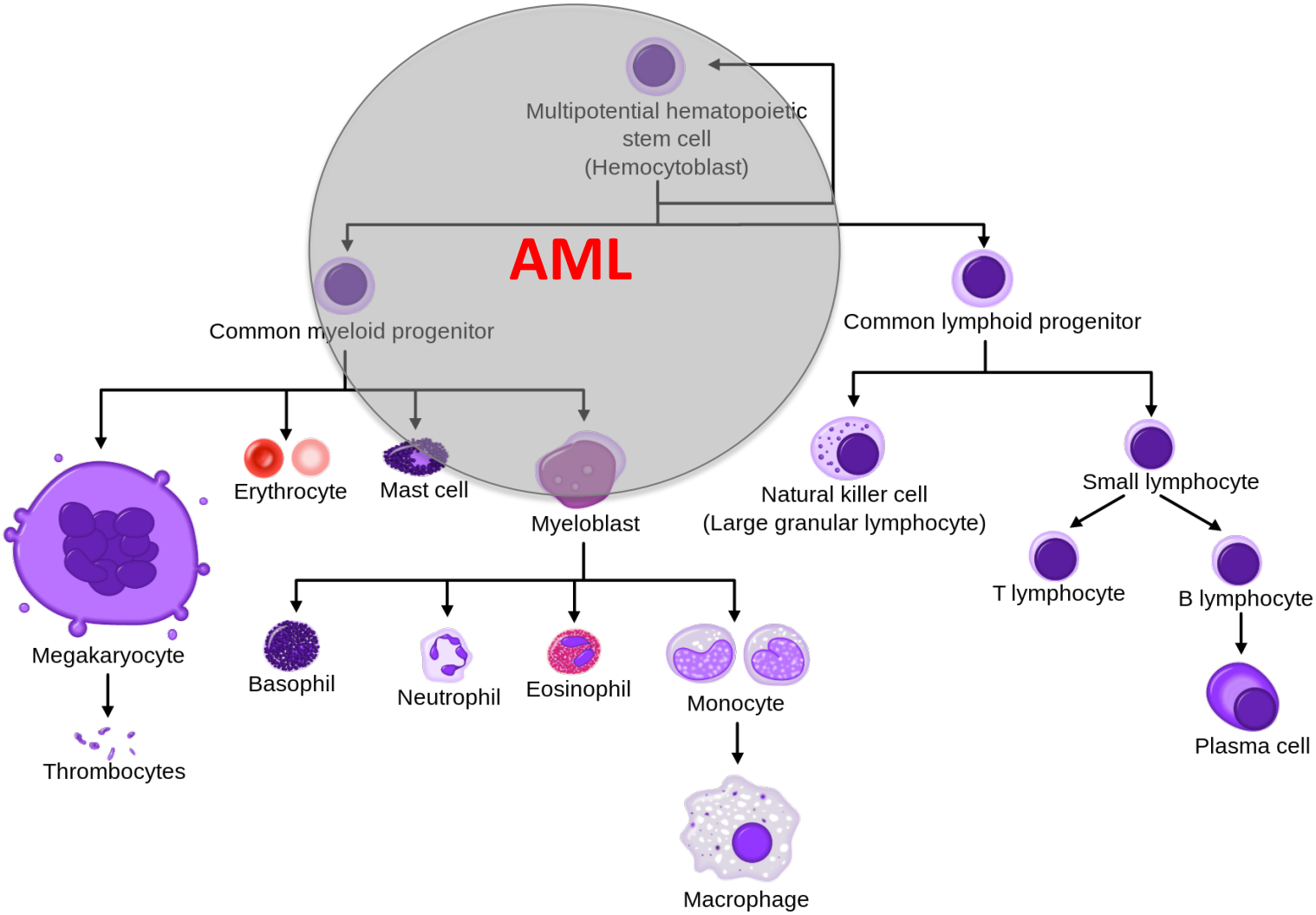
CD200 is an LSC-specific mechanism of immune evasion in AML

