Disclosures for Gary Schiller, MD, FACP

Research Support / P.I.	Celgene, Millennium, Vion, Genzyme, Johnson and Johnson , Antisoma Pharmaceuticals, MGI Pharma
Employee	N/A
Consultant / Scientific Advisory Board	N/A
Major Stockholder	N/A
Speakers' Bureau	N/A

N/A = Not Applicable (no conflicts listed)

Presentation includes discussion of the following off-label use of a drug or medical device: N/A

The Impact of Novel Agents on our Treatment Paradigm

- Drug development informed by concepts of pathogenesis, remission induction, and post-remission therapy
- Clinical trials in which target populations are defined by patient or disease characteristics
- Defining therapies based on distinct disease biology

Mechanisms of Leukemia Ontogeny: Current Models

- Biologic Characteristics of Leukemia
- Biologic Characteristics of Myeloproliferation
- Biologic Characteristics of Maturation Arrest

Hematologic Diseases with Distinct Biologic Characteristics: Potential Targets for Novel Agents

- **Myeloproliferative Disorders**
  - CML, P. vera, AMM, ET
- **Myelodysplastic Disorders**
  - RA, Int-2, or Advanced MDS
- **Clonal Disorders of Hematopoiesis distinct from the above**
  - CMML, PNH, Aplastic Anemia

Molecular Pathogenesis

(Fröhling et al. *JCO* 26: 6285-6295; 2005)

- **Mutational "classes"**
  - Signal transduction activators
    - Associated with myeloproliferation
      - » *RAS*, *KIT,* and *FLT3*, loss of function of NF1, and activating PTPN11
  - Transcription factor or coactivation complexes
    - Associated with blocking differentiation
      - » Core-binding factor complex, RARα, MLL, and transcriptional co-activation complexes CBP, MOZ, TIF2

Mutations in Transcriptional Activators

(Fröhling et al. *JCO* 22:624; 2004 and *NEJM* 351:2370;2004)

- **CBF transcription complexes**
  - CCAAT enhancer binding protein α
    - Upregulation initiates transition to CFU-GM and induces granulocyte development
    - Multiple types of mutations may occur- *TAD1* and *2*; bZIP DNA binding; nonsense, missense mutations upregulating other forms, or decreasing binding

- ***RUNX1* mutations**
  - Familial platelet disorder with propensity to AML

Conventional Cytogenetics and Molecular Pathology Inform Management

- **t(8;21)**
  - RUNX1-CBFα2T1 fusion product
  - Product enhances histone deacetylase with repression of transcription
- **inv (16)**
  - CBFβ-MYH11 fusion product
  - Product enhances histone deacetylase with repression of transcription

The Impact of *flt*3 Mutation Status

- Cytogenetically normal AML characterized by *flt*3 ITD and TKD
  - High relapse rate
  - Offers a therapeutic target
    - Midostaurin
    - Sunitinib (Fiedler et al. Blood116; 1346a; 2010)
    - Aurora kindas inhibitor CCT137690 (Moore et al. Blood 116: 1347a; 2010)

A Phase IIB Trial of Oral midostaurin (PKC412), the FMS-Like Tyrosine Kinase 3 Receptor (FLT3) and Multi-Targeted Kinase Inhibitor, in Patients with Acute Myeloid Leukemia nd High-Risk Myelodysplastic Syndrome with Either Wild-Type or Mutated FLT3

- Complete remission is ahcieved in 70% of younger, and 40-50% older adults with newly diagnosed AML
- There is no specificity to standard therapy, and no convention regarding consolidation therapy
- Long-term disease-free survival is less than 40% in younger, and less than 10% in older adults with AML in CR1
- Mutations of *flt3* are present in 30% of AML cases leading to constitutive activation of the transmembrane protein

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- *Flt3* is an excellent target for the development of a small molcule inhibitor
- Such inhibitors show *in vitro* activity again transformed cell linesand in mice with a mutated MPD
- Phase II trial of midostaurin in humans with AML characterized by mutated *flt3* had reduction in blast counts of the blood and/or marrow by the single agent
- Twice-daily dosing would produce sufficient plasma concentrations to inhibit a target mutant *flt3*

