Tumor Cell and Non Tumor Cell Cross Talk
CSF-1/CSF-1R Axis Targeting

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In the real world there is no real nature versus nurture argument, only infinitely complex, moment-to-moment interactions between genetics and environmental effects.
The Pre-Metastatic Niche

- Specialized microenvironment that supports disseminated tumor cells

Composed of
- Activated stromal cells contribute to enhanced extracellular matrix

- Bone Marrow-Derived Hematopoietic Cells: hematopoietic progenitors, myeloid derived suppressor cells, neutrophils, macrophages

- Signaling cascade within the niche

- Dynamic interchange with residents and recruited cell populations
Identifying the Pre-Metastatic Niche

- Dynamic, specialized microenvironment that supports disseminated tumor cells
- Activated mesenchymal cells with associated extracellular matrix remodeling
- Bone Marrow (BM) Derived Myeloid Cells

Pre-Metastatic Site

- Stroma
- Pericytes & Fibroblasts
- Hematopoietic Cells
- Tumor Cell Secreted Factors
  - Cytokines
  - Chemokines
  - Exosomes
Pre-metastatic Niche: Myeloid Derived Suppressor Cell (MDSC) support immune evasion

Pre-Metastatic Site

Bone Marrow → Pre-Metastatic Niche → Stroma

Stroma: Pericytes & Fibroblasts

Hematopoietic Cells

Tumor Cell Secreted Factors

Early Metastatic Site

Bone Marrow → Early Metastatic Site → Stroma

Stroma: Pericytes & Fibroblasts

Hematopoietic Cells

Tumor Cell Secreted Factors

Pre-metastatic Niche: Myeloid Derived Suppressor Cell (MDSC) support immune evasion
Myeloid Cells Accumulate In the Lungs of M3-9-M-bearing Mice with Increasing Tumor Burden

Immune cells are dysregulated in the metastatic niche
Lymphoid Compartment Contracts In the Pre-Metastatic Lung

Graphs showing changes in cell counts over time post tumor inoculation for CD3+ and CD3+ CD8+ cells. The images on the bottom show the distribution of different cell types in the lung with and without tumor.
Pre-Metastatic Niche: The Early Metastatic Microenvironment
Pexidartinib May Decrease Tumor Growth Through Effects On Tumor Microenvironment

• Small molecule inhibitor of CSF1R, kit, oncogenic FLT3 tyrosine kinases

• Can impact tumor growth through
  • Inhibiting paracrine loops between immune cells (myeloid cells, macrophages, mast cells, microglial cells) and tumors
  • Blocking cell migration and angiogenesis
  • Disrupting osteolytic metastases by targeting osteoclasts that express CSF1R

• Not currently studied in myelodysplastic syndrome
CSF1R Signaling Affects Myeloid Cells

- CSF1R is a type 3 receptor tyrosine kinase
- On myeloid lineage cells: monocytes, macrophages, dendritic cells, osteoclasts
- Binds colony stimulating factor 1 (CSF1)

Effects on myeloid cells
- Production, differentiation, and function of macrophages
- Bone marrow mobilization, migration into target tissues, survival, proliferation
Increased CSF-1R signaling in tumor microenvironment

• CSF-1 is produced by tumor cells
  • Directs integration of macrophages into the tumor parenchyma
  • Modulates myeloid cells and macrophages toward an immunosuppressive phenotype
  • Promotes the production of growth factors and angiogenic factors by TAMs

• Tumor associated macrophages are abundant in pediatric solid tumors

• Inhibition of CSF-1R signaling in mouse models showed tumor regression in solid tumor models (pancreatic, prostate, breast, cervical, thyroid, glial cancers)

• Pigmented Villonodular Synovitis – rare locally aggressive MSK neoplasm with genetic mutation resulting in CSF1 overexpression. Pexidartinib has shown tumor volume reduction and symptom improvement in adults. Phase III trial.
15 Adult Pexidartinib Trials as of 7/2019

