Precision Medicine for the treatment of acute myeloid leukaemia

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Though uncommon, leukaemia is one of the 10 most lethal cancers in Hong Kong.
AML is characterized by an abnormal increase in myeloblasts and patients die of bleeding and infection.
AML is a heterogeneous disease with distinct clinicopathologic characteristics

- Gene mutations
- Family History
  - Antecedent MDS/MPN
  - Previous chemotherapy
- Morphology
- Cytogenetics (karyotypes)
- Immunophenotypes
- Stained Cells
- Fluorescence
- FS & SS
- Laser
AML classification considers clinical, morphological, cytogenetics, genetics and history of MDS and chemo-irradiation.

Classification of acute myeloid leukaemia

- AML with recurrent genetic abnormalities – specific cytogenetics and genetic mutations
- AML with myelodysplasia related changes
- Therapy (chemo-irradiation) related myeloid neoplasms
- AML NOS – morphologic criteria
- Myeloid sarcoma
- Myeloid proliferations associated with Down’s Syndrome

> 20 different types of AML with recurrent features and many more are less characterized.
All AML are treated in the same way despite its heterogeneity.

Diagnosis

Induction

Remission

Consolidation

Close observation

HSCT

Daunorubicin
Idarubicin
Mitoxantrone
Fludarabine
Clofarabine

+ Cytarabine

Cytarabine (High dose)
Different AML subtypes respond differently to conventional treatments (ELN 2017)

<table>
<thead>
<tr>
<th>Risk categories</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>Translocations t(8;21) and Inv(16) Mutations involving NPM1 (with the absence of FLT3-ITD) and CEBPA</td>
</tr>
<tr>
<td>Adverse</td>
<td>Translocations t(6;9), t(v;11)*, Inv(3), t(9;22) Aneuploidy -5/5q; -7; -17/17p Complex and monosomy karyotypes RUNX1, ASXL1, TP53, FLT3-ITD (absence of NPM1 mutation) mutations</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Not in favorable or adverse risk groups</td>
</tr>
</tbody>
</table>

* Except t(9;11)
Even “good risk” young patients showed ~ 40% long-term OS and the outcome of “poor risk” and elderly patients is dismal.
Intermediate risk AML with normal cytogenetics comprise a heterogeneous mix of different mutations.
What are the unmet clinical needs in AML?

- Novel agents
- Personalized treatment (biomarkers driven)
- Personalized treatment (real-time pre-testing)
<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein kinase inhibitors</td>
<td>FLT3 inhibitors (quizartinib, gilteritinib, crenolanib), KIT inhibitors, PI3K/AKT/mTOR inhibitors, Aurora and polo-like kinase inhibitors, CDK4/6 inhibitors, CHK1, WEE1 and MPS1 inhibitors, SRC and HCK inhibitors</td>
</tr>
<tr>
<td>Epigenetic modifiers</td>
<td>New DNA methyltransferase inhibitors (SGI-110), Histone deacetylase (HDAC) inhibitors, IDH1 and IDH2 inhibitors, DOT1L inhibitors, BET-bromodomain inhibitors</td>
</tr>
<tr>
<td>Chemotherapeutic agents</td>
<td>CPX-351, Vosaroxin, Nucleoside analogs, Bcl-2, Bcl-xL, Caseinolytic protease inhibitors</td>
</tr>
<tr>
<td>Mt inhibitors</td>
<td>Bcl-2, Bcl-xL, Caseinolytic protease inhibitors</td>
</tr>
<tr>
<td>Onco-proteins targeting</td>
<td>Fusion transcripts targeting, EVI1 targeting, NPM1 targeting, Hedgehog inhibitors (glasdegib)</td>
</tr>
<tr>
<td>Antibodies and immunotherapies</td>
<td>Monoclonal antibodies against CD33, CD44, CD47, CD123, CLEC12A, Immunocongjugates (e.g., gemtuzumab ozogamicin, SGN33A), Bispecific T-cell engagers (BiTES) and dual affinity re-targeting molecules (DARTs), Chimeric antigen-receptor (CAR) T-cells or genetically engineered T-cell receptor (TCR) T-cells, Immune checkpoint inhibitors (PD-1/PD-L1, CTLA-4), Anti-KIR antibody (lirilumab), Vaccines (e.g., WT1)</td>
</tr>
<tr>
<td>Microenvironment</td>
<td>CXCR4 and CXCL12 antagonists, Anti-angiogenic therapies</td>
</tr>
</tbody>
</table>

