Mouse Models of Genomic Lesions: Utility as Therapeutic Platforms

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  - 80% patients have at least 1 mutation, 6 genes mutated in > 10% patients
  - splicing factor and/or epigenetic regulators are mutated in most patients
    - many recurrent mutations in MDS are very rare

Boulwood 2015
# New mice 1

<table>
<thead>
<tr>
<th>GENE</th>
<th>ALTERATIONS found in MDS patients MDS disease entities (Frequency)</th>
<th>ALTERATIONS STUDIED IN MOUSE MODEL</th>
<th>TYPE OF MOUSE MODEL</th>
<th>MOUSE MODELS DISEASE ENTITIES</th>
<th>HEMATOLOGICAL FEATURES DESCRIBED</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF3B1</td>
<td>MDS, AML with MDS</td>
<td>SF3B1 mutant</td>
<td>Xenograft</td>
<td>MDS-like</td>
<td>HSCs restricted to myeloid lineage with clonal evolution leading to AML (Mian 2015) Increase in myeloid cells. Deregulated expresion of target genes (Mmp9, Puma, Bcl2l1) (SathiaseelanASH2015)</td>
</tr>
<tr>
<td>SRSF2</td>
<td>Mutations in MDS, AML with MDS</td>
<td>SRSF2 mutant</td>
<td>KI</td>
<td>MDS</td>
<td>Impaired Hematopoietic differentiation (Kim 2015) Fetal liver cells had increased apoptosis and decrease of hematopoietic stem/progenitor cells (Komeno 2015)</td>
</tr>
<tr>
<td>U2AF1</td>
<td>Heterozygous mutations in 11% MDS</td>
<td>U2AF1 mutant</td>
<td>Tg</td>
<td>Some features of MDS</td>
<td>Altered hematopoieses and changes in pre-mRNA splicing in progenitors (Shirai 2015)</td>
</tr>
<tr>
<td>EZH2</td>
<td>Inactivating mutations in MDS/MPN</td>
<td>EZH2 Deficiency</td>
<td>KO</td>
<td>MDS/MPN</td>
<td>Thrombocytosis (Mochisuk-Kashio 2015)</td>
</tr>
<tr>
<td>EZH1</td>
<td>Deletion</td>
<td>EZH1 Deletion</td>
<td>KO</td>
<td>No Dysplasia or malignancy</td>
<td>Abolished repopulating capacity of HSCs when combined with EZH2 loss (Mochisuk-Kashio 2015)</td>
</tr>
<tr>
<td>GENE</td>
<td>ALTERATIONS found in MDS patients</td>
<td>ALTERATIONS STUDIED IN MOUSE MODEL</td>
<td>TYPE OF MOUSE MODEL</td>
<td>MOUSE MODELS DISEASE ENTITIES</td>
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<tr>
<td>Tert</td>
<td>BoneMarrow Failure syndromes: DC, AA,MDS</td>
<td>TERT deficiency</td>
<td>Conditional Tert KO</td>
<td>Anemia</td>
<td>Decreased erythroblasts, reduced HSCs, neutrophilia and increased myelopoiesis (Raval 2015)</td>
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<tr>
<td>DNMT3A</td>
<td>Mutated in MPN, MDS,AML</td>
<td>DNMT3A deficiency</td>
<td>Conditional KO</td>
<td>MDS/MPN</td>
<td>Cytopenia, hepatomegaly,expanded stem/progenitors (Guryanova 2015)</td>
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<tr>
<td>TIFAB</td>
<td>TRAF-interacting protein with forkhead-associated domain B</td>
<td>Haploinsufficiency in 5q- MDS</td>
<td>TIFAB deficiency</td>
<td>KO</td>
<td>BM failure</td>
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<tr>
<td>PTEN</td>
<td>Mutated in JMML (MDS/MPN)</td>
<td>PTEN deficiency</td>
<td>KO in NF1 haploinsufficient background</td>
<td>Pediatric MDS/MPN (JMML-like)</td>
<td>Death at days 20-35 days due to organ infiltration with monocytes/macrophages Liu 2016)</td>
</tr>
<tr>
<td>RPS14</td>
<td>Haploinsufficiency 5q- MDS</td>
<td>RPS14 deficiency</td>
<td>Conditional KO</td>
<td>Anemia, megakaryocyte dysplasia</td>
<td>Erythroid differentiation defect, apoptosis, loss of HSC quiescence, high levels of innate immune signaling (Schneider 2016)</td>
</tr>
</tbody>
</table>
## Classification based on Apoptosis

