Dark selection for JAK/STAT-inhibitor resistance in CMML

The Orange team, the future is bright...
Chronic Myelomonocytic Leukemia (CMML)
Inflammatory cytokines are elevated in CMML

| Sample          | TNF alpha | IL-6 | IL-3 | IL-10/CXCL8 | MIP-1/CCL2 | VEGF | SCF | IL-1 beta | IFN-gamma | IL-1ra/IL-1f3 | TNF-α | GM-CSF | IL-2 | M-CSF | IL-4 | IL-7A | IL-17A | RAGE | GM-CSF | IL-2 R alpha | IL-5 | G-CSF | IL-17C | IL-17D | HGF | IL-12/23 p40 | Eotaxin/CCL11 | PDGF-AA | PDGF-BB | EGF | FLT-3 ligand | IL-12p70 | IL-13 | MIF | PDGF-CC | PDGF-DD | TRAIL/TNFSF10 |
|-----------------|-----------|------|------|-------------|------------|------|-----|----------|-----------|---------------|--------|---------|-----|-------|-----|-------|------|-------|--------|-------|-------|---------|-------|-------|---------|-------|-------|---------|-------|-------|---------|-------|-------|---------|-------|-------|---------|-------|-------|

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

- **What are the important cytokines related to:**
  - **How patients feel?**
    - Poorer Outcome: MCP1, PDGFAA, EGF, RANTES, SCF, FLT3, CD44, VEGF, IL12p70, FGFBasic
    - Better Outcome: FLT3, FGFBasic, RAGE, IL12p70, CD44
  - **Spleen size?**
    - Poorer Outcome: IL5, SCF, TRAIL, MIG, IP10
    - Better Outcome: FLT3, FGFBasic, RAGE, IL12p70, CD44
  - **Survival?**
    - Poorer Outcome: IL13, PDGFAA, RAGE, SCF, RANTES
    - Better Outcome: IL10, FGFBasic, IL12.23p40, IL2Ra, Exotaxin

- 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

N=1832
OS=32mo

Proportion Surviving

Time (months) from Randomization

AZA
CCR

15 months
24.4 months
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
Shining light on the paradox of dark selection

Mathematics: Making the Invisible Visible
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

\[
\frac{\partial C}{\partial t} - D \nabla^2 C = S(x, y, \text{STAT3}) - \delta C
\]

\[
S(x, y, \text{STAT3}) = \sum \text{STAT3}(x, y) \mathbb{1}_{\text{Cells}}(x, y)
\]

\[
C = 0 \text{ on } B_2 \text{ and } B_4
\]

\[
C_{|B_3} = C_{|B_1}
\]

Hypoxia

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

Graph: # of cells with heterodimerization pathways vs time.
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

+/- Ruxolitinib

JAK2 V617F

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

After Rx

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

\[ \frac{\partial C}{\partial t} - D \nabla^2 C = S(x, y, \text{STAT3}) - \delta C \]

\[ S(x, y, \text{STAT3}) = \Pi \text{STAT3}(x, y) \cap \text{Comb}(x, y) \]

C = 0 on \( B_2 \) and \( B_4 \)

\[ c_{i,j} = c_{i,j-1} \]

Hybrid Continuous-Discrete Cellular Automaton
Integrating experimental mouse data

JAK2 V617F

+$3K

 +/- 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

x10 patients

$20K
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.
ORANGE TEAM
Thanks
Questions?