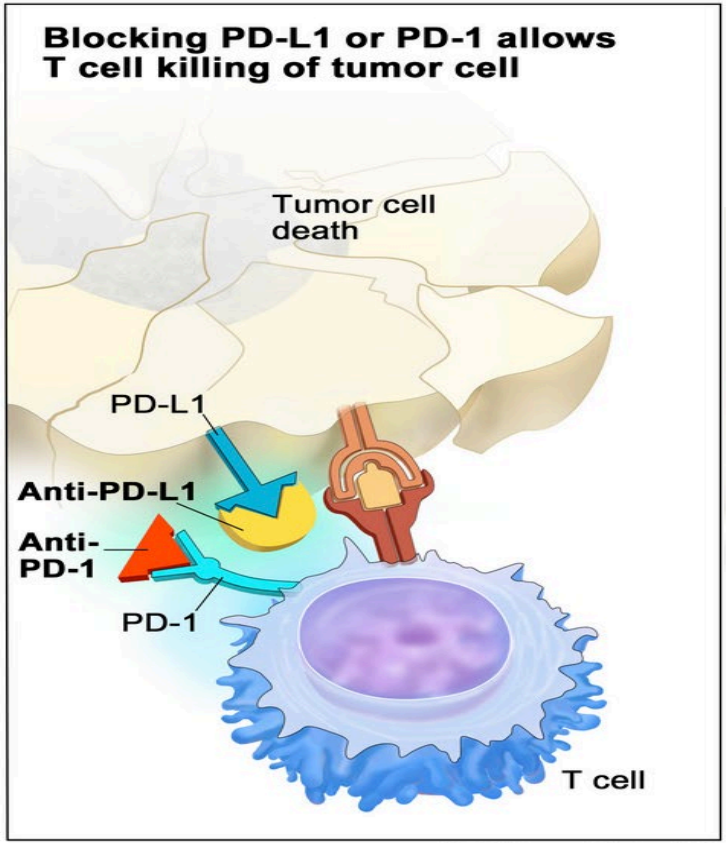
Old and New Paradigms of Treatment

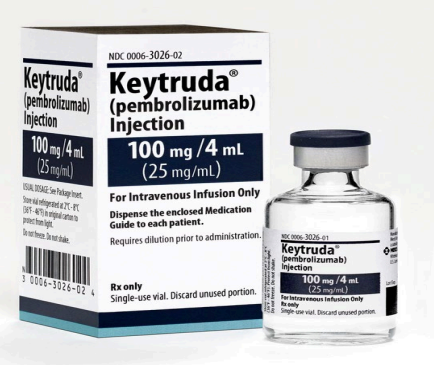


Dr. Siddhartha Mukherjee, TED Talk: "Soon We'll Cure Diseases with a Cell, Not a Pill", 2015

Immunotherapy:
A successful approach in solid tumors





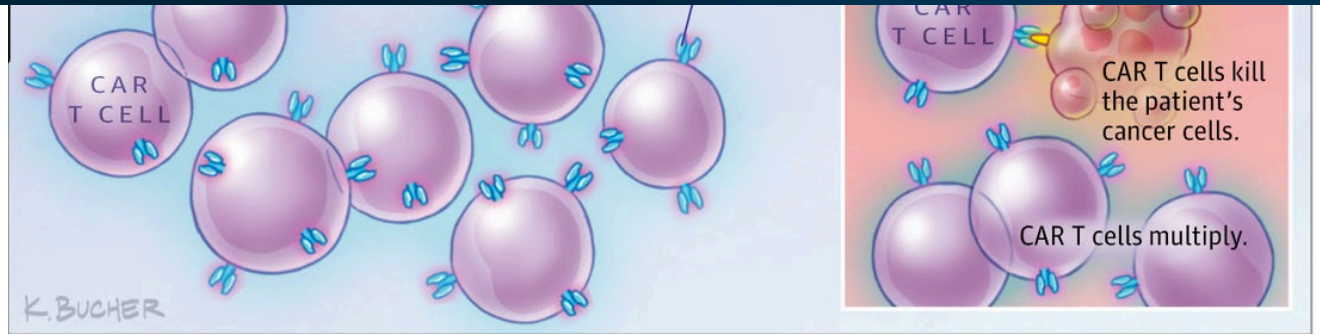


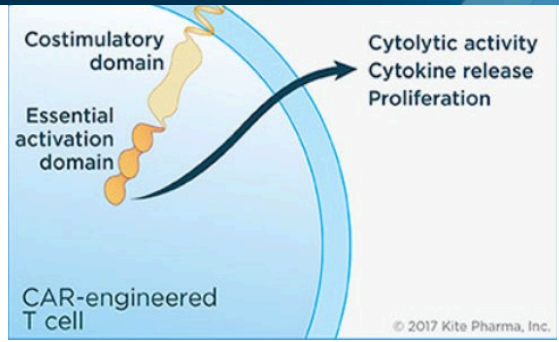
CAR T-Cell Therapy

Cell

ARTICLE | VOLUME 173, ISSUE 6, P1439-1453.E19, MAY 31, 2018

Genetic Inactivation of CD33 in Hematopoietic Stem Cells to Enable CAR T Cell Immunotherapy for Acute Myeloid Leukemia