<table>
<thead>
<tr>
<th>Model</th>
<th>Apoptosis</th>
<th>Classification of Bethesda (BM blasts)</th>
<th>Classification based on Apoptosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BID-deficient</td>
<td>Anti</td>
<td>MPD/CMML(&lt;15%)</td>
<td>MPD/CMML</td>
<td>Zinkel et al 2002</td>
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<tr>
<td>NUP98-HOXD13</td>
<td>Pro</td>
<td>MDS/AML (14%)</td>
<td>MDS</td>
<td>Lin et al 2005</td>
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<tr>
<td>NUP98-HD13+Meis1</td>
<td>?</td>
<td>AML (65%)</td>
<td>?</td>
<td>Pineault et al 2003</td>
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<tr>
<td>SALL4</td>
<td>Pro</td>
<td>MDS-AML</td>
<td>MDS</td>
<td>Ma et al 2006</td>
</tr>
<tr>
<td>NRAS*/BCL-2</td>
<td>Pro</td>
<td>MDS (15%)</td>
<td>MDS</td>
<td>Omidvar et al 2007</td>
</tr>
<tr>
<td>NRAS*/BCL-2</td>
<td>Anti</td>
<td>AML (90%)</td>
<td>AML</td>
<td>Omidvar et al 2007</td>
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<tr>
<td>AML1+EVI 1</td>
<td>?</td>
<td>AML</td>
<td>?</td>
<td>Watanabe-Ocochi et al 2008</td>
</tr>
<tr>
<td>EVI 1</td>
<td>Pro</td>
<td>MDS</td>
<td>MDS</td>
<td>Delwell, pers comm</td>
</tr>
</tbody>
</table>
Overall Aim

- To develop animal models as test platforms for therapeutics
- To develop reagents for improved patient treatment
- To identify biomarkers to predict response to treatment
The NRAS/BCL-2 mediated mouse model of High Risk MDS and AML post-MDS

NRAS: coding for a small GTPase, involved in the regulation of proliferation, differentiation and survival. *Mutation of NRAS*: frequent molecular abnormality in MDS and linked to AML.

Bcl2: regulator of apoptosis. *Overexpression of Bcl2*: observed in AML and MDS.

STEM CELL

NRAS

BCL-2

NRAS/BCL-2

MMTVtTA/TetoBCL-2 → HR-MDS

MRP8BCL-2 → AML post-MDS

INITIATION

ABNORMAL CLONE

CLONAL EXPANSION

LEUKEMIC CLONE

PROGRESSION

EXPANSION
NRAS and BCL2 cooperate to give HR-MDS or AML post-MDS

MDS

-DOX (n=13)

AML

-DOX (n=18)

%blast

8±0.3

12±2

18±5

15±3

89±4

Bone Marrow (LP)  Bone Marrow (HP)  Liver  Spleen

MRP8NRAS

MMTVTA/TBCL-2

MRP8BCL-2

MMTVTA/TBCL-2/NRAS

MRP8[BCL-2/NRAS]

HR-MDS

AML post-MDS

Omidvar Cancer Res 2007
BCL-2 and mutant NRAS co-localize in the bone marrow

**Spleen**

<table>
<thead>
<tr>
<th>Sca-1+</th>
<th>Mac-1+</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB/N</td>
<td>BCL-2</td>
</tr>
<tr>
<td>FVB/N</td>
<td>BCL-2</td>
</tr>
</tbody>
</table>