Completed: 7

• Relapsed or refractory Hodgkin’s lymphoma (II)
• Recurrent GBM (II)
• Advanced metastatic prostate cancer (pilot)
• I SPY 2 TRAIL – neoadjuvant and personalized adaptive novel agents to treat breast cancer
• BRAF-mutated unresectable or metastatic melanoma: combination with vemurafenib (I)
• Two Healthy subject studies

Ongoing: 9 (8 Phase I, 1 Phase III)

• Advanced incurable solid tumors focusing on TGCT (Tenosynovial Giant Cell Tumor): Phase I & Phase III
• Relapsed or refractory AML
• Advanced solid tumors in Asian subjects
• Combination in advanced incurable solid tumors
  • Paclitaxel
  • Pembrolizumab
  • Durvalumab (to open in Met/Advanced Panc and CRC)
• Newly diagnosed GBM: temozolomide and radiation
• Two healthy subject studies- one look at effect of low fat food on PK and one looking at CYP3A4 and CYP2C9 substrates
Key Toxicities from Adult Trials

- Most frequent \( \geq 20\% \) among all treated patients
  - Fatigue, nausea, decreased appetite, diarrhea, vomiting, anemia, constipation, hair color changes, headache, increased AST
- Severe skin reactions have been seen: erythema multiformis, DRESS
  - relationship to Pexidartinib has not been established
- Acute febrile neutrophilic dermatosis in AML subjects
- Severe idiosyncratic liver toxicity/liver failure in three patients with TCGT
- Laboratory changes
  - Liver enzymes
  - Mild decreases in ANC, platelet count, and hemoglobin
- The combination of pexidartinib with other chemotherapeutic or targeted therapies may increase the risk and/or severity of adverse findings associated with the individual agents
Pexidartinib Phase I/II Clinical Trial at NCI

- Phase I/II trial of PLX3397 in children and young adults with refractory leukemias and refractory solid tumors including neurofibromatosis type 1 (NF1) associated plexiform neurofibromas (PN)

- Principal Investigator: Rosandra Kaplan, M.D.

- Lead Associate Investigator: John Gold, M.D., Ph.D.

- Pexidartinib manufactured and supplied by Plexxikon, Inc. then Daychii Sanyko Inc.
Pexidartinib Phase I/II Clinical Trial at NCI

- Daily oral medication given in 30 day cycles
- Phase I
  - Rolling-six phase I design with 3 dose levels (DL) for patients with refractory solid tumors, leukemias, NF-1 related plexiform neurofibromas
  - Includes a Phase I expansion of up to 12 patients at the MTD
  - Maximum 24 patients.
  - Goal to determine toxicities and recommended Phase II dose
- Phase II – was placed on hold with liver toxicity but now open
  - Children and young adults with NF1 and plexiform neurofibromas
  - Maximum 17 patients.
  - Goal to determine the activity of Pexidartinib in this population
## Patient Enrollment

### Phase I

- 12 patients enrolled
  - Peritoneal mesothelioma, osteosarcoma (n=3), Ewings sarcoma, NF1-PN (n=3), CNS PNET, primary brain tumor, MPNST, rhabdomyosarcom
- 11 patients evaluable for MTD (>85% C1)
- NF1 PN patients received 1, 4, & 6 cycles
- Peritoneal mesothelioma patient continues on study in cycle 45 with CR
- All other patients now off study

