A Vision of the Future of MPNs

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

Leonard “Bones” McCoy and his tricorder.
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)

Chung et al.
Science
March 1, 2019

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

Ha-Yuk Chung1,2, Boong Hoong Kim3,4,6,7, Joon Yoon Lee4,6, Jungyup Lee4,6, Zhongqian Xie3,4,6, Erin M. Bler1,2, KunHyuck Lee4,6, Anthony Banu9,10, Ji Yoon Jeong8, Jongwon Kim1,2, Christopher Ogbe10, Dominique Grandje14, Yongjoon Yu4, Hokyung Jung6, Pounya Assem6, Dennis Rye4,6, Jean Won Kwak6, Myeong Namkoong6, Jin Bin Park6, Yehsan Lee6, Do Hooun Kim4, Jolin Rye6, JaneaJeong4, Kevin You6, Bowen Ahn5,6,4,4, Zhunghwan Lin6, Qinger Hao1,2, Xue Feng1,2, Yujin Deng1,2, Yishen Xia1,2, Kyung-In Jung8, Jeongguy Kim9, YiHui Zhang10, Rooshbeh Ghaffar10,11, Casey M. Radd10,12, Molly Schau6,13, Aaron Hamsa12,13, Debra E. Weese-Mayer12,13,14, Youngguang Huang10,11, Seung Min Lee10, Chi Hwan Lee6, Naresh R. Shanbhag6, Amy S. Pallet10,12,13, Suans Xa10,12,13, John A. Rogers10,11,12,13,14,15,17.
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 health 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

- Normal HSC
- Pre-leukemic HSC and clonal hematopoiesis
  - Enhance self-renewal
- Primary mutations (DNMT3A, TET2, IDH1/2)
- Pre-leukemia HSC
- Leukemia stem cells
  - Enhance proliferation/impaired differentiation
- Secondary mutations (FLT3ITD, JAK2, NPM1, NRAS)
- Full leukemia

Legend:
- Normal HSC
- Pre-leukemia HSC
- LSC
- Committed leukemia progenitor
- Blasts
Clonal hematopoiesis of indeterminate potential (CHIP)
a.k.a Age-related clonal hematopoiesis (ARCH)

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

Jaiswal et al, NEJM, 2014
Disease Monitoring
Alex is now 62 and has a new diagnosis of primary myelofibrosis.
2019:
A snapshot in time of ‘mutation landscape’

2030?
Continuous monitoring of mutation dynamics
Refined, Continuous Risk Re-Stratification in MPNs

- Co-Morbidity Status & Infection Risk
- Inherited Risk
- Progression to: MF, AML
- Thrombosis & Bleeding
  - RBC, WBC, platelets
  - Coagulation genomics
  - Platelet RNA expression
  - Endothelial cell biome
  - VWD Factor Activity
  - Bioactivity Profile
- Inherited DNA variants and metabolome
- Lipid/glucose profile
- Metalobome
- Microbiome
- T/B-cell and other immune cell markers
- Co-Morbidity Status & Infection Risk
- Inherited DNA variants and metabolome
- Mutation profile
- Mutation allele burden
- High risk molecular mutations
- Marrow fibrosis
- Flow markers of early blood progenitors
- Single cell analysis
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
Treatment
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

Ex vivo Assay

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

Have Disease
↓
Take Pill
↓
Kill Something

Disease
↓
Medicine
↓
Target

Versus

Environment
↑
Organ, organism
↑
Cells

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

Genetic Inactivation of CD33 in Hematopoietic Stem Cells to Enable CAR T Cell Immunotherapy for Acute Myeloid Leukemia
Gene Editing with CRISPR/CAS9
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
Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy

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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.

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) 

Megakaryocytes/Platelets 

Transcription of genes involved in cell survival, migration and proliferation
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

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

Sandwich-ELISA setup:

2° Ab: a-mouse-HRP
1° Ab: a-CALR (common part)
Analyte: mutant CALR in plasma
Coating Ab: a-mutant CALR

mean: 25.64 ng/mL

CALR = 111
JAK2V617F = 35
triple negative (TN) MF = 2
healthy controls = 11

Mutational status:

- CALR
- JAK2V617F
- TN MF
- healthy
CALR mutated cells secrete mutant CALR that acts in an autocrine and a ‘rogue cytokine’ fashion.

- CALR mutants
- TpoR
- JAK2
- STAT5
- anti-mutant CALR antibody
- small molecule

CALR mutated myeloid/megakaryocytic cells
The calreticulin (CALR) exon 9 mutations are promising targets for cancer immune therapy

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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 (CALRmut)-specific T cells are able to specifically recognize CALRmut cells. First, we established a T-cell culture specific for a CALRmut epitope. These specific T cells were able to recognize several epitopes in the CALRmut C terminus. Next, we established a CALRmut-specific CD4⁺ T-cell clone by limiting dilution. These CD4⁺ T cells recognized autologous CALRmut monocytes and hematopoietic stem cells, and T-cell recognition of target cells was dependent on the presence of CALR. Furthermore, we showed that the CALRmut response was human leukocyte antigen (HLA)-DR restricted. Finally, we demonstrated that the CALRmut-specific CD4⁺ T cells, despite their phenotype, were cytotoxic to autologous CALRmut 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
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)
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