**Anti-Ras**

**Anti-Actin**

**Anti-BCL-2**

**Anti-RAS**

**Merged+DAPI**

**BCL-2/NRAS**

**Omidvar Cancer Res 2007**
RAS:BCL-2 complex localization correlates with apoptosis and prognosis in MDS/AML patients

ME: 11% BM blast/APO- / High RAS:BCL-2 co-loc / Mito+

JM: 4% BM blast/APO+ / Low RAS:BCL-2 co-loc / PM+
Model of Leukemic Progression

INITIATION

ABNORMAL CLONE

CLONAL EXPANSION

LEUKEMIC CLONE

LEUKEMIC EXPANSION

Genomic Instability

NRAS

BCL-2 NRAS*
gH2AX and Phosphorylated ATM on chromatin fibres of BM in NRAS/BCL2 AML post-MDS mice

<table>
<thead>
<tr>
<th></th>
<th>gH2AX</th>
<th>Phos ATM</th>
<th>gH2AX+ Phos ATM</th>
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<tbody>
<tr>
<td>FVBN</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
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<tr>
<td>NRAS</td>
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<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
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<tr>
<td>BCL-2</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
</tr>
<tr>
<td>NRAS + BCL-2</td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
</tbody>
</table>

% +ve fibers:

- 0
- 20-30
- 10-18
- 30-50

(N=4)

Rassool Cancer Res 2007
Is the disease reversible?
Results of Preclinical Studies

- **Antioxidants**
  
  N-Acetyl Cysteine *(Rassool Cancer Res 2007)*

- **Small molecules**
  
  RACi *(Rassool Cancer Res 2007)*

  BCL-2i *(Beurlet Blood 2013)*

- **Immunotherapy**
  
  all-trans retinoic acid (ATRA) – a vitamin A derivative and DNA PML-RARA for APL

  *(Padua Nat Med 2003; Robin Blood 2006; Furugaki Blood 2010; Pokorna Mol Cell Probes 2013)* or a non-specific DNA construct **pVAX14**

  *(Le Pogam Oncotarget 2015; Patel Blood Cancer J 2015)*
Treatment of Mice with N-Acetyl cysteine

Offspring tested for DNA damage and misrepair
Immunostaining for gH2AX in BM MNC Nuclei from N Acetyl cysteine-treated NRAS/BCL2 Mice

<table>
<thead>
<tr>
<th></th>
<th>Foci</th>
<th>Untreated</th>
<th>N=3</th>
<th>NAC Treatment</th>
<th>N=3</th>
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<tr>
<td></td>
<td>2±2</td>
<td>1±1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>10±2</td>
<td>4±3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18±4</td>
<td>10±2</td>
<td></td>
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</tr>
</tbody>
</table>

100 nuclei examined /experiment

FVBN  NRAS  NRAS/BCL2

Rassool Cancer Res 2007
NHEJ Misrepair Frequencies in NRAS/BCL2 Mice Following NAC Treatment in vivo

![Graph showing misrepair frequency in FVB/N, RAS, and RAS/BCL2 mouse spleen cells with and without NAC treatment.]

White Colony PCR products around breakpoint

NRAS

NRAS/BCL2

NRAS + NAC

NRAS/BCL2 + NAC

Rassool Cancer Res 2007
BCL-2 inhibition with ABT-737 prolongs survival in an NRAS/BCL-2 mouse model of myeloid leukemia by targeting leukemia initiating cells

ABT-737 75 mg/kg IP 3x per week for 5 weeks

MDS from onset of disease (3-6 mths)

- Control (n=27)
- ABT n=23

P = 0.0005

AML from birth (3-4 weeks)

- Control (n=60)
- ABT-737 (n=35)