### Phase I expansion

- 3 patients enrolled
  - AML, spindle cell sarcoma, aneurysmal fibrous histiocytoma
- NF1 enrollment was on hold due to concerns about liver injury in adult studies
- Amendment to add pediatric and adult patients with MDS
- Pediatric and young adult patients with relapsed and/or metastatic disease that has been rendered MRD negative.
## Patient Demographics and Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of patients (n=16)</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>Median (range) 16 (4 - 22) years</td>
</tr>
<tr>
<td>Sex</td>
<td>Female/Male 7/9</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White 10</td>
</tr>
<tr>
<td></td>
<td>African American 3</td>
</tr>
<tr>
<td></td>
<td>Asian 1</td>
</tr>
<tr>
<td></td>
<td>Hispanic 2</td>
</tr>
<tr>
<td>Performance Status</td>
<td>Median (Range) 90 (60-90) %</td>
</tr>
<tr>
<td>Tumor Type</td>
<td></td>
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<tr>
<td></td>
<td>Sarcomas 7</td>
</tr>
<tr>
<td></td>
<td>NF1 PN 3</td>
</tr>
<tr>
<td></td>
<td>CNS tumors 3</td>
</tr>
<tr>
<td></td>
<td>MPNST 1</td>
</tr>
<tr>
<td></td>
<td>Acute myeloid leukemia 1</td>
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<tr>
<td></td>
<td>Peritoneal mesothelioma 1</td>
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<tr>
<td>Prior Therapies</td>
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<tr>
<td></td>
<td>Surgery 13</td>
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<tr>
<td></td>
<td>Chemotherapy 12</td>
</tr>
<tr>
<td></td>
<td>Radiation 9</td>
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<tr>
<td></td>
<td>Immunotherapy 5</td>
</tr>
<tr>
<td></td>
<td>Targeted therapy 5</td>
</tr>
<tr>
<td></td>
<td>None 1</td>
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</table>
# Toxicity Profile in Pediatric/Adolescent Trial

Non Dose-Limiting Toxicities Possibly, Probably, and Definitely Related to Pexidartinib in Evaluable Patients During Cycle 1

<table>
<thead>
<tr>
<th></th>
<th>Maximum Grade of Toxicity</th>
<th>% of Patients with Toxicity</th>
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<tbody>
<tr>
<td></td>
<td>DL 1 (n = 3)</td>
<td>DL2 (n = 3)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Hematologic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>White blood cell count decreased</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lymphocyte count decreased</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neutrophil count decreased</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Platelet count decreased</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Prolonged APTT</td>
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<tr>
<td><strong>Constitutional</strong></td>
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</tr>
<tr>
<td>Anorexia</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Fatigue</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
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<tr>
<td>Nausea</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
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</tr>
<tr>
<td><strong>Hepatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT increased</td>
<td>2</td>
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</tr>
<tr>
<td>AST increased</td>
<td>1</td>
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</tr>
<tr>
<td><strong>Metabolism</strong></td>
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<tr>
<td>CPK increased</td>
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<td>Hypoalbuminemia</td>
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</tr>
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<td>Hypercalcemia</td>
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<td>Hypoglycemia</td>
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<td>Hyperglycemia</td>
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<td>1</td>
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<tr>
<td>Hypokalemia</td>
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<tr>
<td>Hypocalcemia</td>
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<td>1</td>
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<tr>
<td>Hypoglycemia</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Serum amylase increased</td>
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<td>1</td>
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<tr>
<td><strong>Neurologic/Psychiatric</strong></td>
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<tr>
<td>Anxiety</td>
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<td></td>
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<tr>
<td>Dizziness</td>
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</tr>
<tr>
<td>Headache</td>
<td>3</td>
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<tr>
<td>Non-cardiac chest pain</td>
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<tr>
<td>Pain</td>
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<tr>
<td>Restlessness</td>
<td>1</td>
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<tr>
<td><strong>Renal</strong></td>
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<tr>
<td>Creatinine increased</td>
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<tr>
<td>Glicosuria</td>
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<td></td>
</tr>
<tr>
<td>Proteinuria</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Dermatologic</strong></td>
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<td></td>
</tr>
<tr>
<td>Bruise</td>
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<tr>
<td>Hair depigmentation</td>
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<tr>
<td>Petechiae</td>
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<td></td>
</tr>
<tr>
<td>Rash</td>
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<td>1</td>
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<tr>
<td><strong>Oral/ENT</strong></td>
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<td></td>
</tr>
<tr>
<td>Dysgeusia</td>
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</tr>
<tr>
<td>Epistaxis</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mucositis</td>
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</tr>
<tr>
<td>Oral Thrush</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
## Reduced Absolute Monocyte Count