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- The trial was conceived as an open-label, randomized, phase II study of midostaurin monotherapy
- Random assignment to 50mg or 100mg orally twice daily
- Eligibility
  - AML reapsed/refarctory, ineligible for standard chemotherapy
  - MDS
  - Adults, WHO 0-2, life expectancy of at least 3 months
  - Treatment with hu up to 7 days prior to starting therapy
  - Renal, hepatic, GI function adequate with no prior transplant within 2 months

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- *Flt3* status assessed prior to starting therapy with wt *flt3* assigned doses of 50-100 mg twice daily, and mutant *flt3* assigned 50-100 mg twice daily
- Patients were enrolled in cohorts of 10 followed by two cohorts of five to a maximum of 20 pts
- Midostaurin continued until disease progression of unacceptable toxicity
- Toxicity could lead to dose reduction to 50mg twice daily. Toxicity at lower ose that did not resolve led to termination of study

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- N=95
- 85 pts with AML, 49 Men, 84 Caucasian, 61 age $\geq$ 65
- *Flt3* mutation identified in 35 pts, mostly ITDs
- 3 mutated and 23 unmutated had never been treated
- 3 pts with wild-type were not evaluated due to protocol violation, and two who failed to complete 8 days due to adverse events
- 92 patients analyzed for response

Phase IIB Trial of Oral Midostaurin (PKC412), the FMS-Like Tyrosine Kinase 3 Receptor (FLT3) and Multi-Targeted Kinase Inhibitor, in Patients With Acute Myeloid Leukemia and High-Risk Myelodysplastic Syndrome With Either Wild-Type or Mutated FLT3

- Table 1 shows patient characteristics in the mutant and wt groups
- Abnormal karyotype was present requently in both groups, although should have been expected more in the wt group
- Older age seemed to be more common among the wt group, and previous treatment more common in the mutant group

A Phase IIB Trial of Oral midostaurin (PKC412), the FMS-Like Tyrosine Kinase 3 Receptor (FLT3) and Multi-Targeted Kinase Inhibitor, in Patients with Acute Myeloid Leukemia nd High-Risk Myelodysplastic Syndrome with Either Wild-Type or Mutated FLT3

- Table 2 shows the Clinical Response
- Both groups showed response, but only one patient had a PR. Blast reductions were seen frequently, and were more common in the *flt3* mutatant vs. the wt group (71% vs. 42%)
- 50% reduction in blasts occurred at a median of 29 days in both groups
- Time to progression was 50 and 56 days, respectively
- Median survival was poor- 130 days

A Phase IIB Trial of Oral midostaurin (PKC412), the FMS-Like Tyrosine Kinase 3 Receptor (FLT3) and Multi-Targeted Kinase Inhibitor, in Patients with Acute Myeloid Leukemia nd High-Risk Myelodysplastic Syndrome with Either Wild-Type or Mutated FLT3

- Pharmacokinetics were collected from 45 and 42 pts in the 50-mg and 100-mg group. Concentrations accumulated in the first 3-5 days and then declined 40-80% reaching a new steady-state 2-3 weeks post-dose.
- These new concentrations were said to be above the MIC-50%
- Responses were limited to biologic responses suggesting that alternative pathways may promote survival even if *flte* signalling is blocked