P < 0.0001

Beurlet Blood 2013; Gorombei, in preparation
RAS:BCL-2 Complex Co-localizes from the Mitochondria to Plasma Membrane

ABT-737

Beurlet Blood 2013
Genes regulated after ABT-737 treatment of MDS and AML
Differential exon specific gene expression – Sca1 + splenocytes

- MDS
  - EPIGENETICS
    - ADA
  - STEM CELL
    - ALDH
  - CELL CYCLE
  - APOPTOSIS
  - IMMUNE TLRs
  - MICROENVIRONMENT
    - ICOS

- AML
  - TRAFFICKING
    - RAB
  - ADHESION
    - RHO
  - EPIGENETICS
    - ADA
  - CELL CYCLE
  - APOPTOSIS
  - STEM CELL
  - IMMUNE TLRs
  - MICROENVIRONMENT
    - ICOS

MDS – 399 genes
AML – 997 genes
Secondary transplants of spleen cells showing that ABT-737 targets leukemia initiating cells.

**HR-MDS + ABT-737**
Post day 33 (n=4)

**AML + ABT-737**
Post day 33 (n=5)

**AML untreated**
(n=5)

<table>
<thead>
<tr>
<th>Bone Marrow (LP)</th>
<th>Bone Marrow (HP)</th>
<th>Liver</th>
<th>Spleen</th>
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<tbody>
<tr>
<td>FVB/N-T NRAS-T</td>
<td>BCL-2-T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCL-2/NRAS-T</td>
<td>R1</td>
<td></td>
<td>R1</td>
</tr>
<tr>
<td>R2</td>
<td>R2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Gr-1**

**Mac1**

**Survival**

**Days (post transplant)**
DNA Vaccination Scheme

Stimulates both CTL and Antibody production

Gene coding for a tumour antigen

Transfection

Perforin granzyme

Intracellular protein

Golgi apparatus

Proteosomes

Antigen presentation cell (APC)

MHC I

MHC II

Secreted antigen

CD8+

CTL

CD4+

B Cell

Modulate immune response

Such as Tregs?

Induction and maintenance of memory?
pVAX14 enhances the survival in HR-MDS Mice and induces stable disease

Le Pogam Oncotarget 2015
pVAX14 enhances the normalizes Signaling proteins
Immune responses: **pVAX14** targets progenitors, Increases memory T-cells, IFNg and MYD88 expression in HR-MDS Mice
These cells shed exogenous or endogenous peptides. At the same time TAA are also present from apoptotic cells or induction of apoptosis.

Activation of lymphocytes:
- CD4+ and CD8+ T cells
- Cytotoxic T lymphocytes
- Secretion of IFN gamma
- Production of memory T cells
- Production of IgG and specific antibodies

Hypothesis: Increase of pre-existing immune response.
Phase I/II Protocol and monitoring on MDS with ongoing reference treatment of 5-azacytidine

High risk MDS Patients Treated with 6 cycles of 5-azacitidine and have stable disease

- 1mg DNA
- 2mg DNA
- 4mg DNA (+ ATRA)

Follow-up Immunomonitoring Exploratory studies

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- Marika Pla
- Satyananda Patel – Past Post doc
- Petra Gorombei – Past PhD student
- Laura Guerenne – Past PhD student
- Stephanie Beurlet – Past PhD student
- Carole le Pogam – Past PhD student
- Pierre Fenaux – Clinical Lead
- Lionel Aides
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Maria-Elena Noguera
Pathology:
Anne Janin
Christophe Leboeuf
Imaging:
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Pascal Merlet

Institut Cochin
Michaela Fontenay

Cardiff University
Nader Omidvar
Robert West

King’s College London
Mohamed Saeed
Ghulam Mufti

Abbott
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University of Texas
MD Anderson
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Michael Andreeff

University of California, San Francisco
Scott Kogan
Mike Bishop
# Gene silencing and altered tumor suppressor genes