### Peripheral blood fold change from Day 1

<table>
<thead>
<tr>
<th>CBC Parameter (Fold Change from Day 1)</th>
<th>6-8 Days from C1</th>
<th>14-16 Days from C1</th>
<th>27-29 Days from C1</th>
<th>Repeated Measures ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P*</td>
<td>Med. Fold Change (95% CI)</td>
<td>N</td>
</tr>
<tr>
<td>WBC</td>
<td>13</td>
<td>0.15</td>
<td>0.89 (0.78, 1.07)</td>
<td>13</td>
</tr>
<tr>
<td>Hgb</td>
<td>13</td>
<td>0.08</td>
<td>1.03 (0.97, 1.08)</td>
<td>13</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>13</td>
<td>0.24</td>
<td>1.10 (0.84, 1.17)</td>
<td>13</td>
</tr>
<tr>
<td>Neutrophils % + bands</td>
<td>13</td>
<td>0.13</td>
<td>1.08 (0.96, 1.13)</td>
<td>13</td>
</tr>
<tr>
<td>Immature granulocytes</td>
<td>9</td>
<td>0.01</td>
<td>0.50 (0.08, 0.67)</td>
<td>9</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>13</td>
<td>0.79</td>
<td>0.96 (0.81, 1.29)</td>
<td>13</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>13</td>
<td>0.002</td>
<td>0.60 (0.40, 0.87)</td>
<td>13</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>10</td>
<td>0.03</td>
<td>0.78 (0.55, 1.04)</td>
<td>11</td>
</tr>
<tr>
<td>Basophils %</td>
<td>12</td>
<td>0.16</td>
<td>0.95 (0.50, 1.13)</td>
<td>11</td>
</tr>
<tr>
<td>ANC</td>
<td>13</td>
<td>0.74</td>
<td>0.90 (0.77, 1.26)</td>
<td>13</td>
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<tr>
<td>Abs Immature Granulocyte</td>
<td>9</td>
<td>0.09</td>
<td>0.50 (0.10, 1.00)</td>
<td>9</td>
</tr>
<tr>
<td>ALC</td>
<td>13</td>
<td>0.49</td>
<td>0.97 (0.68, 1.20)</td>
<td>13</td>
</tr>
<tr>
<td>AMC</td>
<td>13</td>
<td>0.001</td>
<td>0.58 (0.35, 0.81)</td>
<td>13</td>
</tr>
<tr>
<td>AEC</td>
<td>10</td>
<td>0.01</td>
<td>0.76 (0.50, 1.07)</td>
<td>11</td>
</tr>
<tr>
<td>ABC</td>
<td>12</td>
<td>0.06</td>
<td>0.92 (0.50, 1.00)</td>
<td>11</td>
</tr>
</tbody>
</table>

* Two-tailed unadjusted signed rank test p-value for Mu=1
## CSF1 Elevation marker of CSF1R targeting

### Serum cytokine analysis fold change from Day 1

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>N</th>
<th>P*</th>
<th>Med. Fold Change (95% CI)</th>
<th>N</th>
<th>P*</th>
<th>Med. Fold Change (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>12</td>
<td>0.021</td>
<td>1.25 (1.11, 1.44)</td>
<td>8</td>
<td>0.64</td>
<td>1.22 (0.81, 1.30)</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>12</td>
<td>0.79</td>
<td>0.92 (0.80, 1.25)</td>
<td>8</td>
<td>0.023</td>
<td>0.86 (0.66, 1.05)</td>
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<tr>
<td>IL-6</td>
<td>12</td>
<td>0.85</td>
<td>0.91 (0.78, 1.34)</td>
<td>8</td>
<td>0.95</td>
<td>0.98 (0.64, 2.39)</td>
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<tr>
<td>MCP-1</td>
<td>12</td>
<td>0.003</td>
<td>1.28 (1.01, 1.56)</td>
<td>8</td>
<td>0.023</td>
<td>1.27 (1.11, 1.54)</td>
</tr>
<tr>
<td>M-CSF</td>
<td>12</td>
<td>0.0005</td>
<td>3.70 (2.31, 5.24)</td>
<td>8</td>
<td>0.008</td>
<td>4.52 (2.40, 13.3)</td>
</tr>
</tbody>
</table>

