In 2016, approximately 20,000 new cases of AML and 10,000 disease-related deaths occurred in the United States alone. Survival for the last 5 years in US 26.6%

The median age at diagnosis is 67 years and the incidence of the disease increases with age.

Molecular heterogeneity in AML is clinically relevant, has been used for disease classification and treatment selection and is associated with outcome.

Despite molecular risk-based approaches and molecular targeting therapies, only approx. 40% of younger (<60 year/old) and 10% of older (>60 years) achieved cure when treated with currently available chemotherapies.

Allogeneic HCT may improve cure rate, but the long-term success may be somewhat limited by transplant-related toxicity and death.

New and more effective approaches are highly needed.

Current Approaches to AML is based on prognostic and predictive factors:

**Diagnostic work-up for AML**

- **Morphology**
- **Cytogenetics**
- **Immunophenotype**

**Molecular characterization**

Next Generation Sequencing

**2017 ELN risk stratification by genetics**

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Genetic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>RUNX1-RUNX1T1</td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13.1q22)</td>
</tr>
<tr>
<td></td>
<td>or t(16;16)(p13.1q22); CBFB-MYH11</td>
</tr>
<tr>
<td>Mutated NPM1 without FLT3-ITD or with FLT3-ITD&lt;sup&gt;TM&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Mutated NPM1 and FLT3-ITD&lt;sup&gt;TM&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

**Intermediate**

Mutated NPM1 and FLT3-ITD<sup>TM</sup>

Wild-type NPM1 without FLT3-ITD or with FLT3-ITD<sup>TM</sup> (without adverse-risk genetic lesions)

- t(9;11)(p21;q23.3); MLT3-KMT2A

Cytogenetic abnormalities not classified as favorable or adverse

**Adverse**

- t(6;9)(p23;q44.1); DEK-NUP214
- t(8;22)(p14.1;q11.2); BCR-ABL1
- inv(3)(q21.3p26.2) or t(3;3)(q21.3p26.2); GATA2,MECOM[511]
- t(5) or del(5q); t(-7); -17/18(17p)

Complex karyotype, monosomal karyotype

Wild-type NPM1 and FLT3-ITD<sup>TM</sup>

Mutated RUNX1

Mutated ASXL1

Mutated TP53

**Mutations’ Themes in AML**


Molecular classes of AML and concurrent gene mutations in adult patients up to the age of ∼65 years.


Principle of AML Treatment for Younger Patients

Diagnosis

Remission induction

Molecular Targeting Therapeutics

Remission consolidation

Favorable

High-dose Ara-C

Relapse

No CR

Salvage chemotherapy

Principle of AML Treatment for Older Patients

Diagnosis

Molecular Targeting Therapeutics

Remission consolidation

Relapse

No CR

Clinical Trials

Best Supportive Care

Precision Medicine (PM) applied to AML

- Diversified outcomes despite similar “phenotypes”
- PM is the opposite of one-size-fits-all therapy
- PM is an approach for AML treatment that takes into account genetic and epigenetic aberrations and gene and non-coding RNA expression levels in individual patients to select the most appropriate treatment
- Easy tumor sequential sampling
- Characterized mechanisms of normal and malignant hematopoiesis
- Several emerging new drugs
Four new drugs were approved for AML in 2017

- **Vyxeos (CPX-351)** - liposomal cytarabine and daunorubicin at a fixed 5:1 molar ratio
- **Gemtuzumab ozogamicin (Mylotarg)** - humanized antibody against CD33 and conjugated to calicheamicin
- **Midostaurin** - a multi-kinase inhibitory activity against PDGFR, CDK1, KIT and VEGF
- **Enasidenib (AG221)** - IDH2 inhibitor

Promising new drugs expected to receive approval soon

- **Ivosidenib (AG120)** - IDH1 inhibitor; (FDA) has granted orphan drug and fast track designations
- **Venetoclax** - a BCL-2 inhibitor