<table>
<thead>
<tr>
<th>GENE</th>
<th>ALTERATIONS found in MDS patients</th>
<th>ALTERATIONS STUDIED IN MOUSE MODEL</th>
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<tbody>
<tr>
<td>NPM1</td>
<td>t (3,5) translocation NPM/MLF1</td>
<td>NPM1</td>
<td>NPM1+/-</td>
<td>Some features of MDS, MDS/MPN like and Myeloid leukemia (Sportoletti 2008)</td>
<td>Dyserythropoiesis, Dysmegakariopoiesis (Grisendi 2005)</td>
</tr>
<tr>
<td></td>
<td>MDS, AML with MDS &lt;1% (Arber 2003;Yoneda-Kato 1996; Grisendi 2005)</td>
<td></td>
<td></td>
<td></td>
<td>Leukocytosis with organ infiltration (Sportoletti 2008)</td>
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<tr>
<td></td>
<td>NPM1 Mutations MDS/AML(Bains 2011) (4.4%)</td>
<td></td>
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<td></td>
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<tr>
<td>RPS14</td>
<td>Haplo insufficiency 5q- syndrome (Ebert 2008; Eisenmann 2009)</td>
<td>LSCE** Cd74-Nid67region</td>
<td>KO</td>
<td>MDS with isolated del(5q) 5q- syndrome like (Barlow 2009)</td>
<td>Macrocytic Anemia, Mild thrombocytopenia and mild neutropenia, Dyserythropoiesis Dysmegakariopoiesis, Pro-apoptotic profile</td>
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<tr>
<td>SPARC</td>
<td>Haplo insufficiency 5q- syndrome (Eisenmann 2009)</td>
<td>SPARC</td>
<td>KO</td>
<td>MDS with isolated del(5q) Some features of 5q-syndrome (Lehmann 2007)</td>
<td>Thrombocytopenia, No Dysplasia Impaired ability to form BFU-E</td>
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<tr>
<td>APC</td>
<td>Haplo insufficiency 5q- syndrome (Eisenmann 2009) (95%)</td>
<td>APC</td>
<td>Heterozygote KO</td>
<td>MDS/MPN CMML like (Lane 2010; Wang 2010)</td>
<td>Macrocytic anemia, Leukocytosis, Monocytosis, Splenomegaly</td>
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<tr>
<td>BAP1</td>
<td>Heterozygous frameshift mutation RMCD (Dey 2012)</td>
<td>BAP-1</td>
<td>Conditional KO CreERT2</td>
<td>MDS/MPN CMML like (Dey 2012)</td>
<td>Anemia, thrombocytopenia, leukocytosis with monocytosis, splenomegaly, dyserythropoiesis dysmyelopoiesis</td>
</tr>
</tbody>
</table>
### Animal Models

<table>
<thead>
<tr>
<th>Asymmetric stem cell and niche</th>
<th>Dicer 1</th>
<th>No</th>
<th>Dicer</th>
<th>Tg Inducible Osterix</th>
<th>MDS RCMD like (Raaijmakers 2010)</th>
<th>Neutropenia +/-Anemia +/- Thromcytopenia Dysgranulopoiesis Dysmegaripoiesis Pro-apoptotic profile, 2% AML transformation</th>
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</thead>
<tbody>
<tr>
<td>Tet2</td>
<td>Mutations (loss of function) CMML 20% (Teferri 2009), 22% (Delhommeau 2009), MDS 6% (Teferri, 19% (Delhommeau), MPN 23% (Teferri 2009b), MPD 12% (Delhommeau)</td>
<td>Tet2</td>
<td>KO Tet2&lt;sup&gt;lacZ/lacZ&lt;/sup&gt; Conditional KO Mix1Cre</td>
<td>MDS/MPN CMML like (Quivoron 2011) (Incomplete penetrance)</td>
<td>Leukocytosis Monocytosis Anemia Thrombocytopenia Splenomegaly</td>
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<tr>
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<td></td>
<td>Conditional KO VavCre</td>
<td>MDS/MPN CMML like (Moran-Crusio 2011)</td>
<td>Leukocytosis Monocytosis Myeloid dysplasia Splenomegaly</td>
</tr>
</tbody>
</table>