Clinical & Translation Sciences

Candidacy Exam

Acute Myeloid Leukemia



Relapsed AML

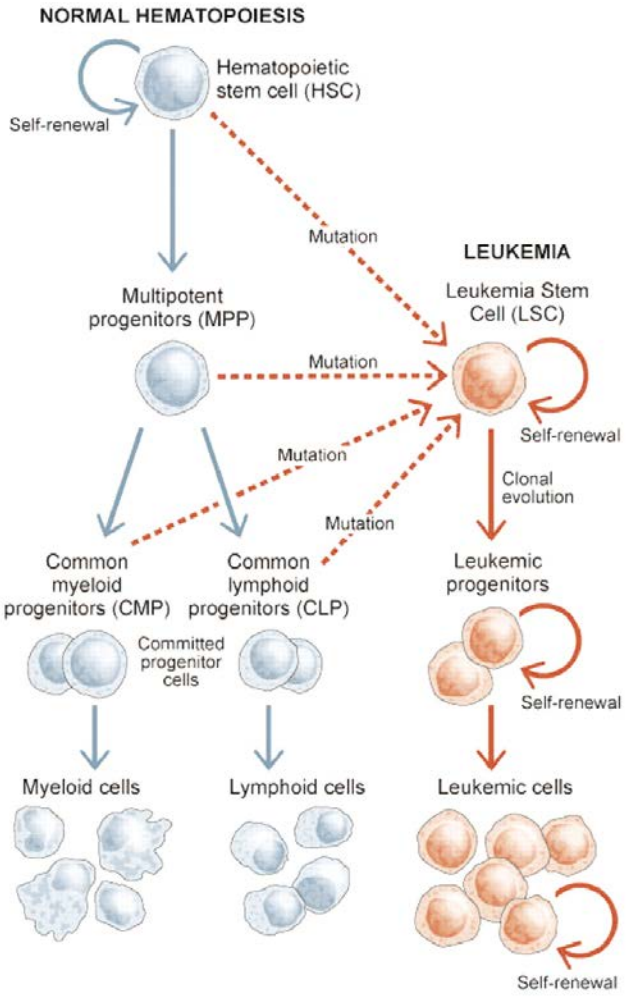
- Most patients achieve remission after front-line chemotherapy
  - 80% for patients <60yo
  - 50% for patients >60yo
- However, the 5-year survival rate of AML is only 25%

→ Most patients relapse
→ Relapsed disease has poor prognosis

Leukemia stem cells

- rare
- capable of both self-renewal and blast production
- functionally defined as cells that can engraft
- quiescent
- chemoresistant

→ LSCs are the source of relapsed disease



Immunotherapy cures AML

- allogeneic stem cell transplant (allo-HSCT) is curative in
  - 35% patients in complete remission
  - 25% relapsed
- cured by graft vs host immune response

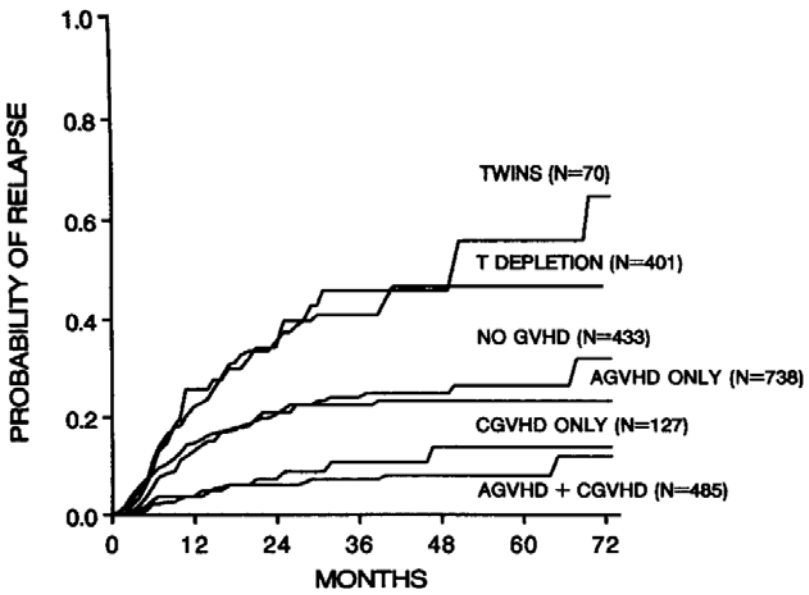
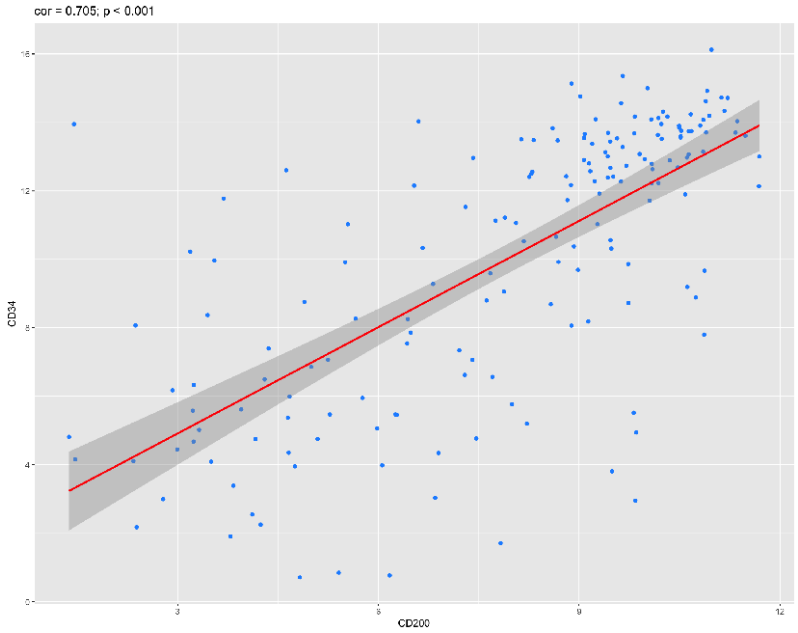


Fig 1. Actuarial probability of relapse after bone marrow transplantation for early leukemia according to type of graft and development of GVHD.

