The origins of blood cancers

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Disclosures

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- **Relationships with commercial interests:**
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Evolution of hematologic malignancies

Sperling and Ebert, *Nature Reviews Cancer* 2017
Clonal hematopoiesis

- Exome sequencing data from peripheral blood of >17,000 individuals
- Unselected for hematologic phenotype

Jaiswal et al., *NEJM* 2014
Somatic mutations and age

25 – 100 mutations per gene by age 70

Jaiswal and Ebert, *Science* 2019
DNMT3A is frequently mutated
Most subjects had only one mutation

<table>
<thead>
<tr>
<th>Number of Mutations</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mutation</td>
<td>688</td>
</tr>
<tr>
<td>2 mutations</td>
<td>49</td>
</tr>
<tr>
<td>3 mutations</td>
<td>2</td>
</tr>
<tr>
<td>4 mutations</td>
<td>2</td>
</tr>
</tbody>
</table>
Clonal hematopoiesis increases the risk of hematologic malignancy
Clonal hematopoiesis: concordant findings from multiple studies

Genovese et al., NEJM 2014
Xie et al., Nat Med, 2014
McKerrell et al., Cell Rep 2015
Prevalence of clonal hematopoiesis

Jaiswal and Ebert, Science 2019
Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Features:

- Absence of definitive morphological evidence of a hematological malignancy

- Presence of a somatic mutation associated with hematological malignancy at a variant allele fraction of at least 2%

- Odds of progression to overt neoplasia are approximately 0.5-1% per year, similar to MGUS

Steensma et al., Blood 2015
CHIP is associated with reduced overall survival

Cox proportional hazards models which included age, gender, and diabetes status as covariates, with results for cohorts analyzed as a fixed-effects meta-analysis.
CHIP is associated with higher risk of heart attack and stroke

Coronary heart disease

- HR 2.0, 95% CI 1.2-3.4, p=0.018

Stroke

- HR 2.6, 95% CI 1.4 to 4.8, p=0.003

Regression models were adjusted for age, sex, BMI, lipids, blood pressure, and smoking
Replication in additional cohorts

Jaiswal et al., NEJM 2017
Experimental examination of CHIP and cardiovascular disease

Vav1-Cre, Tet2^{fl/fl}
Vav1-Cre (control)

BMT

WT
Ldlr^{-/-}

Diet:
1.25% cholesterol

harvest
Aortic root

Tet2 WT

Tet2 KO

Jaiswal et al., *NEJM* 2017
Descending aorta lesion area is larger in *Tet2*<sup>-/-</sup> recipients
Gene expression in *Tet2* knockout macrophages

Jaiswal et al., *NEJM* 2017
Effect of CHIP on cardiovascular disease

Bone marrow myeloid stem cell → Mutation event → Clone of mutant myeloid precursors → Clone of mutant myeloid cells in blood → Heart failure → Proinflammatory mediators → Atherothrombosis
Can CHIP predict the development of therapy-related myeloid malignancies?
CHIP increases the risk of therapy-related myeloid neoplasms

Evaluation of CHIP in lymphoma patients undergoing autologous stem cell transplant

Gibson et al., *JCO* 2017
Summary

CHIP is associated with increased overall mortality
  • Increased risk of hematologic malignancy
  • Increased risk of cardiovascular disease
  • Increased risk of thrombosis (JAK2 mutations)
  • Increased risk of therapy-related malignancy

JAK2 CHIP mutations are associated with thrombosis
  • JAK2 mutations sensitize neutrophils to NETosis

Tet2 mutations accelerate atherosclerosis in vivo
  • Development of xanthomas
  • Altered expression of inflammatory cytokines
Clonal expansions in skin

Mortincorena et al., Science 2015
Thalidomide
- 1957 to 61: Treatment of morning sickness during pregnancy
  >10,000 children born with phocomelia
- 1991: TNF inhibitor
- 1994: Antiangiogenesis: first trials in cancer
- 2006: Approved for multiple myeloma

![Thalidomide molecule]

Pomalidomide
FDA approved for multiple myeloma

![Pomalidomide molecule]
Lenalidomide modulates the activity of the CRL4-CRBN ubiquitin ligase

Lenalidomide targets:
Multiple Myeloma: IKZF1, IKZF3
Del(5q) MDS: CK1α

Lenalidomide activity in mouse cells

Generated Ck1α conditional knockout mouse (Schneider et al., Cancer Cell 2014)
Does lenalidomide target Ck1α haploinsufficient cells? … No effect.

- No toxicity in mice
Human CRBN

Mouse Crbn

Steric bump of mouse isoleucine prevents access of substrates to H bonds on CRBN surface

Kronke, Fink et al., Nature 2015
Crbtn I391V knock-in mouse

WT Len 1 μM vs. DMSO

Crbtn^I391V/I391V^ Len 1 μM vs. DMSO

Crbtn^I391V/I391V^ Pom 1 μM vs. DMSO

Fink et al., *Blood* 2018
**Crbn I391V knock-in mouse: effect on pregnancy**

Thalidomide teratogenicity

<table>
<thead>
<tr>
<th>Species</th>
<th>Ctbavin sequence</th>
<th>Thalidomide teratogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>T E H S W F P G Y A W T</td>
<td></td>
</tr>
<tr>
<td>Orangutan</td>
<td>T E H S W F P G Y A W T</td>
<td></td>
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<tr>
<td>Rhesus Macaque</td>
<td>T E H S W F P G Y A W T</td>
<td></td>
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<tr>
<td>Marmoset</td>
<td>T E H S W F P G Y A W T</td>
<td></td>
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<tr>
<td>Green Monkey</td>
<td>T E H S W F P G Y A W T</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>T E H S W F P G Y A W T</td>
<td></td>
</tr>
<tr>
<td>Bushbaby</td>
<td>T E H S W F P G Y A W T</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>T V H S W F P G Y A W T</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>T V H S W F P G Y A W T</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>T E H S W F P G Y A W T</td>
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Fink et al., *Blood* 2018
Defining a lenalidomide-dependent degron sequence
Saturation mutagenesis of IKZF3 degron
IKZF3 zinc finger 2 is critical for degradation

Sievers, Petzold et al., *Science* 2018
IKZF3 zinc finger 2 is necessary and sufficient for lenalidomide-dependent degradation

Sievers, Petzold et al., *Science* 2018
Structure of zinc finger – pomalidomide - CRBN

Sievers, Petzold et al., Science 2018
Can we target other zinc fingers in the genome?
Proteome-wide C2H2 zinc finger screen

Clone library

Infect

Dose

Sort

Sequence

6,572 unique C2H2 ZFs

eGFP  IRES   mCherry

Thal  Len  Pom
Proteome-wide C2H2 zinc finger screen

Thalidomide

Lenalidomide

Pomalidomide

Sievers et al., Science 2018
Substrate-specific effects of different drugs

Sperling et al., *Blood* 2019
CK1α and GSPT1 have beta-beta fold degrons as well.

Petzold et al., Nature 2016
Summary

- Thalidomide derivatives act as a molecular glue, inducing ubiquitination and degradation of substrates by the CRL4\textsuperscript{CRBN} ubiquitin ligase
- Thalidomide derivatives target a zinc finger degron
- Different thalidomide derivatives target distinct substrates for degradation
- Multiple myeloma: lenalidomide induces degradation of IKZF1 and IKZF3
- Del(5q) MDS: lenalidomide induces degradation of CSNK1A1
- One non-conserved amino acid in mouse and human CRBN determines lenalidomide sensitivity
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