Clinical Application of Anti-CML immunity in the era of TKI therapy

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Honoraria; BMS
Travel accommodation; BMS
Ph1 chromosome and BCR/ABL fusion gene; driver gene alteration for CML

(A) Interphase Nuclear FISH

ABL1 (9q); red

BCR (22q); yellow

(B) Fusion protein

(C) Fusion signal


Fusion signal

ABL1 (9q); red
BCR (22q); yellow
White arrow; BCR/ABL or ABL1/BCR fusion
Tactics of anticancer therapy based on characteristics of cancer cells

Selective TKI therapy: Allogeneic HSCT; the most powerful treatment option, however enforcing pts to live with substantial Treatment-related Toxicity.

Target specific, i.e. less toxic systemically.
The number of HSCTs carried out for CML-CP remarkably decreased.

Since the first trial of imatinib opened

- CML early
  (CP1)
- CML advanced
  (≥ CP2, AP, BC)

Steep decline in transplants for CML early

Remained stable for CML advanced

Data from EBMT

Ponatinib vs SCT for CML With T315I Mutation

<table>
<thead>
<tr>
<th>Disease Group</th>
<th>Ponatinib</th>
<th>SCT</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>NR</td>
<td>103</td>
<td>.013</td>
</tr>
<tr>
<td>AP</td>
<td>NR</td>
<td>56</td>
<td>.889</td>
</tr>
<tr>
<td>BP</td>
<td>7</td>
<td>11</td>
<td>.026</td>
</tr>
<tr>
<td>Ph+ ALL</td>
<td>7</td>
<td>32</td>
<td>.136</td>
</tr>
</tbody>
</table>

Overall response rates to TKIs in pts with CML-CP

(A) IM 1\textsuperscript{st} line Tx. → 60\% durable response → 40\% eligible for IM treatment withdrawal

- 40\% require 2\textsuperscript{nd} TKI
- 50\% require 3\textsuperscript{rd} TKI
- 50\% require alternative treatment

(B) 2\textsuperscript{nd} TKI 1\textsuperscript{st} line Tx. → 70-80\% durable response → Treatment withdrawal under evaluation

- 20-30\% require 3\textsuperscript{rd} TKI
- 50\% require alternative treatment

- Up to 50\% achieve Operational cure
- Around 10-15\% of initial group might benefit from HSCT

Clinical questions in the treatment of CML in the era of TKI

- **Withdrawal of TKI?**
  - How to manage after TKI withdrawal
    - Concept of “Treatment-Free Remission” “Operational Cure”
  - MRD; minimal or measurable? residual disease
  - CML stem cell

- **Required alternative strategy?**
  - To pts with refractory to TKI or advanced disease w/o T315I mutation
    - > 3rd TKIs
    - allo-HSCT
    - Immunotherapy
  - Interfere the disease control after TKI's withdrawal
    - Immunotherapy
“Propose continuation of TKI treatment indefinitely in all responders”

A challenge;
To establish a strategy to avoid the life-long dependency on TKIs.

....Secondary Malignancies, financial issues, recognized and/or unrecognized AEs (for young pts), impairment of QoL (pregnancy issue), ...

e.g. For whom?
Secondary malignancies in Imatinib-treated CML patients

Table 3. Standardized incidence rates of secondary malignancies (excluding non-melanoma skin cancer)