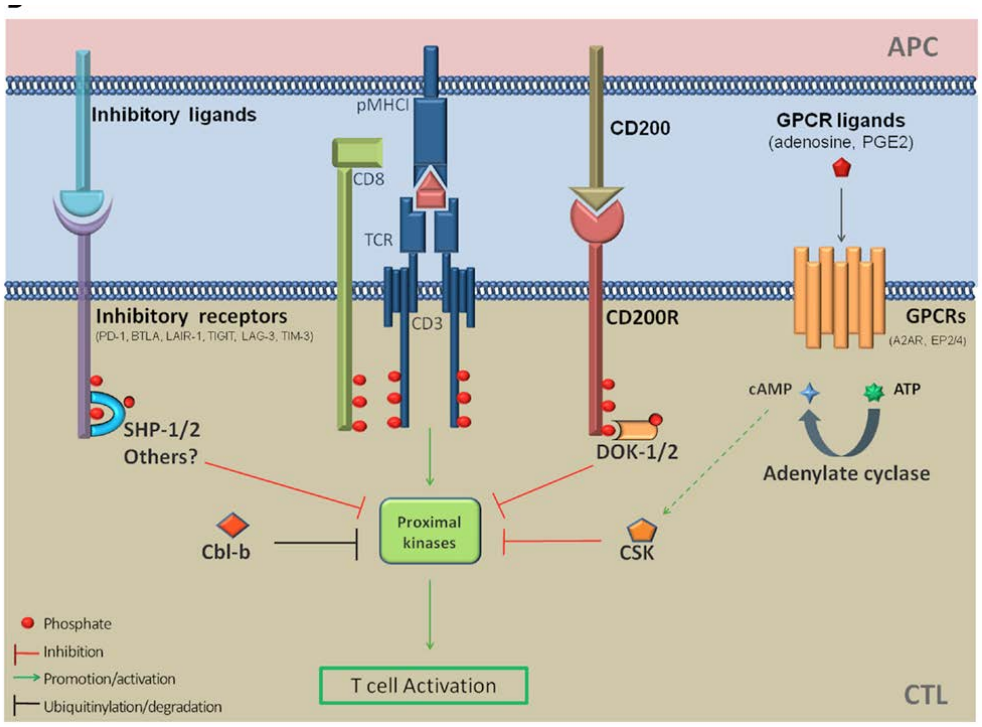
→ LSCs can be cleared by the immune system
→ efficacy of allo-HSCT limited by acute GvHD

CD200 is a stem cell marker

- type-1 transmembrane glycoprotein
- broadly expressed
- binds the CD00 receptor (CD200R)
  - only expressed on myeloid and a subset of lymphocytes
- correlates with epithelial stem cell markers
- significantly enriched in CD34+ AML



CD200 is immunosuppresive

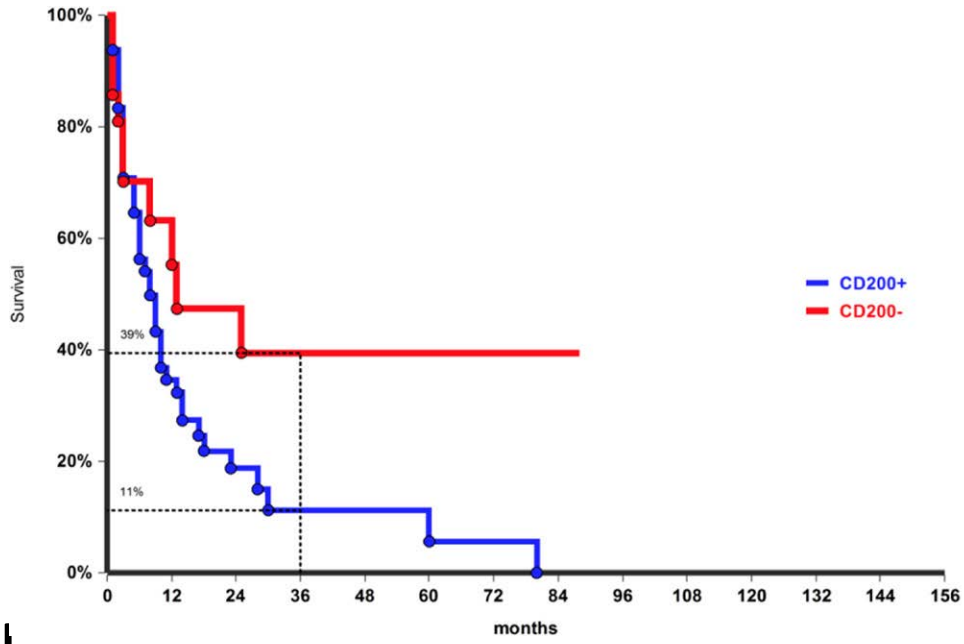


- direct immunosuppressive effects on myeloid cells (macrophages, mast cells), as well as NK and T cells
- shifts cytokine production from Th1 to Th2
- induces accumulation of FOXP3+ regulatory T cells
- induces the secretion the enzyme IDO

Fig. Lloyd, A., Vickery, O. N., & Laugel, B. (2013). Beyond the antigen receptor: editing the genome ofT-cells for cancer adoptive cellular therapies.