**Phase III randomized trial of CPX-351 versus 7+3 in older patients with high risk (secondary) AML.**

- Patients (n=309) aged 60-75 years with untreated AML & a history of prior cytotoxic treatment, antecedent MDS or CMML, or AML with WHO-defined MDS-related cytogenetic abnormalities
- CPX-351 (100 units/m², days 1, 3, 5) vs 7+3
- 153 patients on to CPX-351 and 156 on 7+3
- minimum follow-up of 13.7 months
- CPX-351 had superior CR+CRi response (47.7% vs. 33.3%; P=0.016) and OS (median OS 9.56 vs. 5.95 months; P=0.005)
- 60-day mortality favored CPX-351 (13.7% vs. 21.2%)
- Longer time to neutrophil (36 vs 32 days) and platelet (37 vs 28 days)

(Lancet et al ASCO 2016)
Challenges/Opportunities related to Myelotarg

- Risk of VOD, especially among patients who receive HCT within 3 months
- ALFA-0701 study, GO was combined with DA, which differs from higher-dose cytarabine-based consolidation utilized in USA
- Specific subsets of AML express high levels of CD33: APL, NPM1
- Combinations with other molecular targeting therapeutics need to be explored

RATIFY Study

- 5-year overall survival (OS) from 44.3% to 51.4% (HR 0.78, p=0.009), compared to placebo
- benefit of midostaurin observed in patients with FLT3-ITD low (0.05–0.7) and high allelic ratio (>0.7), as well as in patients with FLT3-TKD
- Approval is restricted only to induction and consolidation therapy and not maintenance
- Companion diagnostic study LeukoStrat CDx FLT3 Mutation Assay (Invivoscribe Technologies, Inc.)

Ratify (CALGB 10603): Prospective Phase III, double-blinded randomized study of induction and consolidation +/- Midostaurin (PKC412) in newly diagnosed patients < 60 years old with FLT3 mutated AML

Not on STUDY: FLT3 WILD TYPE

Study drug is given on Days 8-21 after each course of chemotherapy, and Days 1-28 of each 28 day Maintenance cycle.

Overall Survival.

Challenges/Opportunities related to Midostaurin

- Benefit with maintenance probably will not be studied
- Post-transplant use need to be studied
- Combinations with other chemotherapeutics or molecular targeting therapeutics including other “TKIs”
- Combinations with hypomethylating agents

Other TKI investigated in AML

- Quizartinib
- Crenolatinib
- Gilteritinib

Rationale for Using TKI in CBF AML

- CBF AML: a favorable cytogenetic subset with t(8;21) [RUNX1/RUNX1T1] or inv(16)[CBFB/MYH11]
- KIT mutations, which result in aberrant tyrosine kinase activity and leukemia growth, are present in ~ 25-30% of CBF AML and associate with worse outcomes
- Higher expression of wild-type KIT is found in CBF AML and may associate with worse outcome
- Therefore, novel therapeutic approaches targeting aberrantly activated KIT are being tested

CALGB 10801
A Phase II Study of Induction (Daunorubicin/Cytarabine) and Consolidation (High-Dose Cytarabine) Chemotherapy Plus Dasatinib in Newly Diagnosed CBF AML

- Remission induction
  - Ara-C Daunorubicin + Dasatinib
  - X 1-2 courses
- Remission consolidation
  - High-dose Ara-C + Dasatinib
  - X 4 cycles
- Maintenance
  - Dasatinib
  - X 12 months

PRE-REGISTRATION
SCREENING
REGISTRATION
REGISTRATION
Clinical Outcomes

| N=59 |  
|------|---
| Median Follow-up | 23.8 months (range 1.8-41.7) |
| 30-day Survival Rate | 97% |
| CR Rate | 90% |
| 36-month DFS Rate | 75% (95%CI: 63-89) |
| 36-month OS Rate | 77% (95%CI: 66-89) |