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CML IV Matched German</td>
<td>Observed/expected</td>
<td>CML IV Matched German</td>
<td>Observed/expected</td>
</tr>
<tr>
<td></td>
<td>population</td>
<td>(95% confidence interval)</td>
<td>population</td>
<td>(95% confidence interval)</td>
</tr>
<tr>
<td>Overall, n</td>
<td>38</td>
<td>0.88 (0.63–1.20)</td>
<td>24</td>
<td>1.06 (0.69–1.55)</td>
</tr>
<tr>
<td>Age &gt; 50 years, n</td>
<td>37</td>
<td>0.91 (0.65–1.24)</td>
<td>20</td>
<td>1.02 (0.65–1.54)</td>
</tr>
<tr>
<td>Age &lt; 50 years, n</td>
<td>1</td>
<td>0.42 (0.02–2.06)</td>
<td>4</td>
<td>1.82 (0.58–4.39)</td>
</tr>
<tr>
<td>Prostate, n</td>
<td>9</td>
<td>0.76 (0.37–1.40)</td>
<td>3</td>
<td>1.07 (0.27–2.92)</td>
</tr>
<tr>
<td>Colorectal, n</td>
<td>3</td>
<td>0.49 (0.13–1.34)</td>
<td>2</td>
<td>1.18 (0.20–3.89)</td>
</tr>
<tr>
<td>Lung, n</td>
<td>4</td>
<td>0.66 (0.21–1.58)</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>NHL, n</td>
<td>4</td>
<td>3.33 (1.06–8.04)</td>
<td>3</td>
<td>4.29 (1.09–11.66)</td>
</tr>
<tr>
<td>Breast, n</td>
<td>5</td>
<td>7.8</td>
<td>5</td>
<td>0.64 (0.23–1.42)</td>
</tr>
<tr>
<td>Pancreas, n</td>
<td>2</td>
<td>1.54 (0.26–5.08)</td>
<td>2</td>
<td>2.86 (0.48–9.44)</td>
</tr>
<tr>
<td>Kidney, n</td>
<td>2</td>
<td>1.25 (0.21–4.13)</td>
<td>2</td>
<td>3.33 (0.56–11.01)</td>
</tr>
</tbody>
</table>

Abbreviations: CML, chronic myeloid leukemia; NHL, non-Hodgkin’s lymphoma.

Mirand MB et al. Leukemia, 2016
“Operational Cure”

; Prolonged survival in molecular remission without therapy by Goldman JM et al.  J Natl Compr Canc Netw. 2012

What does MRD in leukemia really mean?

Goldman JM, Gale RP. Leukemia 2014

<table>
<thead>
<tr>
<th>Study</th>
<th>Pt No.</th>
<th>Tx.before discontinuation</th>
<th>Requisite for discontinuation</th>
<th>TFR% (median follow-up time)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M discont trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STIM1</td>
<td>100 FN then M for ≥3y</td>
<td>CMR for ≥2y</td>
<td>39% (55 m.o.)</td>
<td></td>
</tr>
<tr>
<td>STIM2</td>
<td>200 M ≥ 3y</td>
<td>As for STIM</td>
<td>preliminary 46% @ 2y</td>
<td></td>
</tr>
<tr>
<td>EURO-SKI</td>
<td>809 M, NIL, DAS</td>
<td>MR4 for ≥1y</td>
<td>in progress</td>
<td>preliminary 61% @ 0.5y</td>
</tr>
<tr>
<td><strong>M/NIL/DAS discont trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STOP 2G-TKI pilbat</td>
<td>50 NIL or DAS</td>
<td>CMR for median 29m.o.</td>
<td>in progress</td>
<td>preliminary 61.1%</td>
</tr>
<tr>
<td>ENESTFreedom</td>
<td>175 NIL front-line</td>
<td>MR4.5 for ≥1y</td>
<td>in progress</td>
<td></td>
</tr>
<tr>
<td>ENESTop</td>
<td>117 NIL 2nd-line ≥3y</td>
<td>MR4.5 for ≥1y</td>
<td>in progress</td>
<td></td>
</tr>
<tr>
<td>ENESTPath</td>
<td>1058 M ≥2 and NIL</td>
<td>MR4.5 for ≥1y vs MR4.5 for ≥2y RCT</td>
<td>in progress</td>
<td></td>
</tr>
<tr>
<td>Japanese trial*</td>
<td>88 DAS 1y as consoli</td>
<td>DMR, unclearly defined</td>
<td>49% @ 6m.o.</td>
<td></td>
</tr>
</tbody>
</table>

Saußele S et al, Leukemia 2016, modified.
Levels of molecular response and corresponding log-reduction and BCR-ABL transcript levels on the International Scale.