Clinical relevance of CD200

- correlated with 50% reduction in odds of CR
- significantly reduced overall survival in CD200+
- Samalizumab is a CD200 mAB in clinical trials



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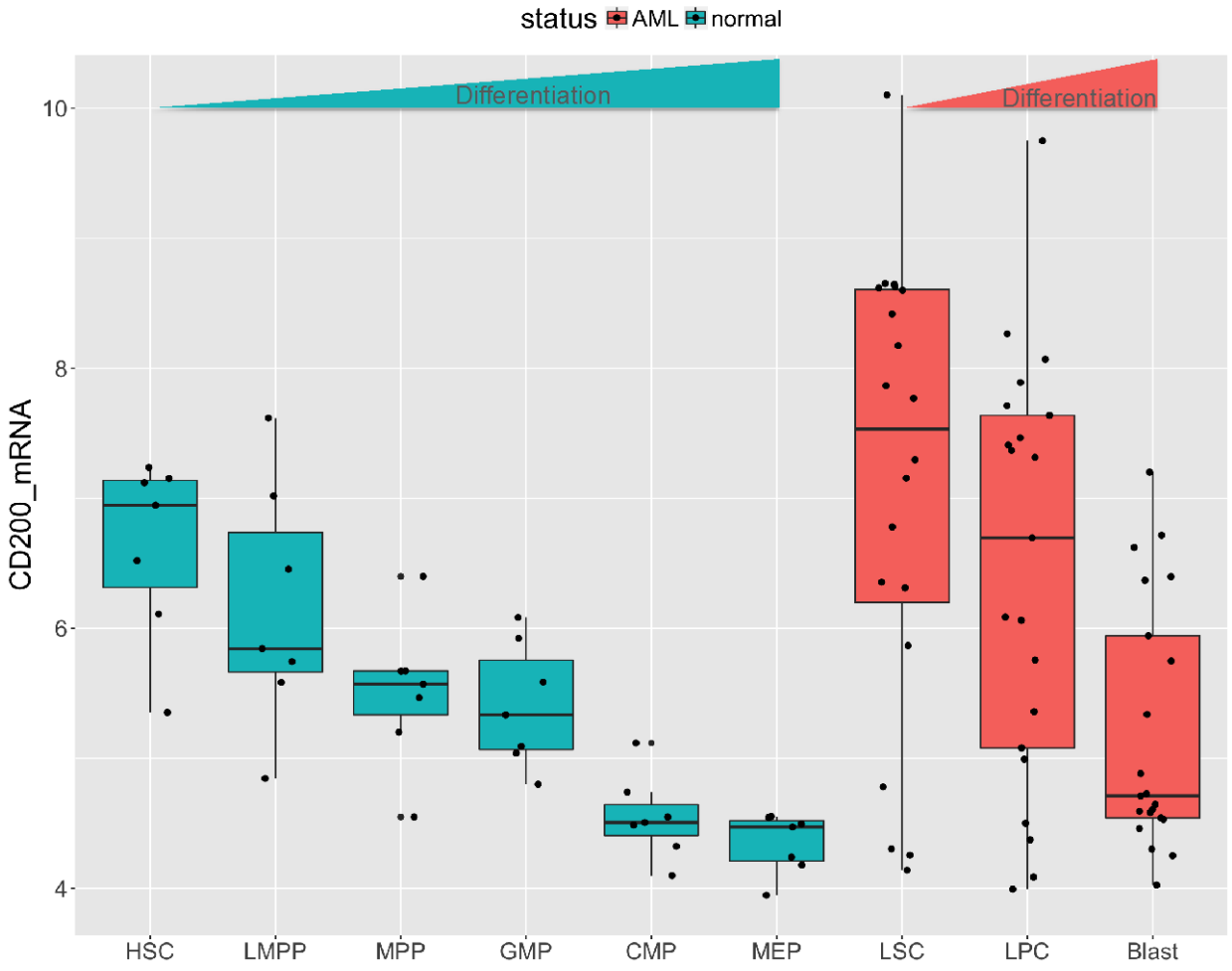