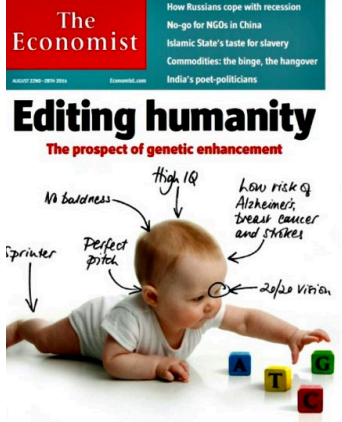


Kim et al, 2018


TIME
The Gene Machine
What the CRISPR experiments mean for humanity By Alice Park

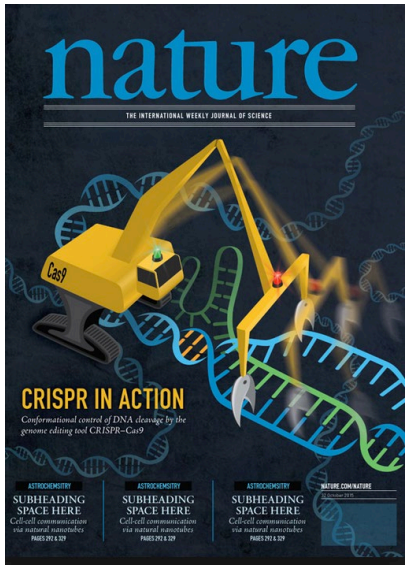

Science
2015
BREAKTHROUGH of the YEAR
CRISPR


nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Dawn of the gene-editing age
CRISPR
EVERYWHERE
A WORLD OF TWO HALVES
FLOWER ARRANGEMENT
THE RIGHT SIZE FOR A LAB


The Economist
How Russians cope with recession
No-go for NGOs in China
Islamic State's taste for slavery
Commodities: the binge, the hangover
India's poet-politicians
Editing humanity
The prospect of genetic enhancement
High IQ
No baldness
Low risk of Alzheimer's, breast cancer and strokes
Perfect pitch
Sprinter
20/20 vision
A
T
G
C


MIT Technology Review
WE CAN NOW ENGINEER THE HUMAN RACE


The war on Boris Rod Liddle / Can I still afford to drive? Melissa Kite
THE SPECTATOR
Eugenics is back
Fraser Nelson on the race for designer babies
ATTACK OF THE RACIST ROBOTS
HUGO RIFKIND
REFERENDUM RAGE
ALEX MASSIE
SEX, ORCHIDS AND MAPPLETHORPE
STEPHEN BAYLEY
THE RABBIT WHO MADE ME A TORY
MATTHEW PARRIS
Against boxing
Simon Barnes


nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Cas9
CRISPR IN ACTION
Conformational control of DNA cleavage by the genome editing tool CRISPR–Cas9
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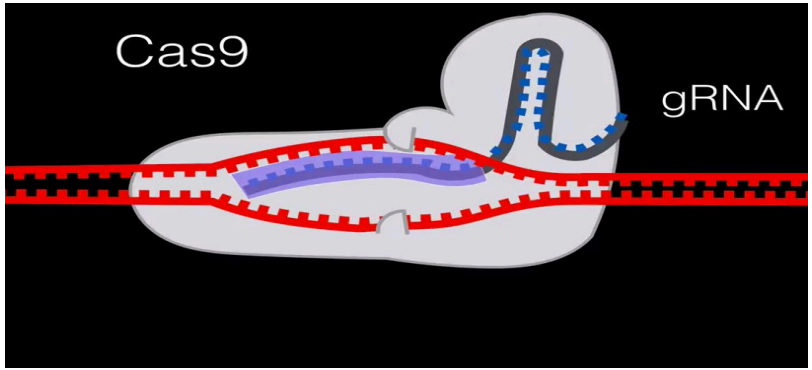
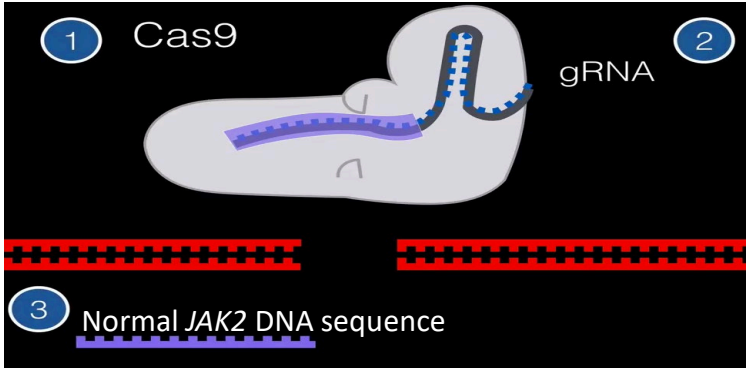
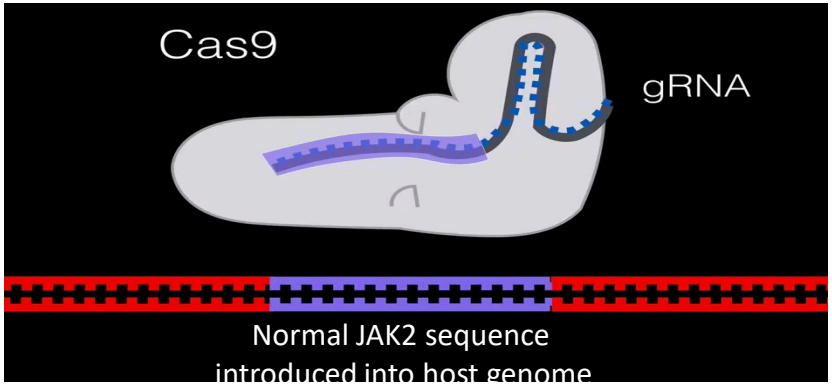
Gene Editing with CRISPR/CAS9







Home / News & Opinion

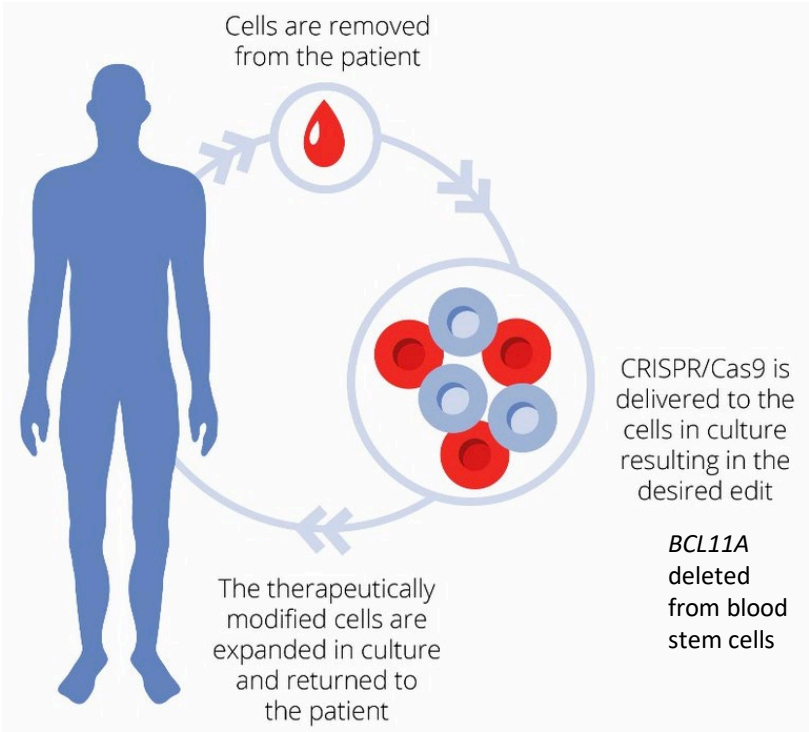
US Companies Launch CRISPR Clinical Trial

The Germany-based study will test an ex vivo genome-editing therapy for the inherited blood disorder β-thalassemia. September 2018

CRISPR Therapeutics and Vertex Pharmaceuticals

CTX001 works by cleaving a gene called *BCL11A*, which suppresses production of fetal hemoglobin

CTX001 could efficiently edit the target gene in more than 90 percent of hematopoietic stem cells to achieve about 40 percent of fetal hemoglobin production, which investigators believe is sufficient to improve a patient's symptoms.



