Dark selection for JAK/STAT-inhibitor resistance in CMML

The Orange team, the future is bright...
Chronic Myelomonocytic Leukemia (CMML)

- Constitutional Symptoms
- Bone Pain
- Cytopenias
- Spleen
- Skin
- Mast Cells
- Pyrullis
- Leukocytosis
- AML
- Splenomegaly

Spleen

Bone Marrow Biopsy

Peripheral Blood Smear
Inflammatory cytokines are elevated in CMML

N=219 CMML n=35 age-matched controls  Blue (10%) percentile Red (90%) percentile
Our analysis revealed prognostic links

<table>
<thead>
<tr>
<th>Poorer Outcome</th>
<th>How patients feel?</th>
<th>Better Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP1</td>
<td>FLT3</td>
<td>IL10</td>
</tr>
<tr>
<td>PDGF AA</td>
<td>CD44</td>
<td>IL10.23p40</td>
</tr>
<tr>
<td>EGF</td>
<td>FLT3</td>
<td>IL2Ra</td>
</tr>
<tr>
<td>RANTES</td>
<td>SCF</td>
<td>Exotaxin</td>
</tr>
<tr>
<td>SCF</td>
<td>FGFB basic</td>
<td></td>
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</table>

What are the important cytokines related to:

- **How patients feel?**
  - MCP1
  - PDGF AA
  - EGF
  - RANTES
  - SCF

- **Spleen size?**
  - IL5
  - SCF
  - TRAIL
  - MIG
  - IP10

- **Survival?**
  - IL13
  - PDGF AA
  - RAGE
  - SCF
  - RANTES

Penalised regression from 48 plasma cytokine values of 161 CMML patients, prior to treatment.
We lack good treatment options for this lethal leukemia.

Fenaux, et al Lancet Oncology 2009
Ruxolitinib: a new hope
Ruxolitinib: a new hope

Spleen pre-/post-treatment
Alas, resistance strikes back
We understand proximal mechanisms
“Normal” resistance: Darwinian selection of resistant (epi)genotypes
Dark Selection: evolution paradox

No obvious impact on stem/progenitors

No impact on clonal architecture

Padron Clin Can Res 2016
Shining light on the paradox of dark selection

Unusual case of Darwinian selection

Non-darwinian selection/emergence

CHOICES
Shining light on the paradox of dark selection
Alternative #1: hidden Darwinian selection
Alternative #1: hidden Darwinian selection
**Hidden Darwinian selection is plausible**

*Left panel:* ODE models on disease burden in the Bone Marrow

*Right panel:* Moran process model on disease output into periphery
Hidden Darwinian selection is plausible

But:

No evidence of genetic changes: must be epigenetic
No evidence for reduced proliferation/increased death
Considering microenvironmental pressure

Oxygen concentration strongly linked to STAT3 expression

- Hypoxia markedly increases STAT3 (and JAK) expression.
- This effect seen both with and without RUX present.

Bone Marrow at low O2 pressure (9-32 mmHg), despite high vascularity

- Oxygen consumption rate (OCR) markedly modulates hypoxia.
- Minor changes in OCR can lead to substantial increases in hypoxia.
- Can minor OCR shifts lead to STAT3 levels > RUX can counteract?

Slight perturbation of oxygen consumption rate leading to slightly different oxygen distributions (Grimes et al 2016, J R Soc Interface) by two vessels at the boundary. This oxygen map was set in accordance with measured O2 levels in bone marrow (Spencer et al, Nature, 2014).
Hypoxia

Hypoxia

<table>
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<tr>
<th>Situation 1</th>
<th>Situation 2</th>
<th>Situation 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.9 mmHg</td>
<td>13.1 mmHg</td>
<td>10.1 mmHg</td>
</tr>
</tbody>
</table>

Drug

No Drug
Alternative #2: Dark selection/emergence

- Stochastic ratcheted phenotypic switch
- Lamarckian selection
- Emerging signaling network behavior
Alt. #2: Molecular basis of dark selection