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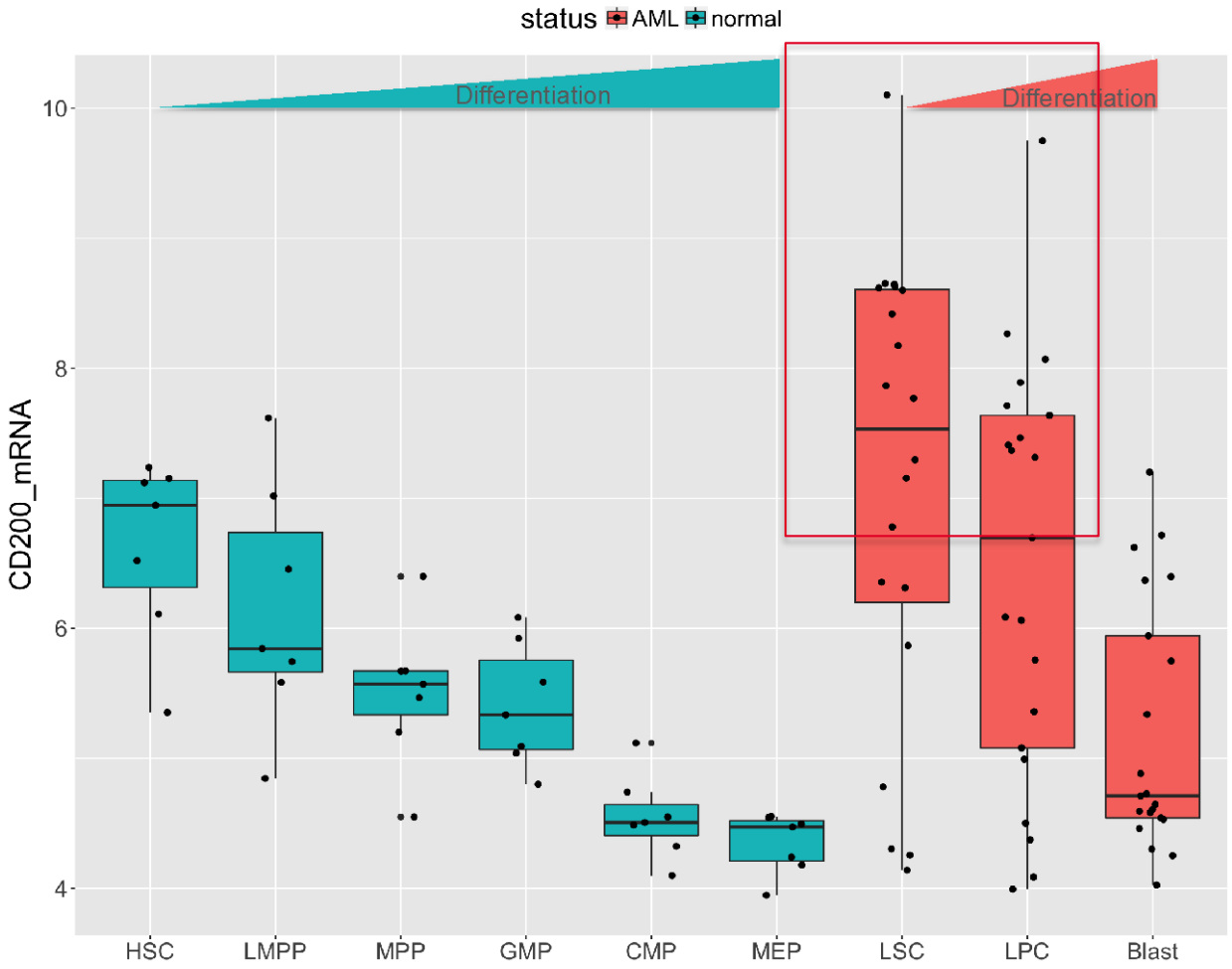
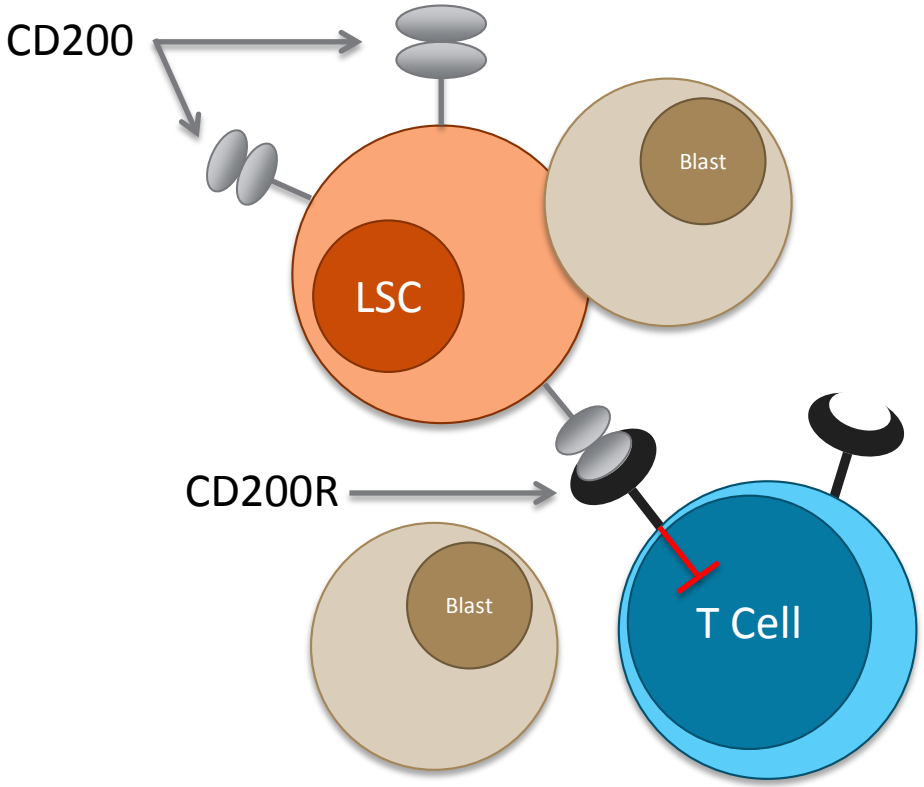