Potential Issues/Concerns

- **Low-risk ET/PV**- is CRISPR needed?
- **Myelofibrosis**– genetically complex
- **Off-target safety concerns**
  - Accidental editing of tumor suppressor genes, oncogenes, or other parts of the genome

RESEARCH ARTICLE

TRANSPLANTATION

Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy

Akanksha Chhabra,[1]* Aaron M. Ring,[2,3,4]* Kipp Weiskopf,[2,3,4]* Peter John Schnorr,[1] Sydney Gordon,[2,3,4] Alan C. Le,[1] Hye-Sook Kwon,[1] Nan Guo Ring,[2,3,4] Jens Volkmer,[2,3,4] Po Yi Ho,[2,3,4] Serena Tseng,[2,3,4] Irving L. Weissman,[2,3,4,5] Judith A. Shizuru[1,2,3†]

Hematopoietic stem cell (HSC) transplantation can cure diverse diseases of the blood system, including hematologic malignancies, anemias, and autoimmune disorders. However, patients must undergo toxic conditioning regimens that use chemotherapy and/or radiation to eliminate host HSCs and enable donor HSC engraftment. Previous studies have shown that anti–c-Kit monoclonal antibodies deplete HSCs from bone marrow niches, allowing donor HSC engraftment in immunodeficient mice. We show that host HSC clearance is dependent on Fc-mediated antibody effector functions, and enhancing effector activity through blockade of CD47, a myeloid-specific immune checkpoint, extends anti–c-Kit conditioning to fully immunocompetent mice. The combined treatment leads to elimination of >99% of host HSCs and robust multilineage blood reconstitution after HSC transplantation. This targeted conditioning regimen that uses only biologic agents has the potential to transform the practice of HSC transplantation and enable its use in a wider spectrum of patients.

Chemotherapy-free Conditioning (Preparatory) Regimen for Transplantation

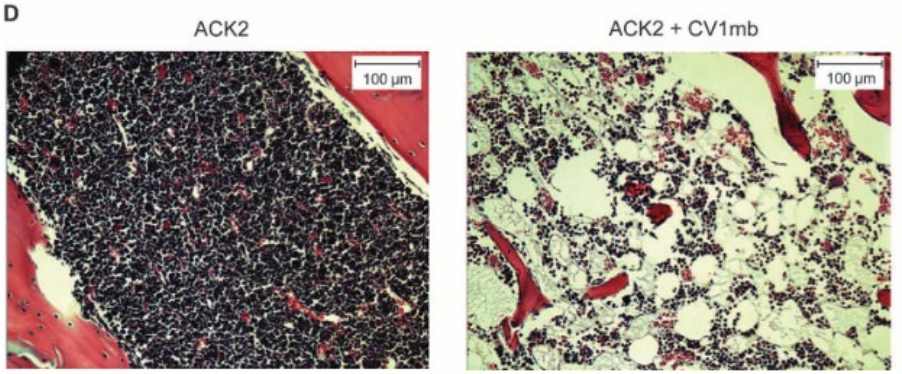
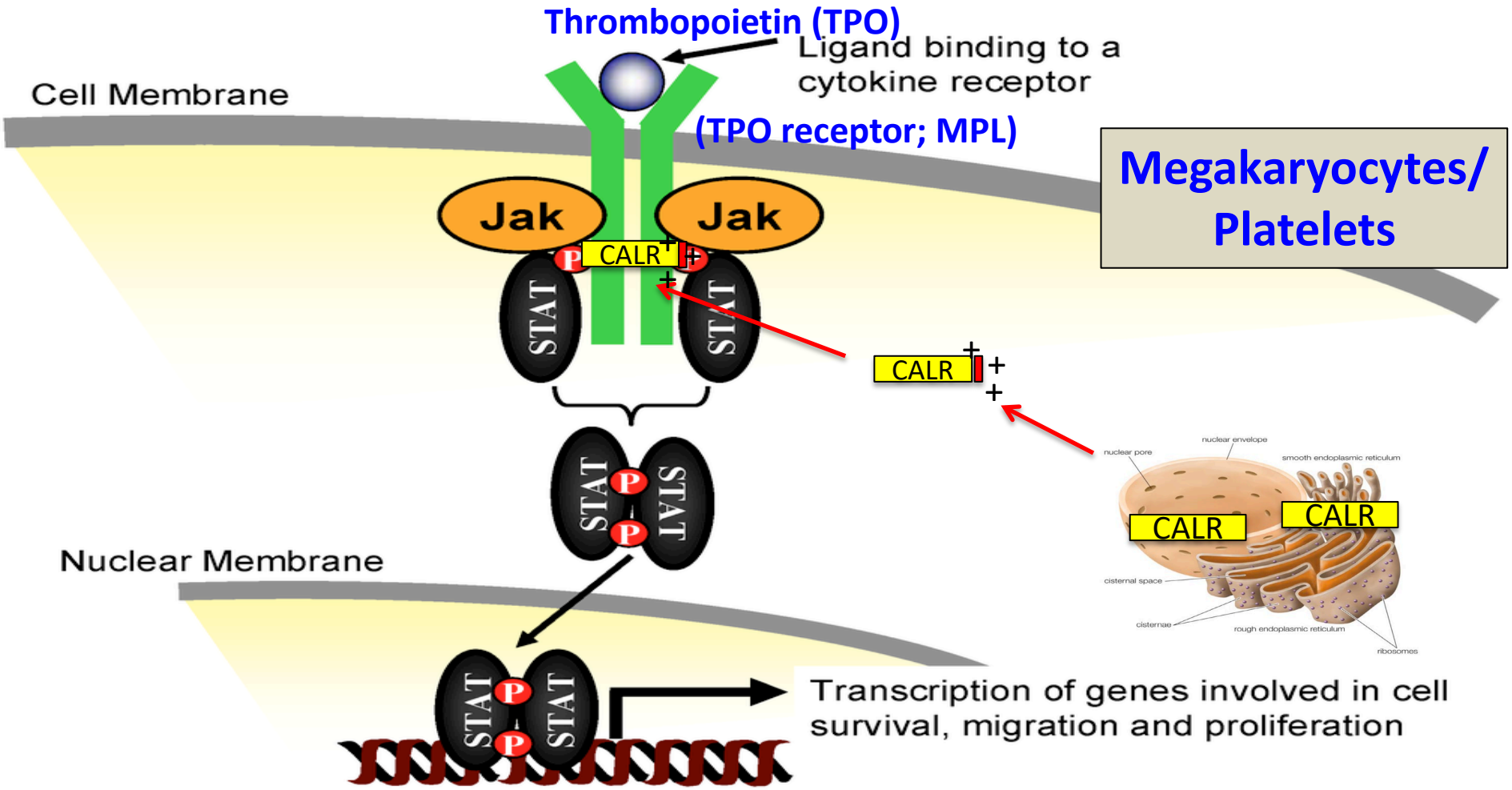


