Inhibitors of Methionine Aminopeptidase-2 in the Treatment of Non-Hodgkin’s Lymphoma

Targeted Cancer Therapies

William Westlin, Ph.D.
Vice President, Preclinical Research
Discovery of Fumagillin and MetAP-2

- Anti-angiogenic activity of fumagillin discovered by D. Ingber (Folkman group)
  - Natural product from Aspergillus fumigatus fresenius

- Methionine aminopeptidase type 2 (MetAP-2) identified as the molecular target of this class of compounds in late 1990s
Fumagillin Class Selectively Bind and Inhibit the Function of MetAP-2

- Mammalian cells express two related MetAP enzymes
  - MetAP-1 & MetAP-2

- Responsible for removal of N-terminal methionine residue from newly synthesized polypeptide chains

- Fumagillin class of molecules have been shown to covalently bind MetAP-2 enzyme

- Binding of MetAP-2 results in anti-proliferative properties
  - Cell cycle arrest in late G1 phase

Liu, et. al., 1998
Fumagillin Class of MetAP-2 Inhibitors

- TNP-470, a semi-synthetic derivative of fumagillin, entered clinical trials in early 1990s
  - Potent inhibitor of endothelial cell proliferation
  - Potent inhibitor of blood vessel growth
  - Active in preclinical tumor models
TNP-470 Clinical Experience

+ Promising initial clinical activity in solid tumors alone or in combination with cytotoxic agents

- Poor pharmacokinetics, short half-life with high patient to patient variability, intravenous

- CNS effects seen at doses above 60 mg/m$^2$ in Phase I studies - reversible
TNP-470 Clinical PK and Safety

- Poor pharmacokinetic properties
  - $T_{1/2}$ 7-8 minutes in clinical trials
  - Metabolic stability <5 minutes

- Dose limiting toxicity was neurological and characterized by:
  - Cerebellar symptoms consisting of:
    - ataxia
    - nystagmus
    - vision abnormalities
    - coordination difficulties
    - nervousness
    - dizziness
    - gait-disturbances
    - emotional lability
    - insomnia
    - vertigo

- Incidence of CNS and visual function related adverse events increased dose-dependently

- Grade 3/4 neurological adverse events occurred after 3-10 weeks of therapy
  - Every case reversible
PPI-2458 is a Methionine Aminopeptidase-2 Inhibitor of the Fumagillin Class

- PPI-2458 was designed to overcome the clinical limitations of TNP-470
- PPI-2458 is structurally related to fumagillin and TNP-470
- PPI-2458 is an irreversible inhibitor of MetAP-2
  - Inhibits angiogenesis and proliferation of sensitive cell types

**Fumagillin**
- Fungal Metabolite
- Anti-proliferative to EC

**TNP-470**
- Potency > fumagillin
- Dose limiting CNS Tox
- Poor PK profile

**PPI-2458**
- Equipotent to TNP-470
- Improved Stability
- Decreased CNS Tox
- Orally Active

- PPI-2458 is in Phase 1 Clinical Development in NHL & solid tumor
PPI-2458 Demonstrates Superior Metabolic Stability

<table>
<thead>
<tr>
<th></th>
<th>PPI-2458</th>
<th>TNP-470</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Plasma Stability, $t_{1/2}$</td>
<td>&gt; 500 min</td>
<td>&lt; 5 min</td>
</tr>
<tr>
<td>Liver Microsome Stability, $t_{1/2}$</td>
<td>12 min</td>
<td>&lt; 1 min</td>
</tr>
</tbody>
</table>

- PPI-2458 shows improved metabolic stability over TNP-470
- PPI-2458 is not an inhibitor or an inducer of P450 enzymes
PPI-2458 has greatly reduced clinical signs of neurological toxicity compared to TNP-470 in the rat.
PPI-2458 Inhibits Endothelial Cell Proliferation and Angiogenesis *In Vitro*

<table>
<thead>
<tr>
<th></th>
<th>PPI-2458</th>
<th>TNP-470</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetAP-2 Inhibition (IC$_{50}$)</td>
<td>1.9 nM</td>
<td>1.2 nM</td>
</tr>
<tr>
<td>BAEC Proliferation (GI$_{50}$)</td>
<td>0.05 nM</td>
<td>0.06 nM</td>
</tr>
<tr>
<td>HUVEC Proliferation (GI$_{50}$)</td>
<td>0.2 nM</td>
<td>1 nM</td>
</tr>
<tr>
<td>Rat Aortic Ring Assay (IC$_{50}$)</td>
<td>~ 1 nM</td>
<td>~ 10 nM</td>
</tr>
</tbody>
</table>

§ No significant direct cytotoxicity up to 100 µM
Inhibition of Human Endothelial Cell Proliferation Directly Correlates with Inhibition of MetAP-2

Primary Human Endothelial Cells (HUVEC)

% relative to vehicle control

- cell growth
- free MetAP-2

Concentration (nM)

Vehicle 0.001 0.01 0.1 1 10 100
Inhibition of Angiogenesis *In Vitro*

- Inhibition of new vessel sprouting in rat aortic rings stimulated with bFGF

![Bar chart showing inhibition of angiogenesis with bFGF](image)
Inhibition of NCI-60 Tumor Cell Line Panel by PPI-2458

Average GI$_{50}$ results from two 6-day exposure experiments per cell line

CHI Targeted Cancer Therapies, September 2005
Fumagillin class of molecules have been shown to covalently bind MetAP-2 enzyme.