Hypothesis

In a subset of AML, high expression of CD200 is an LSC-specific immune evasion mechanism and CD200 blockade will result in the clearance of LSCs



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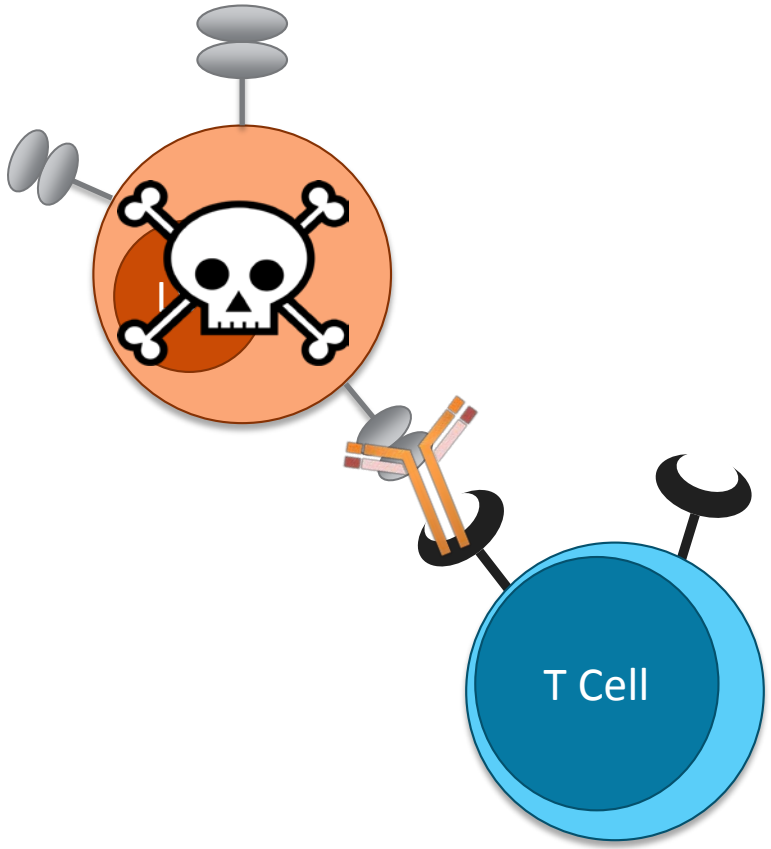
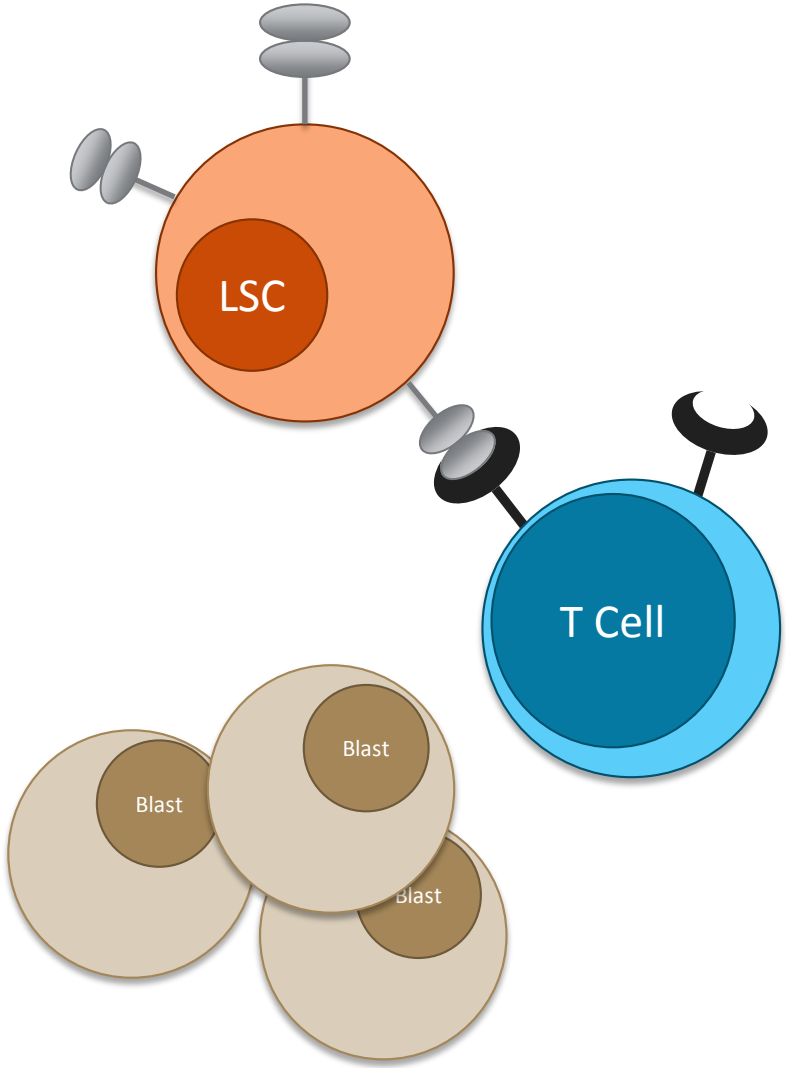