<table>
<thead>
<tr>
<th>Log reduction</th>
<th>MR levels</th>
<th>Time</th>
<th>BCR-ABL %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Standardized baseline</td>
<td>3m</td>
<td>100%</td>
</tr>
<tr>
<td>-1</td>
<td>MR 1.0</td>
<td>6m</td>
<td>10%</td>
</tr>
<tr>
<td>-2</td>
<td>MR 2.0</td>
<td>12m</td>
<td>1%</td>
</tr>
<tr>
<td>-3</td>
<td>MR 3.0</td>
<td></td>
<td>0.1%</td>
</tr>
<tr>
<td>-4</td>
<td>MR 4.0</td>
<td></td>
<td>0.01%</td>
</tr>
<tr>
<td>-4.5</td>
<td>MR 4.5</td>
<td></td>
<td>0.0032%</td>
</tr>
<tr>
<td>-5</td>
<td>MR 5.0</td>
<td></td>
<td>0.001%</td>
</tr>
</tbody>
</table>

‘EMR’ (Early Molecular Response)

‘MMR’ (Major Molecular Response)

‘Deep Molecular Response’ formerly CMR (Complete Molecular Response)

Michele Baccarani, and Simona Soverini Blood
2014;124:469-471
Discontinuation of TKI therapy

Criteria for TKI Discontinuation

1) Age > 18 y.
2) CP with no prior history of AP or BC,
3) TKI Tx > 3 y.
4) Prior evidence of quantifiable BCRABL transcript,
5) Stable MR4; < 0.01% IS for > 2y.
6) No history of resistance to any TKI,
7) Access to a reliable QPCR test with a sensitivity of detection of > 4.5 logs that reports results on the IS and provides results within 2w, monthly molecular monitoring for the first 6 mo. following discontinuation, bimonthly during months 7-24, and quarterly thereafter (indefinitely) for pts who remain in MMR (MR3; <0.1% IS),
8) Consultation with a CML Specialty Center to review the appropriateness for TKI discontinuation, including TKI withdrawal syndrome
9) Prompt resumption of TKI, with a monthly molecular monitoring for the first 6 mo. following resumption of TKI and every 3 mo. thereafter is recommended indefinitely for pts with a loss of MMR. For those who fail to achieve MMR after 6 mo. of TKI resumption, BCR-ABL1 kinase domain mutation testing should be performed, and monthly molecular monitoring should be continued for another 6 mo.

◆ Discontinuation should be as a “clinical trial”

◆ Low risk (sokal score) CML at diagnosis

◆ Although the depth of molecular response seems associated with TFR (Treatment Free Remission) in fact, the correlation between TFR and the “long-term outcome” still remains to be elucidated.

◆ with Higher NK cell number

Bhalla S et al. Clin Lymphoma, Myeloma & Leukemia, 2016, modified
Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival

A primitive subset of CD34+ CML cells survives complete Bcr-Abl inhibition in growth factor–free medium for 12 days.
Dual targeting of p53 and c-MYC selectively eliminates leukaemic stem cells (based on the transcriptome analysis)
Tactics of anticancer therapy based on characteristics of cancer cells

Selective TKI therapy

- EGFR inhibitors
- Cyclin-dependent kinase inhibitors
- Immune activating anti-CTLA4 mAb
- Telomerase Inhibitors
- Selective anti-inflammatory drugs
- PARP inhibitors
- Inhibitors of VEGF signaling
- Inhibitors of HGF/c-Met

Leukemia-specific CD8+ T cells (CTLs) selectively killed leukemia cells.

Being target-specific might reflect the less systemic toxicity (on-target/off-tumor AE).

Immunotherapeutic strategy

CTL: non-labeled moving cells, Leukemia cell : Green labeled
Dead cells: red labeled, Fibroblasts: by-stander cells


Asai H. & Fujiwara H. Biostation IM, NIKON Inc.
Molecular targets of imatinib, nilotinib and dasatinib

<table>
<thead>
<tr>
<th>Imatinib</th>
<th>Nilotinib</th>
<th>Dasatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL</td>
<td>ABL</td>
<td>ABL</td>
</tr>
<tr>
<td>ARG</td>
<td>ARG</td>
<td>ARG</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>BCR-ABL</td>
<td>BCR-ABL</td>
</tr>
<tr>
<td>KIT</td>
<td>KIT</td>
<td>KIT</td>
</tr>
<tr>
<td>PDGFR</td>
<td>PDGFR</td>
<td>PDGFR</td>
</tr>
<tr>
<td>DDR1</td>
<td>DDR1</td>
<td>DDR1</td>
</tr>
<tr>
<td>NQO2</td>
<td>NQO2</td>
<td>NQO2</td>
</tr>
<tr>
<td><strong>Src family</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B-cell development</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Haematopoietic stem cell Differentiation /proliferation</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Colorectal cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cell differentiation / Cancer metastasis</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Molecular targets of imatinib, nilotinib and dasatinib**

Mathematical mode; Autologous immune response might interfere the variation of BCR-ABL transcripts

Based on data from IM first-line Tx.

