## Disclosures for Ayalew Tefferi

<table>
<thead>
<tr>
<th>Principal investigator role</th>
<th>Janssen, Geron, Celgene, Sanofi-Aventis, Gilead Sciences, Incyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employee</td>
<td>None</td>
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<tr>
<td>Consultant</td>
<td>None</td>
</tr>
<tr>
<td>Major Stockholder</td>
<td>None</td>
</tr>
<tr>
<td>Speakers’ Bureau</td>
<td>None</td>
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<tr>
<td>Scientific Advisory Board</td>
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</tr>
</tbody>
</table>

Presentation includes discussion of the following off-label use of a drug or medical device: Hydroxyurea, Interferon-alpha, Busulfan, Thalidomide, Lenalidomide, Pomalidomide, Ruxolitinib, Androgen preparations, Erythropoiesis stimulating agents.
Myeloproliferative neoplasms other than PV, ET and PMF:
CMML: chronic myelomonocytic leukemia
Mastocytosis
Eosinophilic disorders
CNL: chronic neutrophilic leukemia

Ayalew Tefferi, MD
Professor of Medicine and Hematology
Mayo Clinic College of Medicine
Objectives

- Disease definitions
- Diagnosis
- Current prognostication
- Treatment
2016 WHO Classification of Myeloid Malignancies

Acute Myeloid Leukemia (AML)

Myelodysplastic Syndromes (MDS)

Myeloproliferative Neoplasms (MPN)

MDS/MPN overlap

Myeloid/Lymphoid neoplasms with eosinophilia and PDGFR/FGFR1/PCM1-JAK2 mutation

Chronic Myeloid Leukemia (CML)  
BCR-ABL1 100% mutated

Chronic Neutrophilic Leukemia (CNL)  
CSF3R 80-100% mutated

Chronic Eosinophilic Leukemia Not Otherwise Specified (CEL-NOS)

Polycythemia vera (PV)

Essential Thrombocythemia (ET)

Primary Myelofibrosis (PMF)

MPN Unclassifiable (MPN-U)

Mastocytosis no longer under the WHO MPN category

CMML

Molecularly-defined eosinophilia

The JAK2/CALR/MPL mutated MPNs

97% JAK2 V617F  
3% other JAK2 mutations

60% JAK2 mutated  
22% CALR mutated  
3% MPL mutated  
15% triple-negative

60% JAK2 mutated  
23% CALR mutated  
7% MPL mutated  
10% triple-negative
Acquired eosinophilia

Secondary

- Drugs
- Infections
  - Parasites
- Allergy
- Inflammations
  - Kimura’s
  - CSS
  - Well’s
- Neoplasia
  - Hodgkins
  - NHL
  - Solid tumor

Primary

Clonal

- Cytogenetic, molecular or bone marrow morphologic evidence of an otherwise defined myeloid malignancy
  - PDGFRA/PDGFRB mutated (imatinib sensitive)
  - FGFR1 rearranged (urgent transplant)
  - PCM1-JAK2 (ruxolitinib)
  - Abnormal karyotype, non-specific (CEL-NOS)
  - Excess blasts (CEL-NOS)
  - AML/ALL/CML/MDS/CMML/SM/unclassifiable

Idiopathic

- Neither reactive nor clonal

HES

If AEC > 1500/micL × 6 months and organ damage

20% of “HES” pts may display a T cell clone/abnormal phenotype (lymphocytic variant hyper-eosinophilia)
Primary eosinophilia diagnostic algorithm

1st step
Peripheral blood screening for *FIP1L1-PDGFR* using FISH or RT-PCR

2nd step
Bone marrow biopsy with cytogenetics

3rd step
Peripheral blood lymphocyte phenotyping and TCR gene rearrangement studies

Mutation present
- *PDGFRA* mutated clonal eosinophilia (Imatinib 100 mg/day)
- *PDGFRB* mutated clonal eosinophilia (Imatinib 100 mg/day)
- *FGFR1* mutated clonal eosinophilia (Hyper-CVAD + Allo-transplant)

5q33 translocation present
- CEL-NOS (Allo-transplant)

8p11 translocation present
- Other abnormalities or excess blasts present

Abnormal or clonal lymphocytes present
- “lymphocytic” variant hypereosinophilia (Treat like HES)

All the above negative
- Idiopathic eosinophilia including HES

Hyper-eosinophilic syndrome/idiopathic eosinophilia
98 Mayo Clinic patients with WHO-defined HES/IH (Leukemia 2016;30:1924)

NGS revealed 11% harbored pathogenic mutation; 
*TET2*=3, *ASXL1*=2, *KIT*=2, and *IDH2*, *JAK2*, *SF3B1* and *TP53*=1 each.
15% harbored a variant of unknown significance (VUS); 
*TET2*=8, *ASXL1*=2, *SETBP1*=2, and *CALR*, *CEBPA* and *CSF3R*=1 each.