Specific Aims

Aim 1. Characterize CD200 receptor and ligand distribution on AML LSCs, blasts, and immune cell subsets in primary human AML samples using CyTOF

Aim 2. Determine the role of LSC-expressing CD200 on the cytotoxic function of CD8+ T cells

Aim 3. Evaluate the utility of CD200 inhibition as a mechanism for eliminating AML LSCs *in vivo*



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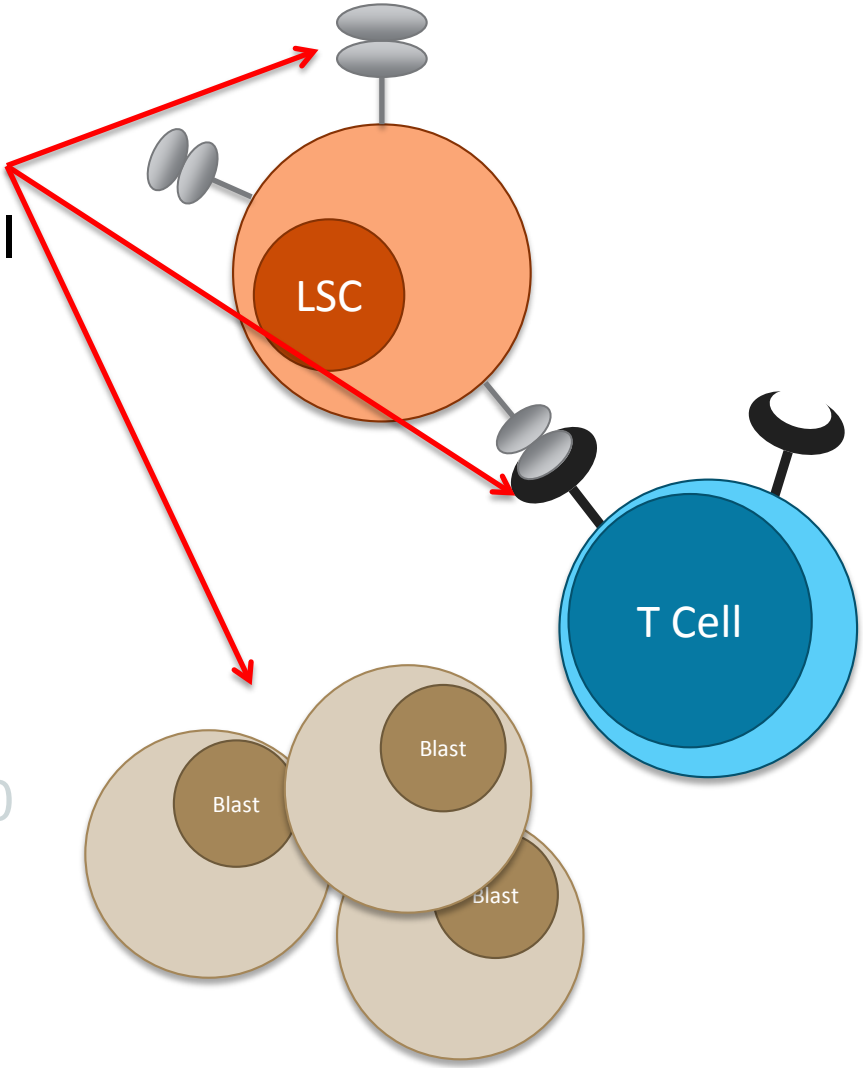