IDH Mutations As a Target in AML

- IDH is a critical enzyme of the citric acid cycle
- IDH mutations occur in a spectrum of solid and hematologic tumors
  - IDH2 mutations: 9-13% of AML and 3-6% of MDS
  - IDH1 mutations: 6-10% of AML and 3% of MDS
- IDH1/2 mutations confer a gain-of-function
  - Increased DNA methylation
  - Impaired cellular differentiation

Normal and Aberrant IDH Activities
Clinical Impact of Distinct IDH Mutations in AML\textsuperscript{1,2}


Pharmacologic Inhibition of IDH mutations

Enasidenib (AG221): IDH2 inhibitor

- orally administered
- CR+CRi 26.6% in patients with refractory/relapsed disease
- Median duration of response was 5.6 months (8.8 months if CR achieved) and OS 9.3 months (19.7 months if CR achieved)

Challenges/Opportunities related to enasidenib

- Differentiation syndrome 14%
- Prolonged periods of time for a clinical response
- Preliminary results suggest a low likelihood of response when mutant NRAS is present
- Post-transplant use
- Combination with other chemotherapeutics or molecular targeting therapeutics including other "TKIs"
- Combination with hypomethylating agents
Targeting Anti-Apoptotic Mechanisms

Background: MOA of Venetoclax (ABT-199/GDC-0199)

Venetoclax

- Increased expression of the pro-survival protein BCL-2 relative to the pro-apoptotic protein BAX is associated with reduced CR rates, earlier relapse and inferior OS in patients receiving intensive chemotherapy for AML
- Venetoclax was only modestly effective as monotherapy in relapsed/refractory AML (19% CR/Cri)
- Phase II studies in elderly patients unfit for intensive chemotherapy have combined venetoclax with either HMAs or LDAC, producing CR/Cri rates of 54–68% and 12-month survival outcomes of 50–70%

Challenges/Opportunities related to venetoclax

- Prolonged neutropenia
- Competition with other “specific” molecular targeting therapeutics
- Best outcome in NPM1 mutant patients
- Combination with other BH3 inhibitors/mimetics
Hypomethylating agents in older patients with AML

OS in Patients With AML-MRC: AZA vs CCR\textsuperscript{1}

Decitabine vs Total TC in AML ≥65 Years\textsuperscript{2}


DNMT3A Mutations As Response Predictors for Hypomethylating Agents in AML

- 46 mostly older AML patients (median age, 74 years) treated with 20 mg/m\textsuperscript{2} decitabine on a 10-day schedule in phase I/II trials.
- CR rate of entire cohort: 41%
- DNMT3A mutations found in 17% of patients
- DNMT3A mutations were associated with a better response to decitabine (P=0.05)

Immunotherapeutics

- Monoclonal antibodies
  - “naked”
  - Chemo-conjugated
  - Bispecific
- CAR T-cells
- Immunocheckpoint inhibitors
**Targeting CD33**

- Antibody-drug conjugate SGN-CD33A
- AML bispecific AMG330
- CAR-T cells

**Clinical trials:**
- single agents
- with "7+3"
- with hypomethylating
- with alloHCT

**Vadastuximab Talirine (SGN-CD33A; 33A)**

**Proposed Mechanism of Action in Combination with HMA**

- Anti-CD33 antibody, engineered cysteines to enable uniform site-specific conjugation
- Cleavable dipeptide linker, highly stable in circulation
- Pyrrolobenzodiazepine (PBD) dimer, binds DNA with high intrinsic affinity

**Best Clinical Response**

<table>
<thead>
<tr>
<th>Efficacy Evaluable</th>
<th>Response Assessment per Investigator</th>
<th>Adverse Cyto. Risk per MRC</th>
<th>Underlying Myelodysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission Rate (CR + CRi)</td>
<td>All N=49</td>
<td>69%</td>
<td>83%</td>
</tr>
<tr>
<td>CR</td>
<td>39%</td>
<td>50%</td>
<td>32%</td>
</tr>
<tr>
<td>CRi (p)</td>
<td>16%</td>
<td>22%</td>
<td>27%</td>
</tr>
<tr>
<td>CRi (n)</td>
<td>14%</td>
<td>11%</td>
<td>9%</td>
</tr>
<tr>
<td>mLFS</td>
<td>4%</td>
<td>-</td>
<td>9%</td>
</tr>
<tr>
<td>PR</td>
<td>2%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ORR (CR + CRi + mLFS + PR)</td>
<td>76%</td>
<td>83%</td>
<td>77%</td>
</tr>
</tbody>
</table>