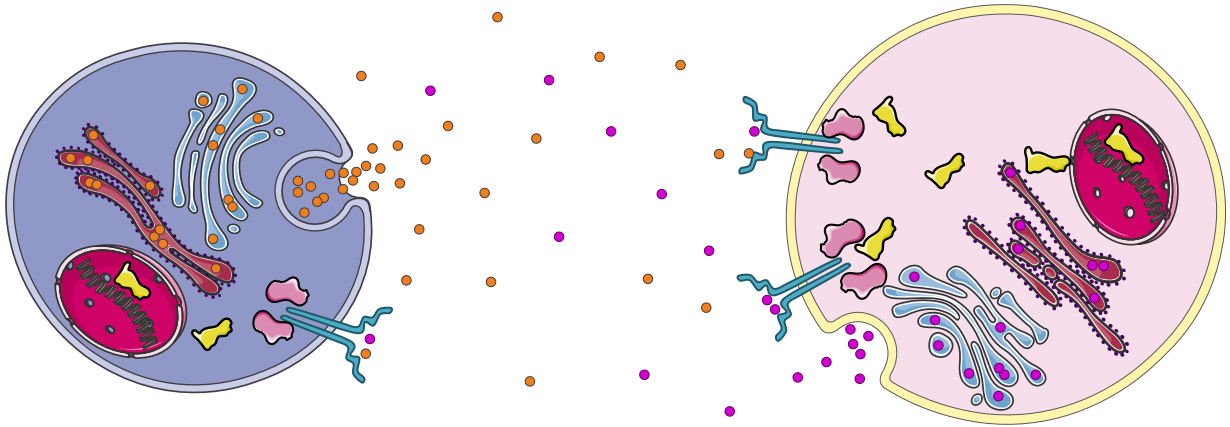
Fig. 3. Combining anti–c-Kit antibodies with CD47 blockade produces profound depletion of HSCs and clearance of the bone marrow niche in immunocompetent mice. (**A**) Total number of

Chhabra et al Sci Trans Med, 2018


Thrombopoietin (TPO)
Ligand binding to a cytokine receptor
(TPO receptor; MPL)
Cell Membrane
Jak
Jak
P
CALR
STAT
STAT
Megakaryocytes/ Platelets
CALR
nuclear envelope
nuclear pore
smooth endoplasmic reticulum
CALR
CALR
cisternal space
cisternae
rough endoplasmic reticulum
ribosomes
Nuclear Membrane
Transcription of genes involved in cell survival, migration and proliferation