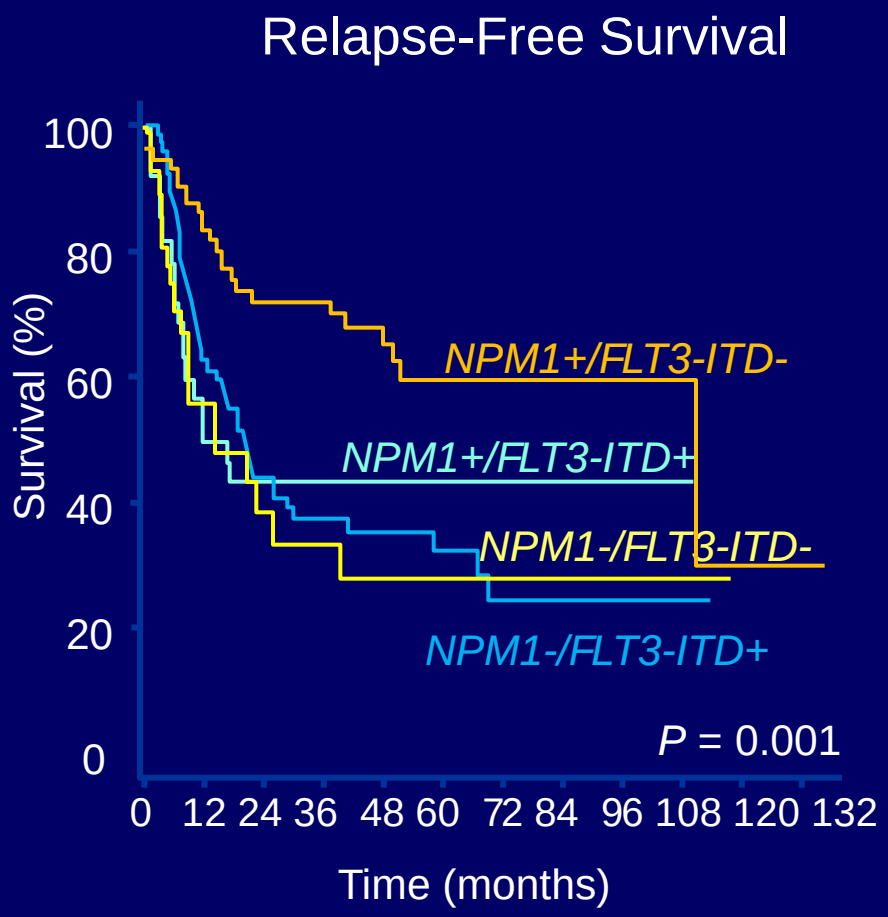
Isocitrate Dehydrogenase Gene Mutations in AML

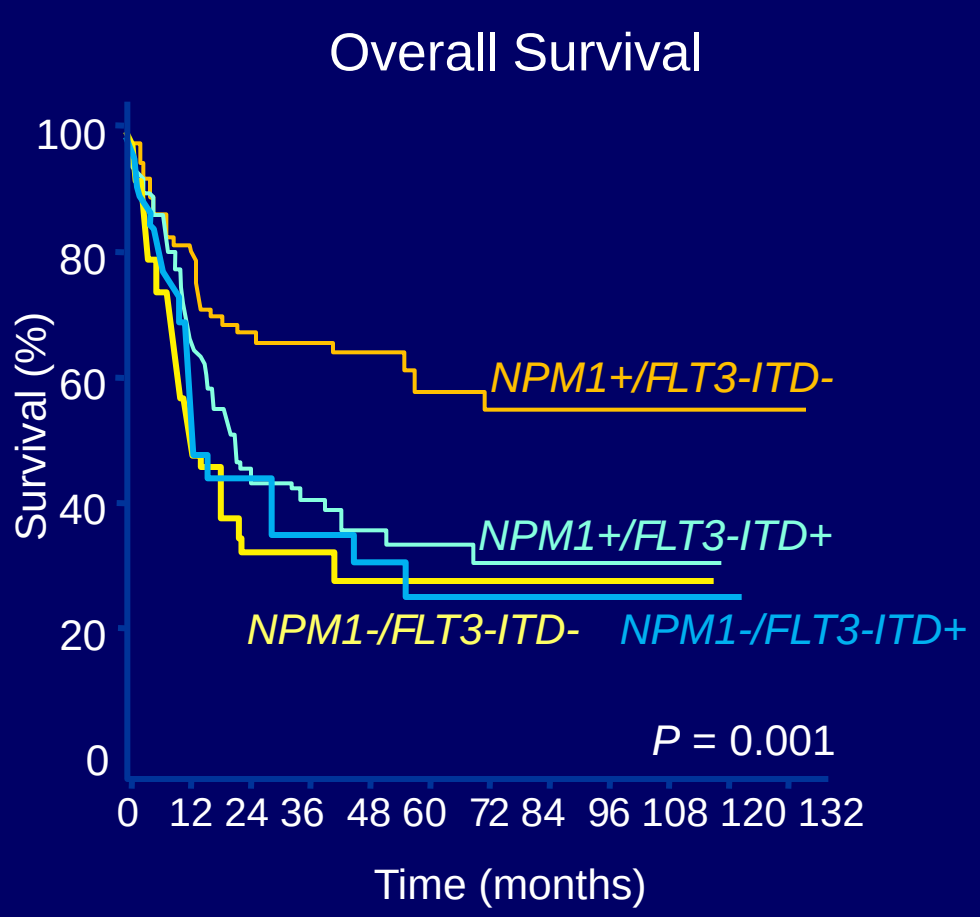
- In younger patients, IDH mutations in NK-AML (NPM1 mutated/*flt*3-ITD-negative) are associated with poor prognosis.
- N=732 AML age $\geq$ 60 treated on two AMSLG trials. N=163 IDH mutations found, and one case IDH2. Clinical association with higher plt count, higher blasts, and mutated NPM1.
- After median follow-up of 3.56 years, no impact on induction outcome or RFS noted, but in NPM1-mutated, IDH1 mutations appeared to have inferior OS (Paschka, et al. Blood 116: abs 101; 2010)