**33A+Standard Induction in Frontline Younger AML**

- Ability to combine active doses of 33A with 7+3
- Acceptable on-target myelosuppression observed
- Non-hematologic toxicity similar to 7+3 alone
- Low 30-day mortality rate
- Single-day dosing of 33A (Day 1) in combination with 7+3 continues enrollment
- A randomized phase 2 trial of 33A+7+3 versus 7+3 alone
- Because of the risk of VOD this program now is under scrutiny
**Targeting CD123**

- Expressed in LSC-enriched AML cell subpopulations more than normal HSC
- Targeted by monoclonal antibodies: SL-401, SGN-123
- CAR T-cells (@COH: Drs Forman and Budde)

### Trial Schema

<table>
<thead>
<tr>
<th>CDH/Agent</th>
<th>Patient Population</th>
<th>Agent</th>
<th>T cells Dose</th>
<th>Planned Enrollments</th>
</tr>
</thead>
<tbody>
<tr>
<td>13272/12/2015</td>
<td>Relapsed/Refractory AML</td>
<td>CD123R(EQ)28/EGFRt+</td>
<td>Bulk T cells</td>
<td>12.5, 50, 200 x 10^6 CAR+ cells</td>
</tr>
</tbody>
</table>

### AML Patient Characteristics and Response

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/sex</th>
<th>Lines of prior treatment</th>
<th>BM Blasts</th>
<th>Cytogenetic/Molecular</th>
<th>Lymph deplet.</th>
<th>CAR T cells</th>
<th>NonHema SAEs &amp; CRS</th>
<th>Response At day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>136</td>
<td>44/F</td>
<td>6; Y (MRD)</td>
<td></td>
<td>20%; -ve to dim</td>
<td>-7 iv(3)</td>
<td>Flu/Cy</td>
<td>SDM donor</td>
<td>None PD 40% blasts</td>
</tr>
<tr>
<td>138</td>
<td>54/F</td>
<td>4; Y (MRO)</td>
<td></td>
<td>18%; dim to mod</td>
<td>IDH1</td>
<td>Flu/Cy</td>
<td>SDM donor</td>
<td>Gr1 CRS Morphologic leukemic free state</td>
</tr>
<tr>
<td>167</td>
<td>43/F</td>
<td>4; Y (MRD)</td>
<td></td>
<td>20%; dim</td>
<td>n1</td>
<td>Flu/Cy</td>
<td>200M donor</td>
<td>Gr3 adeno PNA Gr2 CRS CRI (MRD-ve by Flow on D14)</td>
</tr>
<tr>
<td>178</td>
<td>54/F</td>
<td>7; Y (MUD)</td>
<td></td>
<td>37%; mod</td>
<td>13;7,+21</td>
<td>Flu/Cy</td>
<td>200M Donor (DU)</td>
<td>Gr3 Allergy Gr3 NF &amp; infection SD 20% blasts</td>
</tr>
<tr>
<td>195</td>
<td>42/F</td>
<td>4; Y (MRD)</td>
<td></td>
<td>41%; mod</td>
<td>-7, +8</td>
<td>Flu/Cy decitabine</td>
<td>200M Donor (DU)</td>
<td>Gr1 CRS SD 46% blasts</td>
</tr>
<tr>
<td>200</td>
<td>28/M</td>
<td>6; Y (MRD)</td>
<td></td>
<td>3%; dim</td>
<td>Complex FLT3-TKD N-RAS</td>
<td>Flu/Cy</td>
<td>200M Donor</td>
<td>Gr1 CRS SD 0.10%</td>
</tr>
</tbody>
</table>