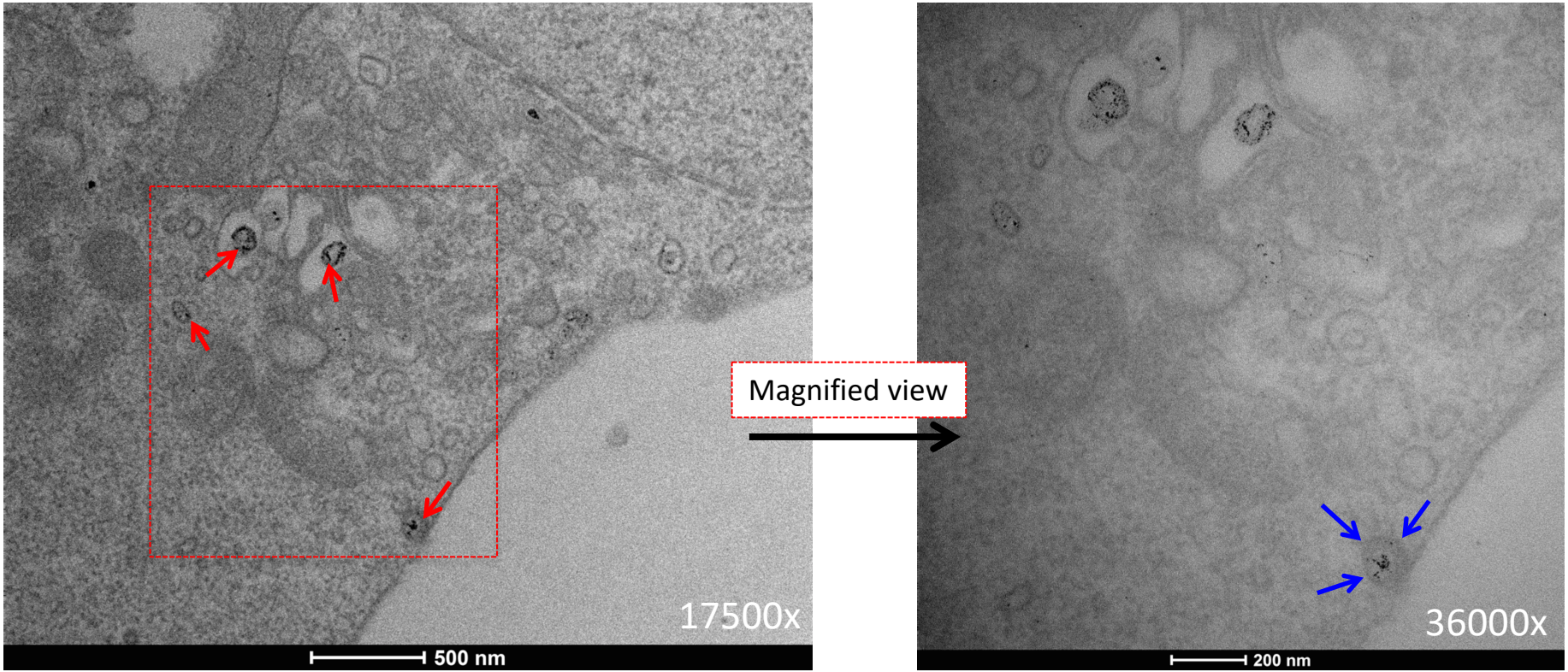
PLENARY ABSTRACT #4

Secreted Mutant Calreticulins As Rogue Cytokines



Christian Pecquet, Thomas Balligand, Ilyas Chachoua, Anita Roy, Gaelle Vertenoeil, Didier Colau, Emanuel Fertig, Caroline Marty, Harini Nivarthi, Jean-Philippe Defour, Erica Xu, Eva Hug, Heinz Gisslinger, Bettina Gisslinger, Martin Schalling, Ilaria Carola Casetti, Elisa Rumi, Daniela Pietra, Chiara Cavalloni, Luca Arcaini, Mario Cazzola, Norio Komatsu, Yoshihiko Kihara, Yoshitaka Sunami, Yoko Edahiro, Marito Araki, Isabelle Plo, William Vainchenker, Robert Kralovics and Stefan N Constantinescu

Mutant calreticulin goes through the secretory pathway and is secreted



Immuno-gold electron microscopy, using an anti-FLAG against FLAG-tagged CALR del52 expressed in the Ba/F3 cell line.