* Two-tailed unadjusted signed rank test p-value for Mu=1
Conclusion

• CSF1/CSF1R axis targeting may be a potential approach to investigate in MDS

• Tumor Microenvironment Targeting including manipulation of Myeloid Cell and Stromal Cell Plasticity may be a potential promising approach in myelodysplastic anemia

• Clinical correlates examined in these trials may reveal new microenvironmental approaches in these diseases
Thank You!

Kaplan Lab
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Linda Vahdat
Shahin Rafii

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Lenny Wexler
Paul Meyers

The patients and their families
To the patients who inspire me and motivate me every day!
C-kit and CSF1R signaling in plexiform neurofibromas microenvironment

- **C-kit in PN Microenvironment**
  - Nf1-/- Schwann cells increase production of stem cell factor (scf-1)
  - Increased response to scf-1 by Nf1+/- mast cells vs wild type mast cells
  - Increased c-kit signaling between mast cells and tumor cells contribute to the inflammatory microenvironment
  - Imatinib (small molecule TKI that targets c-kit) with some clinical activity in the PN treatment

- **Macrophages in PN Microenvironment**
  - ~50% of PN are macrophages - may be inflammatory effectors.
  - Macrophage infiltration correlates with disease progression
  - Targeting c-kit signaling and CSF1R signaling within the PN microenvironment may decrease tumor growth
Hematopoietic Stem and Progenitor Cells (HSPCs) Expand During the Pre-Metastatic Window

Mouse HSPCs are LSK cells: Negative for Lineage markers, expressing Sca1 and cKit

LSK HSPCs are Increased in Circulation of Tumor-Bearing Mice
Circulating HSPCs Develop into Myeloid Derived Suppressor Cells (MDSCs) in Metastatic Tissues

Pre-Metastatic E0771 Breast CA CD45.2+ Recipient

Pre-Metastatic E0771 CD45.1+ Donor

Sorted HSPCs from BM

24 hrs

FACS analysis of Lung

CD11b

CD45.1 Cells in Lung

Control

Breast CA

Giles et al Ca Res 2016
Myeloid Cells and Stromal Cells Maintain Tissue Homeostasis

Myeloid Cells

Stromal Cells

Homeostasis

- Apoptotic cells
- Proteins
- Phospholipids
- Toxins

Clearance

Self-renewal

Crosstalk

TRM

- MSC self-renewal
- Pericyte
- Adipocyte
- Osteocyte
- Connective tissue cell
- Muscle cell
- Lung cell
- Gut epithelial cell

- Immune cells
- Epithelial cells
- Endothelial cells
- Fibroblasts

MoM

Bone marrow

Stem cell

Differentiation

Niche
Bone Marrow-derived Cells are Abundant at the Invasive Edge of the Growing Tumor

B16 melanoma Day 15

GFP+ Bone marrow-derived cells (BMDCs)

DAPI

TE = Tumor Edge
Bone Marrow-derived Cells Form Clusters in Distant Metastatic Sites Such as the Lung

GFP\(^+\) - BMDCs
Identifying the Pre-Metastatic Niche

- Dynamic, specialized microenvironment that supports disseminated tumor cells
- Activated mesenchymal cells with associated extracellular matrix remodeling
- Bone Marrow (BM) Derived Myeloid Cells

Pre-Metastatic Site

- Stroma
- Pericytes & Fibroblasts
- Hematopoietic Cells
- Bone Marrow (BM)

Tumor Cell Secreted Factors
- Cytokines
- Chemokines
- Exosomes
Defining pre-metastatic and metastatic windows