Hazards in Evaluating New Therapies

- **Patient accrual**
  - Heterogeneous disease biology
- **Patient selection bias**
  - Heterogenous clinical characteristics
- **Randomization**
  - Seldom done in current Phase II trials
- **Stratification**

Mutant NPM1 Predicts Favorable Prognosis in Younger Adults With AML and Normal Cytogenetic





Döhner K, et al. *Blood.* 2005;106:3740-3746.

New Approaches in AML Potentially Applicable to the Post-Remission Setting

- New chemotherapeutic drugs
- Modulation of drug resistance
- Sensitization
- Antiangiogenesis
- Modulation of Cell Signaling
- Immunotherapeutic

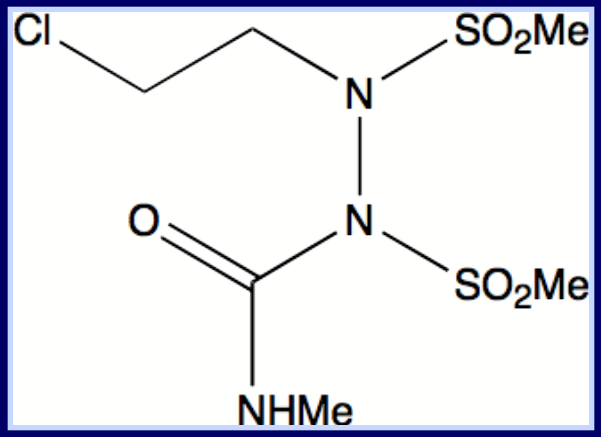
Acute Promyelocytic Leukemia

- **A unique leukemia subtype**
  - Distinct clinical features
    - Disseminated intravascular clothing
  - Distinct histological and cytogenetic features
    - Azurophilic granules, Auer rods, t(15;17(or t(11;17)
  - Distinct molecular features
    - *PML-RARα*
- **Distinct response to distinct therapies**
  - All-trans retinoic acid- intermittent conventional preparations vs. liposomal
  - Arsenic trioxide
  - Anthracycline-based chemotherapy
  - Elimination of cytarabine may contribute to relapse (Ades et al. Blood 116:11a;2010)