CALR mutant proteins are detected in plasma of patients with *CALR* mutations

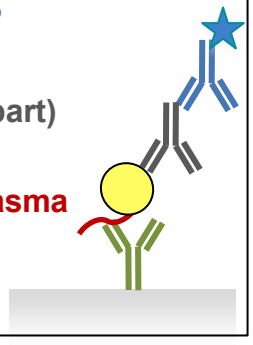
Sandwich-ELISA setup:

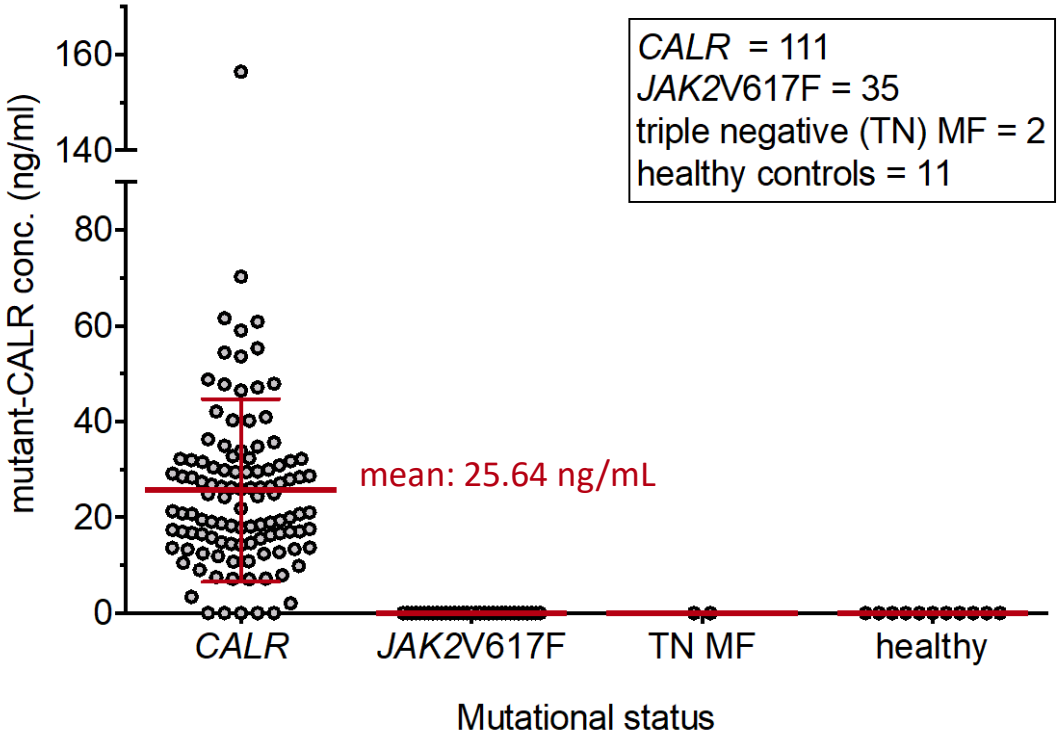
2° Ab: a-mouse-HRP

1° Ab:
a-CALR (common part)