- Whole lung bioluminescence and flow cytometry to quantify total tumor cell burden
- Sequential lung sectioning to identify GFP+ micrometastases
## Characterized Murine Models of Spontaneous Metastasis

<table>
<thead>
<tr>
<th>Type</th>
<th>Orthotopic Primary</th>
<th>Occurence of Spontaneous Metastasis</th>
<th>Location of Mets</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16-F10 Melanoma Subdermal Flank</td>
<td>~ Day 18</td>
<td>Lung, Lymph nodes</td>
<td></td>
</tr>
<tr>
<td>B16-F0 Melanoma Subdermal Flank</td>
<td>none</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>E0771 Breast Carcinoma (Breast CA)</td>
<td>~ Day 20</td>
<td>Lung, Lymph nodes</td>
<td></td>
</tr>
<tr>
<td>76-9 Embryonal Rhabdomyosarcoma (eRMS)</td>
<td>~ Day 35</td>
<td>Liver, Lung, Lymph nodes</td>
<td></td>
</tr>
<tr>
<td>M3-9-M Embryonal Rhabdomyosarcoma (eRMS)</td>
<td>~ Day 35</td>
<td>Lung, Lymph nodes</td>
<td></td>
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<tr>
<td>K7M2/K12 High and Low Metastatic Osteosarcoma</td>
<td>~ Day 30</td>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>KPC16 Pancreatic Carcinoma</td>
<td>~ Day 28</td>
<td>Liver</td>
<td></td>
</tr>
</tbody>
</table>
Normal cells in the tumor microenvironment can be “educated” or subverted by cancer cells to promote malignant progression. Metastatic progression can be enabled by a creation of a dynamic microenvironment.
Activated Stromal Cells Upregulate Fibronectin to Create the Pre-Metastatic Niche

Kaplan et al 2005 Nature
Do GEMys impact metastasis

• Can GEMys impact primary tumor growth and survival of rhabdomyosarcoma bearing mice?

• Do GEMys impact lung metastasis?

• Can GEMys treat established metastatic disease?

• Is chemotherapeutic efficacy impacted by GEMys?

• Do GEMys work in other tumor models?
Can GEMys impact primary tumor growth, survival, and metastasis of Rhabdomyosarcoma tumor-bearing mice?

**Day**
- 0: Inject 5E5 M3-9-M RMS mCherry-FFLUC *i.m.*
- 12: Inject GEMys
- 27: Harvest lungs for IVIS

**Follow for survival (8 per group)**

**Treatment**
- No Treatment
- Non-transduced myeloid cells
- GEMys
- IL-12 Pre-treated myeloid cells
GEMys delay primary tumor growth and extend survival time.
GEMys reduce lung metastasis
Which immune cell types are required for GEMy function?

**Antibody Treatment**

- Inject mice with M3-9-M tumor
- Day 0
- Every 3-5 days for duration of experiment

**Induction**

- GEMys
- Day 9, 11, 12

**Depletion Antibody**

- Isotype
- Anti-CD4
- Anti-CD8
- Anti-NK1.1
What immune cell types are required for GEMy function?

1. CD8+ T cells are necessary for GEMy function.
2. CD4+ T cells contribute to GEMy function.
3. NK1.1+ cells are not required for GEMy function.
Sorting Out the Toys
Recurrent, Metastatic Osteosarcoma Patient Demonstrate Stromal and Immune Plasticity
Activated Stromal Cell Cluster

Key Genes in Cluster 4
PDGFRα
TAGLN
ACTA2
PDGFRβ
RGS5
POSTN
Diverse Myeloid Cell Populations

Key Genes:
- CSF1R
- CD163
- CEBPB
- MMP9
- HIF1a
The Pre-Metastatic Niche: The Process of Building A Metastatic Microenvironment

- Metastatic Tumor Cells
- Bone Marrow-Derived Immune Cells
- Mesenchymal Cells
- Matrix/Fibronectin
CENTRAL ILLUSTRATION: Endothelial to Mesenchymal Transition in Cardiovascular Disease: Key Mechanisms and Clinical Translation Opportunities