### RAS signaling molecules

<table>
<thead>
<tr>
<th>FLT3</th>
<th>FLT3-ITD MDS, CMML 5% (Stover 2005)</th>
<th>FLT3-ITD Endogenous</th>
<th>KI Endogenous</th>
<th>MDS/MPN CMML like (Lee 2007)</th>
<th>Leukocytosis Monocytosis Splenomegaly</th>
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</thead>
<tbody>
<tr>
<td>PDGFRβ</td>
<td>Translocation t(5;12) CMML with eosinophilia Rare (Greipp 2004)</td>
<td>TEL-PDGFRβ BMT LTR-MCSV*</td>
<td>MDS/MPN CMML like (Stover 2005)</td>
<td>Leukocytosis with Neutrophilia and Monocytosis Hepatosplenomegaly</td>
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<tr>
<td>NF1</td>
<td>Loss and mutation Pediatric MDS/MPN 14% (Side 1997)</td>
<td>NF1 Inducible KO Mx1CreXNF1flox</td>
<td>MDS/MPN JMML like (LeDT 2004)</td>
<td>Leukocytosis Monocytosis Splenomegaly</td>
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<tr>
<td>PTPN11</td>
<td>Mutations JMML 34% (Tartaglia 2003)</td>
<td>Ptpn11D61Y Endogenous</td>
<td>MDS/MPN JMML like (Chan 2009)</td>
<td>Anemia Leukocytosis Monocytosis Splenomegaly Hypersensitivity to GM-CSF</td>
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</tr>
</tbody>
</table>