New Chemotherapeutic Drugs: Potential for Post-Remission Therapy

- **Purine Nucleoside Drugs**
  - Clofarabine (Becker et al. Blood 116:466a; 2010) BCLAC may represent an improvement over Fludarabine FLAG
- **Alkylating agents**
  - Laromustine (Schiller, et al. *J Clin Oncol*. 28:815; 2010)
- **mTOR Inhibitors** (Amadori et al. Blood 116:225a; 2010) Temsirolimus and Clofarabine in older patients
- **Antimetabolites**
  - Amonafide (Mohan et al. Blood 116: 883a; 2010) in combination with cytarabine for secondary AML
- **Differentiating Agents, Epigenetic Modifiers** (Cashen AF, et al. *J Clin Oncol.* 28:556; 2010)

Laromustine



- Sulfonylhydrazine alkylating agent
- Prodrug activated to
  - 90CE
    - Generates cationic chloroethylating species that preferentially targets the O^6 position of guanine
  - Methyl isocyanate
    - Carbamoylating species
    - Inhibits alkylguanine alkyltransferase (AGT), a DNA repair enzyme
- Active in L1210 cell lines (BCNU, cyclophosphamide, and melphalan resistant)
- Crosses blood-brain barrier
- Induces remission as a single agent in Phase I and II leukemia studies

Laromustine

- **CLI-043: Phase II study of laromustine in elderly patients with de novo poor-risk AML in 85 evaluable pts with CR/CRp of 32%**
  - Open-label Phase II design
  - Induction with VNP40101M (laromustine)- a Sulfonylhydrazine alkylating prodrug: 600 mg/m2 IV over 60 min.
  - Previously untreated de novo AML by WHO criteria
  - Intermediate or unfavorable cytogenetics
  - ≥60 years old with at least one additional risk factor
    - Unfavorable cytogenetics (47% of pts)
    - ECOG PS = 2 (41%)
    - Age ≥70 (78%)
    - Cardiac or pulmonary or hepatic dysfunction (73%, 3%, 77%)

Schiller G, et al. *J Clin Oncol.* 28:815; 2010.

Laromustine in Elderly with Previously Untreated Poor-Risk AML

- Table 2 illustrates baseline characteristics of patients treated on study
- Table 3 tries to define the severity of illness based on a Hematopoietic Cell Transplantation Comorbidity Index
  - Both try to define a population not suited for conventional therapy. 76% pulmonary dysfunction, 73% cardiac dysfunction (among them, 52% had multiple risks); only 3 (4%) had only one single poor-risk factor.

Laromustine in Elderly with Previously Untreated Poor-Risk AML

- Table 4 looks at adverse events, and attempts to define toxicity signals
  - Distinct signals in pulmonary toxicities
  - Findings included pleural effusion, dyspnea or hypoxia
  - Whether this occurred in the setting of prior pulmonary disease is not stated
  - Response by risk group about the same

Laromustine Induction
CLI-043 Efficacy, Early Death, and Survival

No. Risk Factors	N	CR + CRp	Overall Response Rate	Deaths within 30 days (%)
1-2	21	8	38%	1 (5%)
≥ 3	64	19	30%	11 (17%)
Total	85	27	32%	12 (14%)

- 47% of responders alive at 12 months
- 18 of 27 patients received at least one cycle of cytarabine consolidation
- Median (range) of overall survival for responders is 8.9 months (1.7-16.4)
- __of 27 responding patients alive and continue in follow-up
  - Median (range) of follow up: 17.9 months (11.5 -26.2)

Schiller G, et al. *J Clin Oncol.* 28:815; 2010.