**Endothelial To Mesenchymal Transition**

**Key signaling pathways:**
- Transforming growth factor-β
- Cellular metabolism
- Non-coding RNAs
- Epigenetic
- Oxidative stress and inflammation
- Wnt/β-Catenin
- Fibroblast growth factors
- Other

**Role in homeostasis and disease:**
- Cardiac development
- Atherosclerosis
- Valvular disease
- Fibroelastosis
- Vein graft remodeling
- Cardiac fibrosis
- Pulmonary hypertension
- Other

Myeloid Derived Suppressor Cells
Genetic Changes in Cells Can Cause Cancer
What Causes Genetic Changes

- Heredity
- Viruses
- UV Radiation
- Smoking
- Chemicals
- Cells Dividing

Developmental Abnormality
Cancer is when a cell develops changes in its DNA and grows faster or has a longer life cycle than the other similar neighbor cells.
Investigations into Stromal Cell Populations

Key Pathways:
TGFB
Extracellular Matrix
Remodeling

Key Genes:
SMAa
NG2
PDGFRa
KLF4

Vascular Smooth Muscle Cells
Pericytes
Fibroblasts
Mesenchymal Stem Cells

- Transgelin
- Calponin
- ACTA2
- CD73
- NG2
- RGS5
- CD146
- MYH11
- PDGFRβ
- FAP
- FSP-1
- CD90
- Sca1
- CD105
- CD34
- Nestin
- Gli1
- CD51

Key Genes:
SMAa
NG2
PDGFRa
KLF4
Perivascular Cell Specific KLF4 Deletion Mouse Model to Inhibit Perivascular Cell Plasticity

Stable eYFP labeling and inactivation of the KLF4 gene in perivascular cells

Murgai et al Nature Med 2017
Perivascular Cells Become Activated with Inflammation/Disease and Lose Marker Expression

Contractile Perivascular Cells

Perivascular Markers: ACTA2, MYH11

Key Functions:
- Contraction
- Vessel homeostasis

Activated Perivascular Cells

Perivascular Markers: KLF4, ACTA2, MYH11

Key Functions:
- Proliferation
- Migration
- Extracellular Matrix Remodeling

Inflammation
KLF4 is expressed in perivascular cells in pre-metastatic lung

How does the distant primary tumor induce perivascular KLF4 expression?

Murgai et. al., Nature Med 2017
Perivascular cells proliferate in pre-metastatic lungs

Murgai et. al., Nature Med 2017

HBSS control

Tumor-bearing
Metastasis is a Long Standing Problem

“When a plant goes to seed, its seeds are carried in all directions, but they can only live and grow if they fall on congenial soil.”

–Stephen Paget, The Lancet 1889

Tumor Cell Heterogeneity

Different Microenvironments

Reciprocal Nature of the Process
All tissue is in a state of balance with cell growth and death maintaining the organ structure. Every organ has specialized cells and other important cells such as stromal cells such as fibroblasts, endothelial cells and immune cells including T cell and macrophages.
Metastasis is the spread of tumor cells from one site to another site.
Hematopoietic Stem and Progenitor Cells (HSPCs) Expand During the Pre-Metastatic Window

Mouse HSPCs are LSK cells: Negative for Lineage markers, expressing Sca1 and cKit

Giles et al Ca Res 2016
LSK HSPCs are Increased in Circulation of Tumor-Bearing Mice
Mobilization of HSPCs Enhances Experimental Metastasis

Inject mice:
- PBS
- AMD3100

Inject mice:
- PBS or AMD3100
- BCA (ffluc-eGFP)
tail-vein

Readout:
- IVIS imaging (weekly)
- Survival Curve

HSPCs in Blood
1 hour after PBS or AMD3100

Survival Curve

PBS
AMD3100

Days following tail-vein tumor injection

Percent survival

Days following E0771 f/g t.v.