Döhner et al. BLOOD 2017
AML subtypes not responsive to conventional treatments

**FLT3-ITD**

- Immuno-globulinlike loops
- Transmembrane domain
- Cell membrane
- Tandem duplication (insertion of 3-400 bp)
- Juxtamembrane domain
- Point mutations at D835 / I835
- Insertions between S840 and N841

**Complex/monosomy karyotype**

- TP53
- Genomic instability
  - Nucleotide instability
  - Chromosomal instability
- Clonal expansion
- Aneuploidy
- Gain of oncogenes / Loss of TS

Litzow Blood 2005
FLT3-ITD AML showed very poor outcome with conventional treatments.

All patients

FLT3-WT, N=87

FLT3-ITD, N=45

P=0.005

OS (Years)

FLT3-ITD AML relapsed after 7+3

N=35

OS (Months)

HK AML Working Group
FLT3 inhibitors have been actively tested in clinical trials.
Phase I Study of Quizartinib Administered Daily to Patients With Relapsed or Refractory Acute Myeloid Leukemia Irrespective of FMS-Like Tyrosine Kinase 3–Internal Tandem Duplication Status

Jorge E. Cortes, Hagop Kantarjian, James M. Foran, Darejan Ghirdaladze, Mamia Zodelava, Gautam Borthakur, Guy Gammon, Denise Trone, Robert C. Armstrong, Joyce James, and Mark Levis

Median Survival (weeks)

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponders</td>
<td>9.7</td>
</tr>
<tr>
<td>Responders</td>
<td>35.1</td>
</tr>
</tbody>
</table>

![Graph showing overall survival with lines for nonresponders and responders.](image)

![Comparison of FLT3 and FLT3/ITD expression levels before and at different time points for 18 mg and 60 mg doses.](image)
Sorafenib treatment of FLT3-ITD+ acute myeloid leukemia: favorable initial outcome and mechanisms of subsequent nonresponsiveness associated with the emergence of a D835 mutation

Cheuk Him Man, Tsz Kan Fung, Christa Ho, Heron H. C. Han, Howard C. H. Chow, Alvin C. H. Ma, William W. L. Choi, Si Lok, Alice M. S. Cheung, Connie Eaves, Yok Lam Kwong, and Anskar Y. H. Leung

Departments of Medicine and Pathology, and Genome Research Centre, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong; and Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC

BLOOD 2012
Midostaurin plus chemotherapy improved OS and EFS in FLT3-ITD AML

**Midostaurin**
74.7 mo (95% CI, 31.5–NR)

**Placebo**
25.6 mo (95% CI, 18.6–42.9)

One-sided P = 0.009 by stratified log-rank test
AML subtypes not responsive to conventional treatments

FLT3-ITD

Complex/monosomy karyotype

TP53

Genomic instability

Nucleotide instability

Chromosomal instability

Clonal expansion

Aneuploidy

Gain of oncogenes / Loss of TS

Litzow Blood 2005
Complex/monosomy karyotype AML showed very poor outcome with conventional treatments.
Patients with TP53 mutations showed good response to 10-day course of decitabine. AML with TP53 mutations showed good response to Decitabine.

Responses transient unless consolidated by HSCT.

Clearance of TP53 clones with decitabine

Overall survival (Days)

TP53 VAF (%)
Therapeutic mechanisms of decitabine beyond DNA demethylation

- DNA incorporation and damage
- Endogenous retrovirus expression
- DNA demethylation
- Inhibition of NFkB
What are the unmet clinical needs in AML?