Host immune responses are involved in the clinical course of CML pts treated with TKIs


Christiansson L et al. Mol Cancer Ther., 2015

Naka K and Ichinohe T. Stem Cell Investig 2016
Natural Killer cells in the clinical efficacy of TKIs

Brief Report

**MYELOID NEOPLASIA**

**KIR2DL5B** genotype predicts outcomes in CML patients treated with response-directed sequential imatinib/nilotinib strategy


NK cells seem involved in anti-CML efficacy mediated by TKIs


Adoptively transferred NK cells seemed less effective

Regular Article

**MYELOID NEOPLASIA**

Monoclonal antibody targeting of IL-3 receptor α with CSL362 effectively depletes CML progenitor and stem cells

Nievergall E, et al, Blood, 2014

ADCC mediated by NK cells might be able to kill CML stem cells
Dasatinib inhibits the majority of imatinib-resistant BCR-ABL mutations, but not T315I mutation, tested in vitro.
46 y.o. male; CML-CP+ myeloid sarcoma (rt.hip)

MRI

Ph chr. in BM
G-banding

Dasatinib 140mg/day

Major bcr- abl mRNA(IS)

Copy / assay

Major bcr- abl mRNA(TMA)

FISH

Dasatinib 100mg/day

MR3.0 (MMR)

Loss of CCyR

Ineligible for the Ponatinib clinical trial

Registration to JMDP

MSD; N/A 6/6 MUD; N/A
Tactics of immunotherapy for CML

- **Induction or Adoptive transfer of High quality effector cell**

- **Removal of immuno-regulatory elements**

**Sources:**
- Science 2013
- Nature 2014
- Trends in Immunology 2016
- Blood 2015;125:4017
Tactics of immunotherapy for CML-2

**CML specific Ag**
- p210 BCR-ABL
- BCR-ABL1 derived antigens

**CML related Ags**
- PGPs; PR3 (PR1), NE (PR1), Cath-G (CG-1)
- WT1, AURKA

**Overexpressed protein**
- CD123

**CML cell**
- Driver mutation = *BCR-ABL1*

**CML stem cell**

**CML cell**
- HLA^1
- HLA^2
- A gene
- B gene

**CML related Ags**
- HLA^1
- HLA^2

**Anti-CML-CTL/HTL**
- Treg.

**Anti-CML-CAR-T**
- MDSC

**Drawn by author**
Primary Granular Proteins as therapeutic targets of cellular immunotherapy for CM-CP: lineage specific Ags

Myeloblast
No cytoplasmic granules

Promyelocyte
First azurophilic granules being secreted in Golgi apparatus

Myelocyte
Moderate number of azurophilic granules and initial production of specific granules in Golgi zone

Metamyelocyte
Abundant specific granules and dispersed azurophilic granules; Golgi apparatus reduced

Copyright © The McGraw-Hill Companies, Inc. All rights reserved.
CTLs specific for PR1 peptide which derived from both PR3 and NE display tumoricidal activity against CML cells (CML-BM-CD34+ cells).

Molldrem J.J. et al., Nat Med, 2000

Fujiwara H. et al., Blood, 2004
Generation of PGPs-specific T cells

HL60 → RNAs to cDNAs / PR3, NE, CG → electroporation → pcDNA3/ PGP

Target gene

PGPs producing CD40L-B

GFP, IE1-pp65
(as control)

Artificial APC

Fujiwara H et al, Clin Cancer Res, 2005
Successful generation of PGPs-specific T cells derived from CML patient’s CD3+T cells at day 90 after allo-HSCT using PCP gene-modified autologous CD40LBs

- CML-CP (case# 5)-

Fujiwara H et al, Clin Cancer Res, 2005
Up-dated strategy to target PGPs

Immunogenic determinant epitope derived from AURKA


KPEN motif  D-box activating motif  D-box (PxxRxxL)