NO DIFFERENCE IN MUTATED VS NON-MUTATED IN PHENOTYPE
MUTATED PATIENTS HAD INFERIOR SURVIVAL IN UNIVARIATE ANALYSIS

Risk factors for survival:

*Hepatosplenomegaly (3 points)*
*Advanced age (2 points)*
*Hgb <10 g/dl (one point)*
*Cardiac involvement (one point)*

Low risk: 0-1 risk points
N=60
5-year survival 98%

High risk: 2 or more risk points
N=38
5-year survival 62%
HES treatment algorithm

Urgent treatment needed

Prednisone 1-2 mg/kg/day with taper

Treatment not urgent

Do you even need to treat?
- Asymptomatic
- AEC < 50 x 10^9/l
- Serum troponin normal
- Echocardiogram normal

HES

Low-dose prednisone (10 mg/day or less)
Low-dose prednisone + hydroxyurea
Low-dose prednisone + pegasys
Imatinib 400 mg/day trial x 1 month
Mepolizumab (Nucala®; biweekly injections)
Benralizumab (Fasenra®; monthly injections)
Midostaurin 100 mg BID

T clone present

CSA
MTX
Cytoxan
Novel targeted therapies for eosinophilic disorders

- **Anti-IL-5**
  - Mepolizumab
  - Reslizumab
  - Anti-IL-5Rα
  - Benralizumab

- **Anti-CD52**
  - Alemtuzumab

- **Anti-IgE**
  - Omalizumab

- **Multi-targeted TKI**
  - Midostaurin

- **Eotaxin receptor**
  - LMW CCR3 inhibitors

- **Eosinophil inhibitory receptor**
  - Anti-Siglec-8 Ab

- **Prostaglandin D₂ receptor**
  - LMW CRTH2 inhibitors

When should you suspect mastocytosis?

- Urticaria pigmentosa
- Mast cell mediator symptoms
  - Anaphylactoid
  - Diarrhea
  - Flushing/urticaria
- Osteopenia/unexplained fractures
Diagnostic Evaluation in Systemic Mastocytosis

- **serum tryptase**
- **Bone marrow biopsy with tryptase stains**
- **Bone marrow mast cell flow cytometry**
  - Normal mast cells — CD117+, CD25-, CD2-
  - Abnormal mast cells — CD117+, CD25+, CD2 ±

- Regions with activating mutations
- Phospho-Tyr binding proteins
- Structure
  - Domains
    - Ligand binding
    - Dimerization
    - Transmembrane
    - Juxtamembrane (JM)
    - Kinase (ATP binding)
    - Kinase insert
    - Kinase (Phosphotransferase)
    - Carboxy-terminal tail region

- C-kit mutations
  - Asp816Val (kinase domain)
  - Val560Gly (JM domain)

References:
- Sattler & Salgia, Leukemia Research, 2004
- Pardanani et al. BJH 2003;120:691
Practical classification of mast cell disease

1. Cutaneous mastocytosis (skin-only disease)

   Both can manifest mast cell mediator release symptoms

2. Systemic mastocytosis (SM)

   i. Indolent SM
   ii. Aggressive SM (cytopenia, bone disease, organomegaly, etc.)

   1. SM without associated 2nd myeloid neoplasm
   2. SM with associated 2nd myeloid neoplasm
   3. Mast cell leukemia

Hartmann U, Henz B. Br J Dermatol 2001;144:682
Medicine 1988;67:345
Leukemia Research 2001;25:603
Survival for 342 systemic mastocytosis patients classified by disease type compared with the expected age and gender matched US Population’s survival.