Molecular Therapies in AML- Nonmyelosuppressive Therapies

- **RAS farnesyl transferase inhibitors**
- **Histone deacetylase inhibitors (phenylbutyrate, etc)**
- **DNA-hypomethylation agents**
- **Promoters of apoptosis (BCL-2 antisense)**
- **Tyrosine kinase inhibitors**
- **HSP90 inhibitors (geldanamycin, etc.)** (Can be inhibited by pandeacetylase inhibitors such as Panabinostat- Uy et al. Blood 116:464a; 2010)

Multicenter, Phase II Study of Decitabine for the First-Line Treatment of Older Patients with Acute Myeloid Leukemia

- Methylation of cytosine in CpG dinucleotides bye DNA methyltransferase leads to trascriptional silencing of genes. This may be a mechanism for loss to tumor suppressor gene expression

- Decitabine is incorporated in DNA during S phase and irreversibly inhibits DNA methyltransferase leading to loss of methylation and Reactivation of silenced genes. It is also cytotoxic at high doses

Multicenter, Phase II Study of Decitabine for the First-Line Treatment of Older Patients with Acute Myeloid Leukemia

- FDA approved dose is 15 mg/sq.m every 8 hours for three days
- In the MDS trial, 18% of pts had criteria for AML and some responded
- Eligibility determined by age >60, previously untreated, with no mention of comorbid condition limiting access to conventional therpy
- Exclusion for active infection, CNS leukemia, good-risk cytogenetic AML, APL

Multicenter, Phase II Study of Decitabine for the First-Line Treatment of Older Patients with Acute Myeloid Leukemia

- Open-label, single-arm, tricenter Phase II trial of a novel dose and schedule
- Hydroxyurea was allowed for those with blasts > 30,000/mcl
- Treatment could be delayed at the discretion of the investigator with repeat cycles every four weeks
- Treatment stopped for progression, intercurrent illness preventing therapy, pt withdrawal, other
- Primary endpoint was morphologic CR. Sample size statistical considerations listed on pp557 of manuscript

Multicenter, Phase II Study of Decitabine for the First-Line Treatment of Older Patients with Acute Myeloid Leukemia

- N=55 age 61-87 (median 74)
- 42% AML secndary to MDS or prior therapy, but only one was treated
- Median baseline BM blasts were 50% but 12 pt had blasts of 20-30%
- Table 2 lists baseline cytogenetics- 45% with adverse features, and median presenting wbc 2.7 (1-111)

Multicenter, Phase II Study of Decitabine for the First-Line Treatment of Older Patients with Acute Myeloid Leukemia

- Table 3 illustrates response to treatment by intent-to-treat groups; median of 3 cycles given (1-25), and 64%pts received >3 cycles
- Highest response rate in those with transformed or secondary AML??
- No response in those with >10,000 blasts at presentation
- Best response in those with <1000 blasts at presentation

Multicenter, Phase II Study of Decitabine for the First-Line Treatment of Older Patients with Acute Myeloid Leukemia

- Independent response assessment 25% (13.2-37%) morphologic CR and one with Cri
- 5 of 34 w/ cytogenetic abnormality achieved cytogenetic CR
- Median time to achieve CR 126 days (48-238d)
- Of n=14 in CR, six relapsed
- Another 16 pt had stable blasts in bone marrow

Multicenter, Phase II Study of Decitabine for the First-Line Treatment of Older Patients with Acute Myeloid Leukemia

- Median survival 7.7 m (5.7-11.6m)
- Median EFS 5.8 m (3d to 23.6m)
- Fig. 2 shows survival curve and Table 5 the adverse events (grades 3 or higher)
- Dyspnea occurred in 18% of patients, and pneumonia in 11%
- Dose delays in 13 pts (24%)

Impact of Novel Chemotherapies on the Standard Treatment Paradigm

- **Acute Myelogenous Leukemia**
  - Isolate molecular and cytogenetic features as a guide to therapy, on or off protocol
  - Isolate clinical features for decisions regarding myelosuppressive therapies vs non-intensive maintenance with arsenic trioxide, retinoids, epigenetic modifiers, low-dose cytarabine, or monoclonal antibodies with consideration of incorporating new agents in consolidation and maintenance

Novel Therapeutic Approaches applied to Post-Remission Therapy

- Identification of new pathways may be useful for identifying and screening new treatments and understanding biology of leukemia
- Clinical trials may enroll based on molecular features of disease
- Combination therapies are likely to continue and where new agents show promise in induction, studying them in the postremission setting may prove useful