**Figure X.**

A bone marrow biopsy (H&E) showing residual acute myeloid leukemia highlighted by B. CD34 immunohistochemical stain. C-D Day 14 bone marrow biopsy (H&E), Wright Giemsa stain post CD123 CAR T-cell therapy showing no evidence of disease. E, F Day 29 bone marrow biopsy (H&E) post CD123 CAR T-cell therapy showing no evidence of disease. G, J Day 54 bone marrow biopsy (H&E), Wright Giemsa stain post CD123 CAR T-cell therapy with no evidence of disease.
**Myelodysplastic Syndromes**

- MDS are clonal stem cell neoplasms stem hallmarked by ineffective hematopoiesis, BM dysplasia, propensity for transformation to AML
- The treatment of MDS patients is largely based on stratification into lower and higher risk disease the IPSS and the revised IPSS (IPSS-R) score systems
- However, one of the limitation of prognostication is that most MDS patients are elderly with high risk of co-morbidities
- Gene mutations linked to worse outcome are TP52, ETV6, RUNX1, EZH2, ASXL1 TP53, DNMT3A

**Pathogenesis of MDS is complex**

Low-Risk MDS: what is the treatment?

- Aggressive supportive care
- Growth factors
- Lenalidomide for patients with 5q abnormalities (specially if without thrombocytopenia)
- Hypomethylating agents but probably with a reduced treatment duration (respect to high-risk MDS) because the potential prolonged treatment-related cytopenias

High-risk MDS: Hypomethylating Agents

- Azacitidine and Decitabine inhibit DNA methyltransferases
- The AZA-001 study found a survival benefit among patients treated with azacitidine compared with those treated with best supportive care. However, the overall response rate was 40% to 50%, and the complete response (CR) rate was only 10% to 20%.
- Mutations in TET2, was found to be associated with the HMA response in a few studies, in particular, in the absence of a concomitant ASXL1 mutation, which has been associated with a poorer prognosis
- TP53 mutations correlated highly with improved clinical responses with Decitabine: 100% (n= 21) patients with TP53 mutations achieving CR or CRi compared with 41% (n=32) with wild-type TP53 (P < .001)
- AlloHSCT is the only curative treatment of HR-MDS, with about 30–50 % prolonged DFS and a longer life expectancy (36 months) compared to non-transplanted patients
New Treatment for MDS

- Mutations in components of the spliceosome occur in ~50% of patients with MDS and include mutations in
  - SF3B1, SRSF2, U2AF1 mutations
- SF3B1 mutations: most common found in > 25% of patients, highly associated with ring sideroblasts and a favorable prognosis
- SRSF2 mutations: found in 10% to 15% of MDS patients and is frequently mutated in MDS/MPN; associated with a poor prognosis
- U2AF1 mutations: found in 8% to 12% of patients with MDS; associated with poor prognosis
- H3B-8800, an SF3B1 modulator is currently under clinical investigation

Spliceosome Mutations

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- U2AF1 mutations: found in 8% to 12% of patients with MDS; associated with poor prognosis
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Conclusions

- Exciting time in the treatment of AML (a “bit” less for MDS) because of new molecular classification, novel molecular targets, new drugs and new transplant modalities that may lead to implementation of truly “personalized” therapies
- Challenge is how to best incorporate into clinical practice:
  - Novel “predictive” biomarkers
  - Other non-mutation biomarkers (epigenetics, ncRNAs, transcriptome, proteomics, signaling pathways)
  - New drugs
  - MRD
  - Combinatory or sequential use of novel molecularly targeting drugs with AlloHSCT

Targeting Toll-like Receptor Signaling in MDS

- Aberrations of the TLR signaling cascade are frequently associated with the development of MDS
- OPN-305 a fully humanized antagonistic IgG4 kappa monoclonal antibody to TLR2 in a phase I/II trial of lower risk MDS patients
- CX-01, which disrupts HMGB1 (high-mobility group box protein 1, a stress-related endogenous ligand of TLR4) interactions with TLR4, is under initial investigation in MDS in combination with Azacitidine