**Beurlet Hematologica 2013**
## Animal Models

<table>
<thead>
<tr>
<th>RAS signaling molecules</th>
<th>NRAS</th>
<th>NRASG12D</th>
<th>NRASG12D</th>
<th>NRASG12D</th>
<th>NRASG12D</th>
<th>NRASG12D</th>
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<th>NRASG12D</th>
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<tbody>
<tr>
<td>rT/BMT LTR- LK *</td>
<td>AML with maturation or MDS/MPN Atypical CML like (Mackenzie 1999)</td>
<td>High BM blasts with monocytic-monoblastic differentiation Leukocytosis with Monocytosis Hepatosplenomegaly</td>
<td></td>
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</tr>
</tbody>
</table>
| AML with maturation or MDS/MPN Atypical CML like (Mackenzie 1999) | High BM blasts with monocytic-monoblastic differentiation Leukosplenia Monocytosis
<p>| rT/BMT LTR- LTR-MCSV*  | AML (monocytic/monoblastic) (Parikh 2006) Or MDS/MPN CMML like |
| Tg MRP8 promoter Cooperation with BCL-2 | MDS (Omidvar 2007) RAEB-1 like or AML post-MDS (Omidvar 2007) |
| LSL-NRASG12D Conditional KI Mix1Cre | MPD (Li 2011) |
| LSL-NRASG12D Conditional KI Mix1Cre + MOL4070LTR | AML-like (Li 2011) |
| LSL-NRASG12D Conditional KI Mix1Cre + MOL4070LTR | Myeloblasts M4/M5 subtype |
| LSL-NRASG12D Conditional KI Mix1Cre + MOL4070LTR | AML-likeli |
| KRAS | MDS, CMML, JMML | 20 - 25% (Braun 2004; Chan 2004) | MDS/MPN JMML or CMML like (Braun 2006; Van Meter 2007) |
| KRASG12D | MDS/MPN | Leukosplenia with Monocytosis |
| KRASG12D | MDS/MPN CMML like (Parikh 2007) | Leukosplenia Monocytosis Hepatosplenomegaly |</p>
<table>
<thead>
<tr>
<th>Nuclear and Transcription Factors</th>
<th>EVI1</th>
<th>Inv(3), t(3;12), t(3;21) MDS, MDS/AML, t-MDS/AML (Rare) (Mitani 1994;Mochizuki 2000;Suzukawa 1994) Overexpression (Common)(Russell 1994)</th>
<th>rT/BMT LTR-MSCV*</th>
<th>MDS RCMD like(Laricchia-Robbio 2006)</th>
<th>Pancytopenia Dyserythropoiesis Dysmegakariopoiesis, Pro-apoptotic profile</th>
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<td>EVI1-MDS1</td>
<td>Ev1</td>
<td>MDS, MDS/AML, t-MDS/AML</td>
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<td>Overexpression (Common)(Russell 1994)</td>
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<td>RUNX1/AML1</td>
<td>Mutations</td>
<td>MDS subgroup MDS/AML (5 to 20%)(Dicker 2010) t-MDS/AML (40%)(Harada 2009)</td>
<td>rT/BMT LTR-Retrovirus* (unspecified)</td>
<td>AML with MDS related changes, MDS RAEB-2 (Watanabe-Okochi 2008)</td>
<td>Multilineage Dysplasia, Leukocytosis Hepatosplenomegaly, High blasts count in BM Multilineage Dysplasia, Pancytopenia BM blasts&lt;20%</td>
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<td>NUP98</td>
<td>Translocations</td>
<td>MDS, MDS/AML, t-MDS/AML 1 - 2 % (Lam 2001)</td>
<td>NUP98-HOX</td>
<td>Tg VAV promoter</td>
<td>AML (Kuo 2008) AML with monocytic differentiation Multilineage differentiation impairment</td>
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<td>CBFβ</td>
<td>Translocation Inv 16 t-MDS/AML 1.5% (Leroy 2002)</td>
<td>CBFβ-MYH11</td>
<td>Conditional KI Mx1Cre promoter</td>
<td>AML (Kuo 2008) AML with monocytic differentiation Multilineage differentiation impairment</td>
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<td>MLL</td>
<td>Translocations t-MDS, t-MDS/AML 12% (Block 2002; Rowley 1997; Taki 1997) Amplification MDS Mostly RAEB, t-MDS, AML with MDSrc &lt;1% (Anderson 2001)</td>
<td>MLL-AF9</td>
<td>KI Endogenous promoter</td>
<td>AML (Johnson 2003) AML with Mac-1+/c-KIT+ phenotype No MDS features AML with Mac-1+/c-KIT+ phenotype</td>
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<td>MLL-AF9</td>
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<td>AML (CHEN 2008) No MDS features AML with Mac-1+/c-KIT+ phenotype</td>
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<td>MLL-ENL</td>
<td>Accelerated to AML with caffeine, an inhibitor of DNA damage response kinases ATM and ATR with increase in Mac-1/c-KIT/GR-1 phenotype</td>
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<td>C/EBPα</td>
<td>Mutations</td>
<td>MDS, CMML Rare, AML 5 to 14% (Fuchs 2008; Gombart 2002)</td>
<td>C/EBPα mutant p30</td>
<td>KI Endogenous Promoter</td>
<td>AML(Kirstetter 2008) No MDS features AML with Mac-1+/c-KIT+ phenotype</td>
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<td>SALL4</td>
<td>Overexpression AML and MDS 60% (4B isoform)(Ma 2006)</td>
<td>SALL4B isoform</td>
<td>Tg CMV promoter</td>
<td>MDS RCMD like(Ma 2006) Neutropenia Multilineage dysplasia 50% AML transformation Pro-apoptotic profile in young mice</td>
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*Beurlet Hematologica 2013*
RAS and BCL-2 co-localization in MDS/AML patients correlates with WHO classification

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<th>Patient</th>
<th>BM blasts (%)</th>
<th>RAS:BCL-2 Col-loc</th>
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