Blood 2009;113:5727.
Mutation-augmented prognostic scoring system (MAPSS) in 94 patients with advanced mastocytosis

Risk factors:
- Platelet count <150
- Albumin <3.5
- Age >60
- ASXL1/CBL mutated
- Hgb <10

ISM (n=44)
- KIT 73%
- TET2 7%
- No other mutations

ASM (n=25)
- KIT 84%
- TET2 20%
- ASXL1 16%

AHN (n=80)
- KIT 75%
- TET2 45%
- ASXL1 26%
- CBL 19%
- JAK2
- DNMT3A
- U2AF1
- RUNX1
- SF3B1
- Others

One risk factor other than platelet count
2-3 risk factors or low platelet count
4 or more risk factors or low platelet count

Low-risk: n=26 (16 events), median OS=86 months
Intermediate-risk: n=41 (38 events), median OS=21 months
High-risk: n=27 (25 events), median OS=5 months

P<0.0001
Treatment for Systemic mastocytosis

**Indolent**
- H1 and H2 blockers
- Cromolyn
- Phototherapy
- Topical steroids

**Aggressive**
- Cladribine
  - 5 mg/m2 x 5 days; monthly x 4
  - (first choice)
- Midostaurine
  - 100 mg BID
  - (second choice)
- Avapritinib (BLU-285)
  - 300 mg once-daily
  - 32 treated
  - OR 72% at 9 months
  - 56% CR/PR
  - Edema, fatigue, GI toxicity
  - 50% grade ≥3 AEs

**Associated with MDS or CMML**
- Treat as MDS or CMML

**Mast cell leukemia**
- Midostaurine or Avapritinib or Cladribine or AML-like therapy followed by
  - Transplant?

Additional notes:
- Cladribine 5 mg/m2 x 5 days; monthly x 4 (first choice)
- Midostaurine 100 mg BID (second choice)
- Avapritinib (BLU-285) 300 mg once-daily
  - 32 treated
  - OR 72% at 9 months
  - 56% CR/PR
  - Edema, fatigue, GI toxicity
  - 50% grade ≥3 AEs
- If this fails, OK to try IFN-α or cladribine

Reference:
J Clin Oncol. 2014;32:3264
Phase-2 study of midostaurin 10-year follow-up (N=26; responders = 18 (69%; major response 50%)
SM-AHN = 17 (13 responders); ASM = 3 (1 responder), MCL = 6 (4 responders)

Median OS 40 months
92% treatment discontinuation rate at median 5 months of treatment
Nauseas 88%
Vomiting 69%
Diarrhea 27%
Fatigue 35%
Headache 31%
Edema 35%

Bonus effects:
- Complete resolution of eosinophilia in 7 of 7 evaluable patients
- Improvement in monocytosis in all 14 patients with baseline increase

<table>
<thead>
<tr>
<th>Study status/reason for discontinuation (# of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ Continuing treatment (n=2)</td>
</tr>
<tr>
<td>* Adverse event: Grade 3 or 4 thrombocytopenia (n=2)</td>
</tr>
<tr>
<td>** Serious adverse event: sepsis (n=3), inflammatory mixed neuropathy/myopathy and altered mental status (n=1)</td>
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<tr>
<td>¥ Withdraw consent (n=1)</td>
</tr>
<tr>
<td>^ Unsatisfactory therapeutic effect; discontinued per investigator discretion (n=5)</td>
</tr>
<tr>
<td>✱ Disease progression (n=4)</td>
</tr>
</tbody>
</table>

* Data through 3/1/2017; best response at any time on therapy
Solid bars are KIT D816 mutation-positive, two patterned bars are KIT D816 mutation-negative

Median duration of treatment (months)
<table>
<thead>
<tr>
<th>median</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>2-132</td>
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</tbody>
</table>
Allogeneic hematopoietic stem-cell transplantation (alloHCT or HCT) outcomes in 57 patients with advanced systemic mastocytosis (SM): 38 SM-AHNMD; 12 MCL and 7 aggressive SM.
**CSF3R mutations**

Chromosome 1p34.3
17 exons
813 amino acids

Extracellular domain

Membrane proximal

Cytoplasmic domain

Kosmider et al. Leukemia
## WHO diagnostic criteria for CNL

1. Leukocytosis $\geq 25 \times 10^9$/L
   - Neutrophils plus bands $\geq 80\%$
   - Neutrophil precursors $< 10\%$
   - Myeloblasts rarely observed
   - No dysgranulopoiesis

2. Hypercellular bone marrow
   - Neutrophil granulocytes increased
   - Neutrophil maturation normal
   - Myeloblasts $< 5\%$

3. Not meeting WHO criteria for BCR-ABL1+ CML, PMF, PV or ET

4. No PDGFRA/PDGFRB/FGFR1/PCM1-JAK2

5. CSF3RT618I or other activating CSF3R mutation

or

no identifiable cause of reactive neutrophilia

## WHO diagnostic criteria for aCML

- Leukocytosis with $\geq 10\%$ precursors
- Dysgranulopoiesis
- Basophils $< 2\%$
- Monocytes $< 10\%$
- Hypercellular bone marrow with dysgranulopoiesis
- $< 20\%$ blasts in the blood and bone marrow
- No PDGFRA/PDGFRB/FGFR1/PCM1-JAK2
- Not meeting WHO criteria for BCR-ABL1+ CML, PMF, PV or ET
Risk-stratified Kaplan–Meier survival curves for 19 CSF3R-mutated CNL patients.