- Novel agents
- Personalized treatment (biomarkers driven)
- Personalized treatment (real-time pre-testing)
Personalized treatment for AML based on genomics and drug sensitivity testing

Heterogeneity between and within individual AML

Personalized regimens for individuals/groups of AML patients

Therapeutic regimens most effective for individual AML samples

Validation by clinical trials

Lam et al.  BLOOD REVIEWS 2017
Optimization of drug sensitivity analysis platform is technically cumbersome

Flow cytometry
- Proliferation
- Apoptosis
- Differentiation
- Intracellular protein/signalling

Fluorometric/Colorimetric Assays
- Cell number/viability
- Proliferation

In vitro combination testing on patient samples

In vivo trial of drugs with patient-specific xenograft

Cytokines
- IL-3
- G-CSF
- SCF
- FLT3L
- TPO

AhR antagonists
- Endothelial cells
- Osteoblast
- Mesenchymal stromal cells

Drugs
- Singly
- Combination

Myeloblasts
- Purified/MNC fraction
- Density
*In vitro* drug testing and NGS have led to identification of novel therapeutic agents for specific AML subtypes.
Timeline for the development of homoharringtonine

- 1963: Paudler et al. identified harringtonine
- 1969: Demonstrated clinical efficacy of HHT in CML & AML in China
- 80: Phase 1/2 trial of HHT in the US
- 83: Mechanisms of HHT on protein synthesis
- 85: Semi-synthetic HHT (OME)
- 87: FDA approval of OME for CML
- 89: Powell et al. determined HHT structure and demonstrated anti-leukemia effects in mouse cell lines
- 95: Demonstrated efficacy of HHT in CML in the U.S.
- 96: Demonstrated efficacy of HHT in CML in the U.S.
- 2012: Synergies with TKIs, PI, HDACi, HMA, ATRA in the treatment of haematological malignancies
Homoharringtonine is a protein translation inhibitor
Synergism between HHT and sorafenib in FLT3-ITD AML in vitro
Combination of sorafenib and HHT resulted in clearance of blasts in most R/R FLT3/ITD AML
Outcome of R/R FLT3/ITD patients

- CR/CRi, N=28
- No CR/CRi, N=11

- $P=0.02$

- HSCT (n=12)
- No HSCT (n=16)

$p=0.002$
港大研發 平均延壽至5個月
新法治急性血癌 延命候骨髓

醫健

本港每年約有300宗急性髓性白血病新症，其中三成病人屬FLT3–ITD基因突變，死亡率達七成。港大醫學院內科研究人員全球首次發現，以高三尖杉酯醣配合標靶藥治療，可助患者壽命由數周延伸至4至5個月，讓他們有更多時間候骨髓移植。

提取植物成分配標靶藥 抑癌細胞

港大醫學院內科學系臨牀教授梁如鴻稱，急性髓性白血病是由患者體內有過量髓性白血病細胞增生引起，最初會有發燒、瘀血及骨痛等症狀。補充目前會為病人做化療，但未必有效，一旦治療無效，病人可於數周內死亡。

港大醫學院內科於2012年展開研究，分析96名急性髓性白血病的細胞及25種藥物的配對，發現從三尖杉屬植物中提取的「高三尖杉酯醣」，配合標靶藥FLT3抑制劑，可針對抑制相關癌細胞的蛋白生長，以清除癌細胞，病人接受治療後平均壽命可達4至5個月。三尖杉俗稱狗尾松、山榧樹，多見於中國西南、河南、湖北、浙江、四川等地。上述研究已刊於醫學期刊

治療24人 6人存活

梁如鴻說，此療法的副作用較傳統化療少，病人或會出紅疹，但毋須用止癢藥，適用於較年長者，但因此方法較新，醫生仍會先為病人做化療。

年11月回港後再有感冒病徵求診，當時腳有瘀傷，獲醫生處方抗生素，但病情轉差，腳上瘀傷亦增加，需再求醫，經檢查證實是急性髓性白血病。她最初在屯門醫院接受化療但無效，轉至瑪麗醫院接受高三尖杉酯醣配合標靶藥治療，成功抑制癌細胞，並已獲姐姐捐骨髓移植。她期望於明年回返新西蘭繼續學業。梁如鴻說，病人移植
Optimization of drug sensitivity analysis platform is technically cumbersome

- Myeloblasts
  - Purified/MNC fraction
  - Density
- Stromal cells
  - Endothelial cells
  - Osteoblast
  - Mesenchymal stromal cells
- Cytokines
  - IL-3
  - G-CSF
  - SCF
  - FLT3L
  - TPO
- AhR antagonists
  - Endothelial cells
  - Osteoblast
  - Mesenchymal stromal cells

Flow cytometry
- Proliferation
- Apoptosis
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Fluorometric/Colorimetric Assays
- Cell number/viability
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In vitro combination testing on patient samples

In vivo trial of drugs with patient-specific xenograft
High throughput liquid handler facilitates rapid testing of drug sensitivity and resistance
High throughput liquid handler facilitates rapid testing of drug sensitivity and resistance.
What are the unmet clinical needs in AML?