Liu, et. al., 1998
Development of a Pharmacodynamic Assay

ELISA to detect free, uninhibited MetAP-2 enzyme

Sample contains mix of 2458-inhibited and uninhibited MetAP-2

Biotinylated-2458 interacts specifically with free, uninhibited MetAP-2

Incubate with biotinylated-2458 reagent
Effects of PPI-2458 on Monkey Spleen Histology

Histologic findings consisted of lymphoid depletion or an absence of germinal centers in lymph nodes and spleen of the 0.3, 1.0, and 3.0 mg/kg animals.

Lymphoid depletion of germinal centers were characterized by a reduction in size and a decrease in the density of lymphoblasts in the germinal center.
Clinical Rationale

High Expression of Methionine Aminopeptidase Type 2 in Germinal Center B Cells and Their Neoplastic Counterparts

Takayuki Kanno, Hideya Endo, Kengo Takeuchi, Yasuyuki Morishita, Masashi Fukayama, and Shigeo Mori

Division of Pathology (TK, HE, SM), Institute of Medical Science, and Department of Pathology (KT, YM, MF), Faculty of Medicine, University of Tokyo, Tokyo, Japan

SUMMARY: Methionine aminopeptidase type 2 (MetAP2) is a bifunctional protein that plays critical roles in the regulation of protein synthesis and post-translational processing by (a) protecting the alpha subunit of eukaryotic initiation factor 2 from inhibitory phosphorylation by eukaryotic initiation factor 2 kinases and (b) removing the amino-terminal methionine residue from nascent protein. MetAP2 is also known as the molecular target of the angiogenesis inhibitor TNP-470. In addition, it has been recently suggested that MetAP2 has an antiapoptotic function in mesothelioma. To know the pattern of expression of MetAP2 in normal and neoplastic tissues, we raised two specific rabbit polyclonal Abs and examined the pattern of MetAP2 expression in various normal and pathologic specimens. Unexpectedly, we found a very high and selective expression of MetAP2 in germinal center B cells. In the germinal center, dark zone B cells tended to express more MetAP2 than light zone B cells. When 200 malignant lymphomas of various subtypes were studied, a high level of MetAP2 expression, equivalent to that observed in germinal center B cells, was noted exclusively on B-cell lymphoma subtypes that are currently regarded as the neoplastic counterparts of germinal center B cells. The expression of MetAP2 in diffuse large B-cell lymphomas correlated well with that of BCL6 (p < 0.05) but not with that of either CD10 or BCL2. These data suggest that MetAP2 has specific function(s) in germinal center B cells and that the function is shared by neoplastic counterparts of germinal center B cells. (Lab Invest 2002, 82:893–901).
MetAP-2 is Highly Expressed in Malignant Cells Derived from Germinal Center B Cells

Table 2. Expression Levels of Methionine Aminopeptidase Type 2 Protein in Malignant Lymphomas

<table>
<thead>
<tr>
<th>Type of Lymphoma</th>
<th>++</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-cell neoplasms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precursor B-cell neoplasm: precursor B-lymphoblastic leukemia/lymphoma</td>
<td>26</td>
<td>67</td>
<td>57</td>
<td>150</td>
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<tr>
<td>Mature B-cell neoplasms</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Chronic lymphocytic leukemia/small lymphocytic lymphoma</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Mantle cell lymphoma</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>10</td>
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<tr>
<td>Follicular lymphoma</td>
<td>3</td>
<td>11</td>
<td>2</td>
<td>16</td>
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<tr>
<td>Mucosa-associated lymphoid tissue lymphoma</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Nodal marginal zone B-cell lymphoma</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Plasma cell lymphoma</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>20</td>
<td>51</td>
<td>29</td>
<td>100</td>
</tr>
<tr>
<td>Burkitt's lymphoma</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>T-cell and NK-cell neoplasms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precursor T-lymphoblastic leukemia/lymphoma</td>
<td>0</td>
<td>4</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Mature T-cell and NK-cell neoplasms</td>
<td>0</td>
<td>10</td>
<td>15</td>
<td>25</td>
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<tr>
<td>Mycosis fungoides</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Peripheral T-cell lymphomas, unspecified</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>8</td>
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<tr>
<td>Angioimmunoblastic T-cell lymphoma (AILD)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Adult T-cell leukemia/lymphoma (ATL/L)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Anaplastic large cell lymphoma (ALCL)</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
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<tr>
<td>Hodgkin's lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular lymphocyte predominant Hodgkin's lymphoma</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Classical Hodgkin's lymphoma</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Nodular sclerosis classical Hodgkin's lymphoma</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mixed cellularity classical Hodgkin's lymphoma</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>91</td>
<td>83</td>
<td>200</td>
</tr>
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Laboratory Invest, July 2002