Risk points: platelet count <160 x 10^9/L (2 points); leukocyte count >60 x 10^9/L (1 point); ASXL1 mutation (1 point)

Treatment:
- High-risk: ASCT
- Low risk: Hydroxyurea (first) Ruxolitinib (second) Transient response

Log rank p = 0.0016 HR 95% CI

n = 9

n = 10

Survival probability (%)

Time (months)
CMML

2016 WHO Diagnostic Criteria

- AMC >1 x 10^9 /L and monocytes >10%
- Dysplasia in one or more myeloid lineages
- Not meeting WHO criteria for CML, PV, ET or MF
- No PDGFRA/PDGFRB/FGFR1/PCM1-JAK2
- <20% blasts in the blood and BM

In the absence of dysplasia, a diagnosis of CMML can still be made if:
- An acquired clonal cytogenetic or molecular genetic abnormality can be documented. 
  these include ASXL1, TET2, SRSF2 and SETBP1 mutations
- Monocytosis has persisted for >3 months
- Other causes of reactive monocytosis have been ruled out
Differential diagnosis of Monocytosis

Reactive
1. Viral infections.
2. Recovering bone marrow.
3. Connective tissue disorders.
4. Sarcoidosis
5. Tuberculosis, Brucellosis, Leishmaniasis.
6. SABE.

Clonal
1. CMML
2. JMML
3. AML with monocytic differentiation.
4. MDS/MPN overlap syndromes - unclassifiable.

BJH 2014
CMML- Peripheral Blood and Bone Marrow Findings

Peripheral Blood Smear

Bone Marrow Aspirate

Core Biopsy

Dual Esterase Stain
WHO CMML Subcategories

**CMML-0**
- Blasts + promonocytes <2% in PB.
- Blasts + promonocytes <5% in BM.

**CMML-1**
- Blasts + promonocytes 2-4% in PB.
- Blasts + promonocytes 5-9% in BM

**CMML-2**
- Blasts + promonocytes 5-19% in PB.
- Blasts + promonocytes 10-19% in BM.
- Presence of Auer rods, irrespective of blast count.

*Blood 2016*
Survival data for 435 patients with WHO defined chronic myelomonocytic leukemia stratified by “Proliferative” versus “Dysplastic” sub-types.

- CMML patients with dysplastic phenotype, $n=226$, median survival ~30 months.
- CMML patients with proliferative phenotype, $n=209$, median survival ~19 months.

Figure 2: Heat map of an unsupervised differential gene expression profile in peripheral blood CMML samples, demonstrating two predominantly unique clusters, segregating dysplastic (cluster 1) from proliferative (cluster 2) CMML subtypes.
CMML – Genomics

- Epigenetic regulators – *TET2* (~60%), *IDH1, IDH2, DNMT3A*
- Chromatin modeling – *ASXL1* (~40%), *EZH2*
- Spliceosome components – *SRSF2* (~45%), *SF3B1, U2AF1, ZRSR2*
- Transcription factors – *RUNX1* (~15%)
- Signal pathways – *JAK2, KRAS, NRAS, CBL, PTPN11* (*RAS* pathway ~30%)
- Others – *SETBP1* (~15%), *PHF6, BCOR, Tp53*

> 90% CMML patients have ≥ 1 somatic mutations
Cytogenetic abnormalities in CMML

- Cytogenetic abnormalities seen in 20-40% of cases
- Most common are +8, chromosome 7 abnormalities and 12p deletions

Spanish Cytogenetic Risk Stratification.
- Low: Normal, -Y
- Intermediate: all others
- High: +8, chromosome 7 abnormalities and complex changes

5 year OS- 35%, 26% and 4%.

Haematologica 2011
AJH 2013
## CMML Prognostic Models

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mayo Model</th>
<th>Molecular Mayo Model</th>
<th>GFM Model</th>
<th>CPSS-Molecular</th>
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<tbody>
<tr>
<td>HB &lt; 10 gm/dl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Red blood cell transfusion dependance</td>
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<tr>
<td>High WBC</td>
<td></td>
<td></td>
<td>+ (&gt;15)</td>
<td>+ (&gt;13)</td>
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<tr>
<td>AMC &gt;10</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Platelets &lt; 100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Circulating IMC</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
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<tr>
<td>BM blasts</td>
<td></td>
<td></td>
<td>+ (&gt;5%)</td>
<td></td>
</tr>
<tr>
<td>Age &gt;65</td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>Cytogenetic risk groups</td>
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<td>Molecular genetics</td>
<td>ASXL1</td>
<td>ASXL1</td>
<td>ASXL1/ NRAS/ RUNX1 and SETBP1</td>
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</table>
Survival data for 420 patients with CMML stratified by the Molecular Mayo Model.

