Adaptive capabilities of the PI3K/Akt/mTOR axis in Acute Myeloid Leukemia revealed by pathway selective inhibition and phosphoproteome analysis

University of Modena and Reggio Emilia

Cell Signaling Unit, ChiMOMo
Sandra Marmiroli
Jessika Bertacchini
Marianna Guida
Laura Mediani

Hematology Department
Mario Luppi
Patrizia Barozzi

University of Padova

Oncohematology Laboratory
Giuseppe Basso
Benedetta Accordi

George Mason University
Manassass Virginia USA

Center for Applied Proteomics and Molecular Medicine
Lance Liotta
Emmanuel Petricoin
84 samples with >80% blast cells analyzed by RPPA, then validated by western blotting.

Fresh peripheral blood and bone marrow specimens with newly diagnosed AML.

Patients are diagnosed according to blast content, FAB classification and cytogenetic analysis.
Overlapping profiles of peripheral blood (PB) or bone marrow (BM) derived blast cells from the same patients

18 blood and bone marrow specimens from the same patient were available.

Overlapping profiles of fresh and cryopreserved blast samples from the same patient

Samples derived from the same patient before and after cryopreservation in 10% DMSO were analyzed, with similar results.
Sypro Ruby staining for protein quantification

Reverse Phase Protein Array

Automated sample printing on nitrocellulose-coated glass

Statistical analysis

Detection and quantification of specific endpoints
Through RPPA technology 90 endpoints were analyzed, involved in different signaling pathways: survival, apoptosis, oxidative-stress and metabolism. Areas with pathways hyper-activated in limited clusters of patients are highlighted.
Correlation of protein profiles with FAB groups:

- p-Akt, p-PKA, p-PKC, p-mTOR, p-GSK, p-p70S6K, p-p90RSK and p-p38 cluster preferentially in M4/M5 FAB subtype;
- pro-apoptotic proteins cluster preferentially in M1/M2 FAB subtype.
C-Kit receptor expression correlates with hyperphosphorylation of the PI3K/Akt pathway.
Overnight treatment with Akt inhibitors leads to sustained phosphorylation of Akt in 70% primary blast samples
Overnight treatment with Akt inhibitors leads to sustained phosphorylation both of Akt and its direct targets.
The Akt inhibitor paradox:

- sustained apoptosis
- sustained Akt activation
pAkt S473 short time inhibition might account for apoptosis

Fold change upon treatment

LY294002  TCN  Perifosine  Akti VIII
Fold change upon treatment

- **Perifosine**
  - hr 2: -1.2
  - hr 4: -1.1
  - hr 20: 2.0

- **Akti VIII**
  - hr 2: 3.1
  - hr 4: 3.0
  - hr 20: 3.2
Is the mTOR kinase responsible for sustained Akt phosphorylation?

Perifosine, AktiVIII, TCN, LY249002

Rapamycin

Torin 1
**mTORC1 mTORC2 inhibition, 20 h**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fold Change Upon Treatment</th>
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<tbody>
<tr>
<td>Akt T308</td>
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<tr>
<td>Akt S473</td>
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<tr>
<td>PRAS40 T246</td>
<td></td>
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<tr>
<td>GSK3 S21/9</td>
<td></td>
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<tr>
<td>P70 S6K T389</td>
<td></td>
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<tr>
<td>4EBP1 S65</td>
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<tr>
<td>IRS-1</td>
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- **Rapamycin**
- **Torin1**
Phosphorylation profile of receptor tyrosine kinases in human primary blast cells
fold change upon treatment

-5 -4 -3 -2 -1 0 1 2 3 4

pAkt S473

- VIII
- TORIN 1
- VIII + TORIN
- SUNITINIB
- SUNITINIB + VIII
- QUIZARTINIB
- QUIZARTINIB + VIII
- LINSITINIB
- LINSITINIB + VIII
Activation of PI3K/Akt pathway in AML is modulated by feedback.

Akt inhibitors activate Akt, possibly through stabilization of IRS-1 and FOXO, and increased IR or other RTKs expression/activation.

Akt inhibition may trigger signaling via mTORC1.

RTKs inhibition prevents re-induction of Akt and sensitizes tumor cells to inhibitors of mTOR.
The results highlight the limitations of these drugs if used as monotherapy, and suggest that combined treatment with Akt inhibitors and RTKs inhibitors is much more potent that either alone.

Our data support the development of targeted treatment paradigms for PI3K/Akt/mTOR-altered adult leukemias and also demonstrate that therapies must be tailored to the specific RTKs and phosphorylome context.

All together, this study demonstrates that RPPA analysis is a very valuable tool to profile patients and help the clinician to the therapy.