CHI Targeted Cancer Therapies, September 2005
Germinal Center B-Cell Derived Tumor Lines Maintain Sensitivity to MetAP-2 Inhibition

<table>
<thead>
<tr>
<th>B Lymphoma Cell Line</th>
<th>Classification</th>
<th>Growth Inhibition by PPI-2458 Relative to Vehicle Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU-DHL-16</td>
<td>DLBCL</td>
<td>Maximum Inhibition 60% GI$_{50}$ 1.9 nM</td>
</tr>
<tr>
<td>DB</td>
<td>DLBCL</td>
<td>42% -</td>
</tr>
<tr>
<td>H2</td>
<td>FL</td>
<td>59% GI$_{50}$ 0.16 nM</td>
</tr>
<tr>
<td>D10</td>
<td>FL</td>
<td>59% GI$_{50}$ 0.42 nM</td>
</tr>
<tr>
<td>ST486</td>
<td>BL</td>
<td>53% GI$_{50}$ 0.22 nM</td>
</tr>
<tr>
<td>Ramos</td>
<td>BL</td>
<td>-</td>
</tr>
</tbody>
</table>

DLBCL – diffuse large B cell lymphoma, FL – follicular lymphoma, BL – Burkitt’s lymphoma

GI$_{50}$ = 1.9 nM
SR Human Lymphoma Cells Are Highly Sensitive to MetAP-2 Inhibition by PPI-2458 *In Vitro*
PPI-2458 is efficacious in human SR lymphoma xenograft *In Vivo*

![Graph showing tumor volume over time for different treatments](image_url)

- **Vehicle**
- **10mg/kg 2458**
- **100mg/kg 2458**

Initiated dosing

- *p < 0.05
- **p < 0.001
PPI-2458 Therapy is Synergistic with CHOP Chemotherapy in the Human SR Lymphoma Model

The diagram illustrates the percent survival over treatment days for different treatment regimens:

- Vehicle
- 2458 alone
- CHOP alone
- CHOP + 2458 d6
- CHOP + 2458 d-1
Rationale for PPI-2458 in Non-Hodgkin’s Lymphoma

- The molecular target of PPI-2458, MetAP-2, is expressed selectively at high levels in germinal center B cells and in the neoplastic counterpart of germinal center B-cells, diffuse large B cell lymphomas

- Treatment of rats and monkeys with PPI-2458 results in a reversible depletion of germinal centers in lymph node and spleen

- PPI-2458 is active *in vivo* in preclinical models of tumor growth

Therefore, MetAP-2 may play a role in the growth and survival of malignant B-cell lymphomas and PPI-2458 may be a useful therapeutic intervention in the treatment of specific B-cell malignancies
PPI-2458: Clinical Rationale

- Potential for Multiple Oncology Indications
  - Molecularly targeted therapy
  - Direct cytostatic antiproliferative activity combined with angiogenesis inhibition
  - Novel mechanism of action relative to available therapies
    - Potential for combination therapy
  - TNP-470 clinical experience provides positive support for this class of compounds
PPI-2458 Shows Activity in a Broad Range of *In Vivo* Tumor Models

**Xenograft**
- UACC-62 melanoma
- SR lymphoma
- MDA MB-435 breast cancer
- U251 glioblastoma
- HL-60 leukemia
- MV-522 lung
- LNCaP prostate
- DU-145 prostate
- MDA PCa2b prostate

**Murine Models**
- B16F10 melanoma
- L1210 leukemia
- M5076 sarcoma
- e-end-1 endothelioma
PPI-2458: Summary

- PPI-2458 is an orally available, selective MetAP-2 inhibitor

- A pharmacodynamic marker is available to guide the clinical program
  - Initial doses active on molecular target

- Strong preclinical, CMC and IP positions

- Potential across a broad range of proliferative diseases
  - Oncology clinical program ongoing
  - Nonclinical safety package supports early clinical development plan in both oncology and autoimmune disease
PPI-2458: A “Great Investigational Drug”

PPI-2458 — irreversibly inhibits metAP-2 enzyme

PPI-2458 is a novel, proprietary molecule that acts by irreversibly inhibiting the enzyme methionine aminopeptidase type 2. PPI-2458 is based on the fumagillin class of compounds, which has been shown to prevent abnormal cell growth and the formation of new blood vessels that contribute to the growth of aberrant tissues in diseases such as cancer and rheumatoid arthritis. Dose-limiting toxicity associated with certain fumagillin derivatives has largely prevented their clinical development. In preclinical, PPI-2458 has demonstrated the potent activity fumagillins while displaying an improved toxicity profile.