MMM low risk patients, n= 64, median survival 97 months.
MMM intermediate-1 risk patients, n= 128, median survival 59 months.
MMM intermediate-2 risk patients, n= 118, median survival 31 months.
MMM high risk patients, n= 110, median survival 16 months.

Survival data for 420 patients with CMML stratified by the GFM Model.

GFM low risk patients, n= 188, median survival 65 months.
GFM intermediate risk patients, n= 154, median survival 28 months.
GFM high risk patients, n= 78, median survival 17 months.

P=<0.0001
CMML Therapeutics

Supportive care
- Transfusions
- Hydroxyurea
- ESA
- Iron chelation therapy

Directed Therapies
- Hypomethylating agents
- Allogeneic SCT
- Clinical trials
## Hypomethylating (HMA) Agents in CMML

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Median Age (years)</th>
<th>Phase</th>
<th>Drug used</th>
<th>Response rates (%)</th>
<th>Median survival (months)</th>
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<tbody>
<tr>
<td>Wijermans 2008</td>
<td>31</td>
<td>71</td>
<td>II</td>
<td>Decitabine</td>
<td>CR-10 PR-16 HI-19</td>
<td>15</td>
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<tr>
<td>Costa 2011</td>
<td>38</td>
<td>70</td>
<td>II</td>
<td>Azacitidine</td>
<td>CR-11 PR- 3 HI- 25</td>
<td>12</td>
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<td>Braun 2011</td>
<td>39</td>
<td>71</td>
<td>II</td>
<td>Decitabine</td>
<td>CR-10 PR-20 HI-8</td>
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<td>Thorpe 2012</td>
<td>10</td>
<td>66</td>
<td>II</td>
<td>Azacitidine</td>
<td>CR-20 HI-40</td>
<td>NR</td>
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<tr>
<td>Ades 2013</td>
<td>76</td>
<td>70</td>
<td>II</td>
<td>Azacitidine</td>
<td>CR-17 PR-1 HI-17</td>
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<td>Wong 2013</td>
<td>11</td>
<td>65</td>
<td>II</td>
<td>Azacitidine</td>
<td>CR-9 PR-9 HI-9</td>
<td>17</td>
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<tr>
<td>Fianchi 2013</td>
<td>31</td>
<td>69</td>
<td>II</td>
<td>Azacitidine</td>
<td>CR-45 PR-3 HI-6</td>
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## Role for Allogeneic SCT in CMML

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Age median (range)</th>
<th>Donor Source</th>
<th>Conditioning regimen</th>
<th>Relapse rate and TRM</th>
<th>Outcome OS &amp; DFS</th>
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<tbody>
<tr>
<td>Kroger 2002</td>
<td>50</td>
<td>44 (19-61)</td>
<td>MRD-43 MUD-7</td>
<td>MAC-50</td>
<td>RR-28% TRM-52%</td>
<td>5 yr DFS-18% 5 yr OS-21%</td>
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<tr>
<td>Symeonidis 2010</td>
<td>283</td>
<td>50</td>
<td>MRD-160 MUD-85</td>
<td>MAC-152 RIC-87</td>
<td>RR-25% TRM-37%</td>
<td>5 yr DFS-38% 5 yr OS-42%</td>
</tr>
<tr>
<td>Eissa 2011</td>
<td>85</td>
<td>51 (21-66)</td>
<td>MRD-38 MUD-47</td>
<td>MAC-58 RIC-27</td>
<td>RR-27% TRM-35%</td>
<td>10 yr DFS-40% 10 yr OS-40%</td>
</tr>
<tr>
<td>Park 2013</td>
<td>73</td>
<td>53 (27-66)</td>
<td>MRD-41 MUD-32</td>
<td>MAC-30 RIC-43</td>
<td>RR-35%</td>
<td>3 yr DFS-29% 3 yr OS-32%</td>
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</tbody>
</table>
Novel Agents- Clinical Trials.

• MEK inhibitors.
• Hedgehog pathway inhibitors.
• GM-CSF monoclonal antibody (KB003).
• Neddylation inhibitors.
• MAP kinase inhibitor.
• P38/Tie-2 inhibitor.
• Aminopeptidase inhibitors.