Analyte:
mutant CALR in plasma

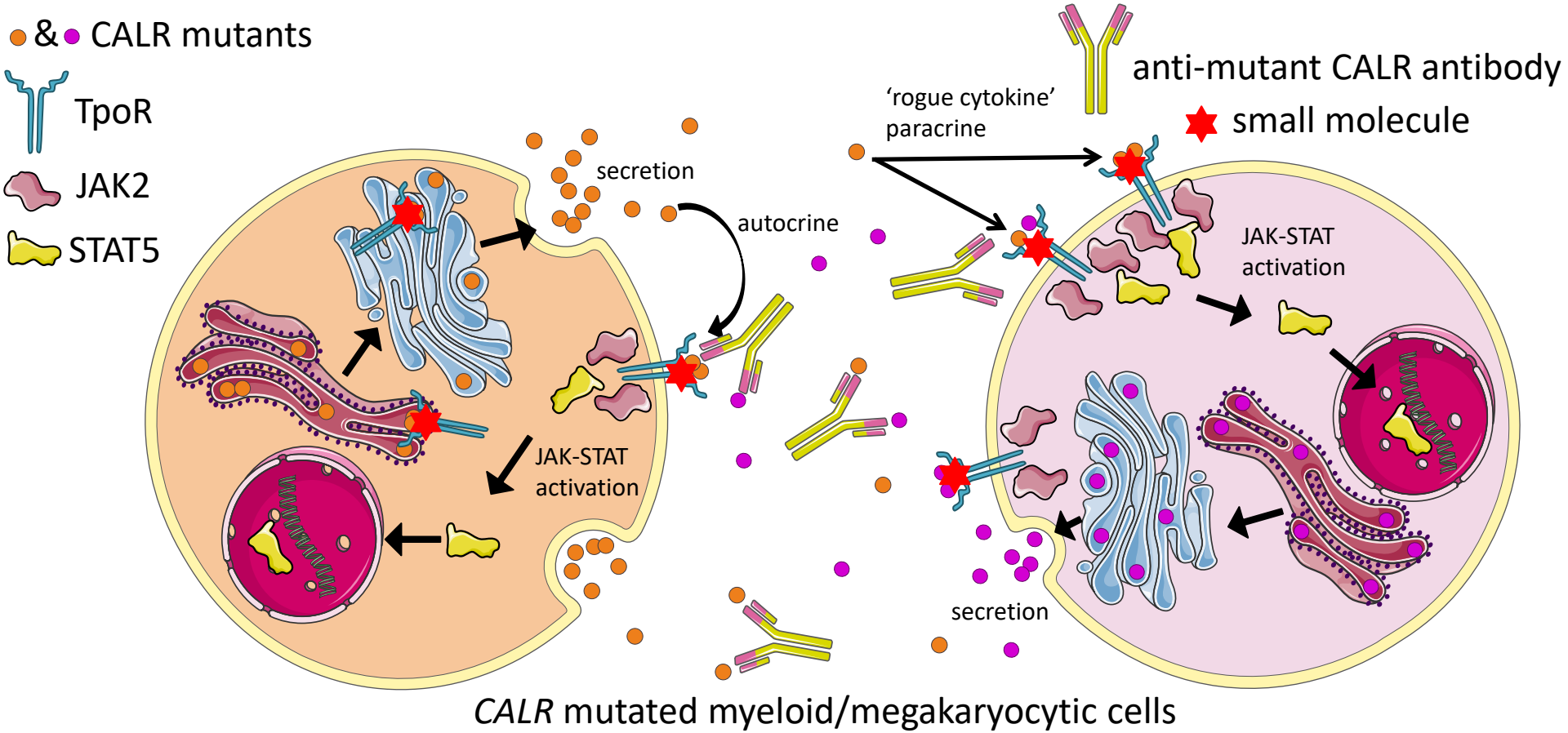
Coating Ab:
a-mutant CALR





By sandwich ELISA using antibodies directed against the mutant end-tail / common part of CALR proteins

CALR mutated cells secrete mutant CALR that acts in a autocrine and a ‘rogue cytokine’ fashion



CALR mutated myeloid/megakaryocytic cells

Peptide Vaccination Targeting CALR

ORIGINAL ARTICLE

The *calreticulin* (*CALR*) exon 9 mutations are promising targets for cancer immune therapy

MO Holmström[1,2], E Martinenaite[2], SM Ahmad[2], Ö Met[2,3], C Friese[2], L Kjær[1], CH Riley[4], P thor Straten[2,5], IM Svane[2,3], HC Hasselbalch[1] and MH Andersen[2,5]

The *calreticulin* (*CALR*) exon 9 mutations are found in ~30% of patients with essential thrombocythemia and primary myelofibrosis. Recently, we reported spontaneous immune responses against the *CALR* mutations. Here, we describe that CALR-mutant (*CALR*mut)-specific T cells are able to specifically recognize *CALR*mut cells. First, we established a T-cell culture specific for a *CALR*mut epitope. These specific T cells were able to recognize several epitopes in the *CALR*mut C terminus. Next, we established a *CALR*mut-specific $CD4^+$ T-cell clone by limiting dilution. These $CD4^+$ T cells recognized autologous *CALR*mut monocytes and hematopoietic stem cells, and T-cell recognition of target cells was dependent on the presence of *CALR*. Furthermore, we showed that the *CALR*mut response was human leukocyte antigen (HLA)-DR restricted. Finally, we demonstrated that the *CALR*mut-specific $CD4^+$ T cells, despite their phenotype, were cytotoxic to autologous *CALR*mut cells, and that the cytotoxicity was mediated by degranulation of the T cells. In conclusion, the *CALR* exon 9 mutations are targets for specific T cells and thus are promising targets for cancer immune therapy such as peptide vaccination in patients harboring *CALR* exon 9 mutations.

Leukemia advance online publication, 15 August 2017; doi:10.1038/leu.2017.214

Holmstrom *et al*, *Leukemia*, 2017

Summary: The Future of MPNs

- The future diagnosis and monitoring of MPNs will be real-time, wireless, personalized, and data-rich

- Treatment: harnessing the immune system to kill cancer

- The potential of genome editing is exciting, but will need to proceed with caution to understand safety issues and disease-specific role(s)

Acknowledgements