H₂N   Kinase domain   COOH

1  51  132  383  402

191 207 215 230

PNILRLGYF HDATRV YLILEYAPL GTVYR ELQKLSKFDE

Relative expression of AURKA mRNA

Leukemia cells

- ALL BMMCs (n=7)
- AML BMMCs (n=17)
- CML BMMCs (n=10)
- Normal PBMCs (n=4)
- PHA blasts (n=3)

%Specific lysis

0  20  40  60

0  10  20  30  40  50  60
Development of AURKA-specific siTCR-T cells

**Cancer Cell**

- HLA class I
- Immunogenic epitope

**TCR gene-modified (CD8+) T cells**

**Endogenous TCR**

**Introduce Therapeutic TCR**

**AURKA-siTCR retroviral vector**

Targeting leukemia stem cells by AURKA-specific CTLs

1. e.g. CML stem cells subset overexpressing AURKA mRNA


2. e.g. Side population (SP) of GANMO-1

**PD-1 Pathway Overview**

<table>
<thead>
<tr>
<th>Type</th>
<th>PD-1</th>
</tr>
</thead>
</table>
| Type         | Co-inhibitory receptor  
|              | Can be induced through T-cell activation¹                           |
| Ligands      | PD-L1 and PD-L2²                                                     |
| Expression   | Receptor expression: On a range of immune cells, including activated  
|              | T cells³                                                            |
|              | Ligand expression: On tumors and immune cells and low levels in normal  
|              | tissues³                                                           |
|              | Amplification of 9p24 results in increased ligand expression⁴        |
| Activation   | Through interaction of tumor and T cell at tumor site³              |
| Blockade     | Blockade results in target cell killing and inhibition of Akt signaling and PI3K activity³,⁵ |

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APC, antigen-presenting cell; Akt, protein kinase B; PD-1, programmed death-1; PD-L1, PD ligand-1; PD-L2, PD ligand-2; PI3K, phosphoinositide 3-kinase.
PD-1 is up-regulated on CD8+ T cells of CML patients in the chronic phase of the disease.


©2009 by American Society of Hematology
PD-L1 expression in human leukemia cells

**Via IFN-\(\gamma\) stimulation**

MOLM-13 (AML-M5a)

DMSO

CD274-PE (PD-L1)

MFI: 360 vs. 399

DMSO + IFN-\(\gamma\)

MFI: 393 vs. 1641

**Constitutive expression**

MOLM-13 (AML-M5a)

DMSO

CD274-PE (PD-L1)

MFI: 360 vs. 4689

DMSO + IFN-\(\gamma\)

MFI: 393 vs. 5250

Maruta M, Fujiwara H, Unpublished data
Combined therapy using inhibitor of PD-1/PD-L1 binding and adoptive transfer of CML-specific CTLs. 

- Murine model -

**PD-L1 expression by CML-SCs induced by CTL-IFN-γ**

(a)

**Increasing PD-1 expression on CTL**

(b)

Combined inhibition of PD-1/PD-L1 binding by mAb or siRNA improved the survival of CML mouse. During this process, CML-CSs were preferentially suppressed.

(c)

(d)

Riether C et al. Leukemia, 2015
To generate the long-lasting anti-leukemia memory immunity in patients

- Overt leukemia
- CR/MRD
- Relapse/ Progression

**LSC targeting Molecular target Tx.**

**LSC targeting Vaccine (Th1 helper) Tx.**

**Immune Checkpoint Inhibition**

**TCR-T /CAR cells targeting LCS**

Robust oligoclonal TCR/CAR-T cells bearing different targets

Minimum required chemo-radio Tx

Functional Cure

Cure

LSC: non-LSC

LSC: LSC

Fujiwara H. IJH, 2014, modified
In the era of TKIs, the prognosis of pts with CML-CP has beautifully improved. Studies of clinical responses and underlying mechanisms again emphasized the importance of host immune responses.

Immunological strategies would be beneficial not only for the enhancement of efficacy of TKI therapy, e.g., maintenance after withdrawal of TKIs via suppression of CML-SCs, but also for the breakthrough strategy of TKI-resistant and aggressive CML, particularly for pts ineligible for allo-HSCT.

Furthermore, immunotherapy could be applied for disease relapse after allo-HSCT.

Accumulated knowledge regarding anti-CML immunity during TKI treatment will have to provide a scientific rationale not only for the treatment of leukemias, but also for solid tumors.
Acknowledgement

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