- Novel agents
- Personalized treatment (biomarkers driven)
- Personalized treatment (real-time pre-testing)
Decision on the best therapeutic strategy for individual patients based on real-time drug sensitivity and resistance testing (DSRT)

Pemovska et al. Cancer Discovery 2013
**In vitro** drug sensitivity testing leads to novel therapeutic regimens testable in clinics

<table>
<thead>
<tr>
<th>Patient</th>
<th>DSRT-guided treatment</th>
<th>Treatment duration (days)</th>
<th>Disease state at treatment start</th>
<th>Treatment response</th>
<th>Additional information</th>
<th>Time to progression (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>252</td>
<td>Dasatinib</td>
<td>59</td>
<td>Relapsed, resistant disease</td>
<td>RD</td>
<td>Bone marrow blasts: 65-40-75%</td>
<td>8</td>
</tr>
<tr>
<td>560</td>
<td>Dasatinib-temsirolimus</td>
<td>34</td>
<td>Relapsed, remission</td>
<td>RD</td>
<td>Induction w. plerixafor-MAC</td>
<td>4</td>
</tr>
<tr>
<td>560</td>
<td>Dasatinib-sunitinib</td>
<td>5</td>
<td>Relapsed, resistant disease</td>
<td>RD</td>
<td>Blood blasts 34-0%</td>
<td>N/A</td>
</tr>
<tr>
<td>600</td>
<td>Dasatinib-sunitinib-temsirolimus</td>
<td>44</td>
<td>Relapsed, resistant disease</td>
<td>CRi</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>718</td>
<td>Sorafenib-clofarabine</td>
<td>63</td>
<td>Relapsed, resistant disease</td>
<td>Morphologic leukemia-free state</td>
<td>Hypoplasia</td>
<td>Lost to follow-up</td>
</tr>
<tr>
<td>784</td>
<td>Dasatinib-sunitinib-temsirolimus</td>
<td>13</td>
<td>Resistant disease</td>
<td>RD</td>
<td>Bone marrow blasts: 70-35-85%</td>
<td>Not evaluable</td>
</tr>
<tr>
<td>800</td>
<td>Dasatinib-clofarabine-vinblastine</td>
<td>6</td>
<td>Resistant disease</td>
<td>Morphologic leukemia-free state</td>
<td>Hypoplasia</td>
<td>Hypoplasia, no disease progression</td>
</tr>
<tr>
<td>1145</td>
<td>Ruxolitinib-dexamethasone</td>
<td>48</td>
<td>Relapsed, resistant disease</td>
<td>RD</td>
<td>Hematologic improvement</td>
<td>6</td>
</tr>
</tbody>
</table>

Pemovska et al. Cancer Discovery 2013
Axitinib effectively inhibits BCR–ABL1(T315I) with a distinct binding conformation

Tea Pemovska¹, Eric Johnson², Mika Kontro³, Gretchen A. Repasky¹, Jeffrey Chen²†, Peter Wells², Ciarán N. Cronin², Michele McTigue², Olli Kallioniemi¹, Kimmo Porkka³§, Brion W. Murray²§ & Krister Wennerberg¹§

Nature 2015
Conclusions

AML is a heterogeneous group of diseases for which treatment has been uniform and outcome unsatisfactory.

Novel agents targeting the most difficulty AML subtypes are emerging.

High throughput drug sensitivity testing may provide biomarker driven regimen and real-time guidance to personalized treatment.
Prognostically defined AML subtypes reflect their differential responses to the current treatments

* Induction / consolidation chemotherapy ± Allogeneic HSCT