Stochastic ratcheted phenotypic switch & Lamarkian inheritance
Alternative #2: Dark selection/emergence

Lamarckian

non-cell autonomous
Alternative #2: Dark selection/emergence

- Lamarckian
- change in variance
- concave vs convex
- Non-cell autonomous

The graph shows the number of cells with heterodimerization pathways over time. The y-axis represents the number of cells, and the x-axis represents time. The red and blue lines represent different trends in cell behavior, with the red line indicating a Lamarckian change, and the blue line showing a non-cell autonomous trend. The graph illustrates a concave vs convex pattern in variance.
Different sources of “dark selection” lead to different predictions

Stochastic ratcheted phenotypic switch

Lamarckian selection

Emerging signaling network behavior

Hidden Darwinian selection
Experiments to differentiate between the scenarios

JAK2 V617F

+/- Ruxolitinib

Analyze in the Bone Marrow:

JAK2/Tyk heterodimers (by proximity ligation)
Proliferation
Apoptosis

Blood:

Cytokines

(a) Tumour foci in the metaphysis but not in other regions of long bone (b), tumour foci in epiphysis and metaphysis but not in mid-diaphysis
(c). 5x magnification. Figure from Holstead Jones et al, Nature, 2006
Alternative #2: Dark selection/emergence

Emerging signaling network behavior

Before Rx

Activated ➔ Desensitized ➔ Activated

After Rx

Sensitized ➔ Inactivated ➔ Activated
Alternative #2: Dark selection/emergence
Alternative #2: Dark selection/emergence
Integrating the continuous and CA models

\[ \frac{\partial C}{\partial t} + D \nabla^2 C = S(x, y, \text{STAT3}) - \delta C \]
\[ S(x, y, \text{STAT3}) = \text{STAT3}(x, y) \times C_{\text{max}}(x, y) \]
\[ C = 0 \text{ on } B_2 \text{ and } B_4 \]
\[ C_{\text{in}} = C_{\text{out}} \]

Hybrid Continuous-Discrete Cellular Automaton
Integrating experimental mouse data

JAK2 V617F

$+$ Ruxolitinib

Analyze in the bone marrow:

Spatial analyses:
Blood vessels
STAT3 activation
Experiments to differentiate between the scenarios

Analyze in the bone marrow
- Before treatment
- During remission
- During response

JAK2/TYK heterodimers
- Proliferation
- Apoptosis

Blood (weekly):
- Cytokines

x5 patients

$10K
Scales of Cancer
SA1: To determine whether the emergence of ruxolitinib resistance is a Darwinian, Lamarckian, or non-cell autonomous process.
SA2: To determine the kinetics of cytokine expression and resultant symptomatology in patients treated with ruxolitinib.
To summarize

Suite of novel integrated mathematical models test different evolutionary hypotheses and provide experimentally-testable insights.

Qualitative cytokine dynamics observed in the clinic can be captured in a simulation that models emergence of resistance.

Critically, the resistance paradox may depend on the tumor microenvironment, hidden selection, and network selection.
Questions?
Hypothesis 1: Subcellular Selection
Hypothesis 2: Physical Interactions
Hypothesis 3: Hypoxia

Statistics

Statement of Problem

Treatment Strategies

Treatment Strategy

Clinical Impact
Ruxolitinib for CMML

Potent JAK1/2 inhibitor FDA approved for the treatment of primary myelofibrosis

In myelofibrosis, improvements in symptoms, spleen size, and cytokine levels are seen in the majority of patients.

Even in responding patients and mouse models, no changes in tumor burden or mutational frequencies are seen while on therapy.

Within months on therapy, symptoms return, spleen enlarges, and disease progresses.
Importance: Our approach addresses an important and unresolved evolutionary question that is applicable to CMML but also to a wide range of cancers.

Originality: Suite of novel mathematical tools to explore a potentially novel mechanism of treatment resistance.

Feasibility: Padron and Marusyk’s lab and the young talent at #teamOrange
CMML: therapy does not visibly impact relevant population

Progenitors/stem cells

Differentiated cells