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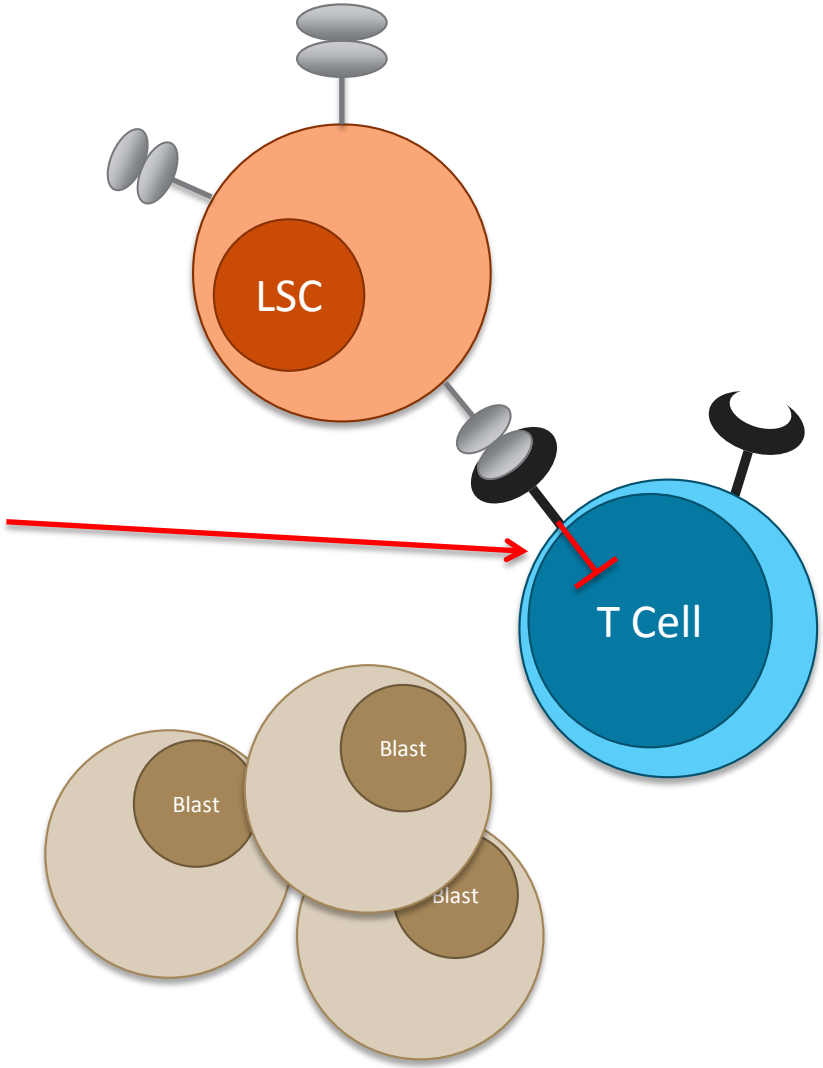

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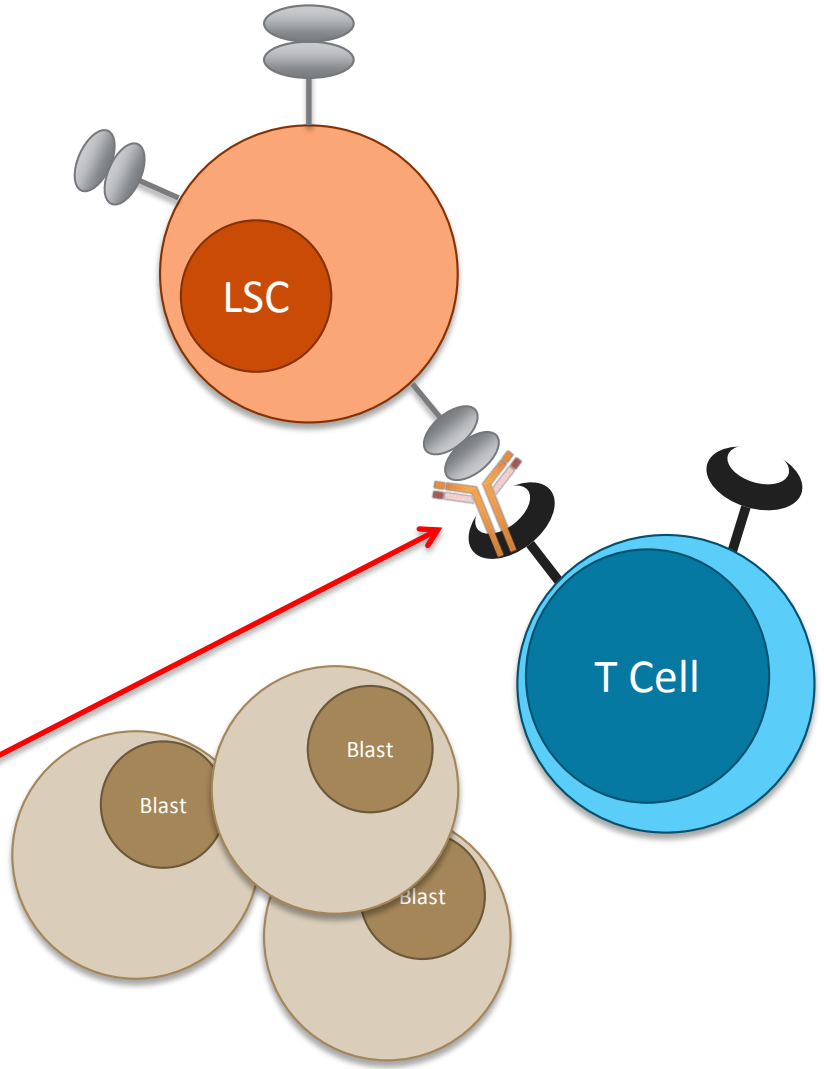
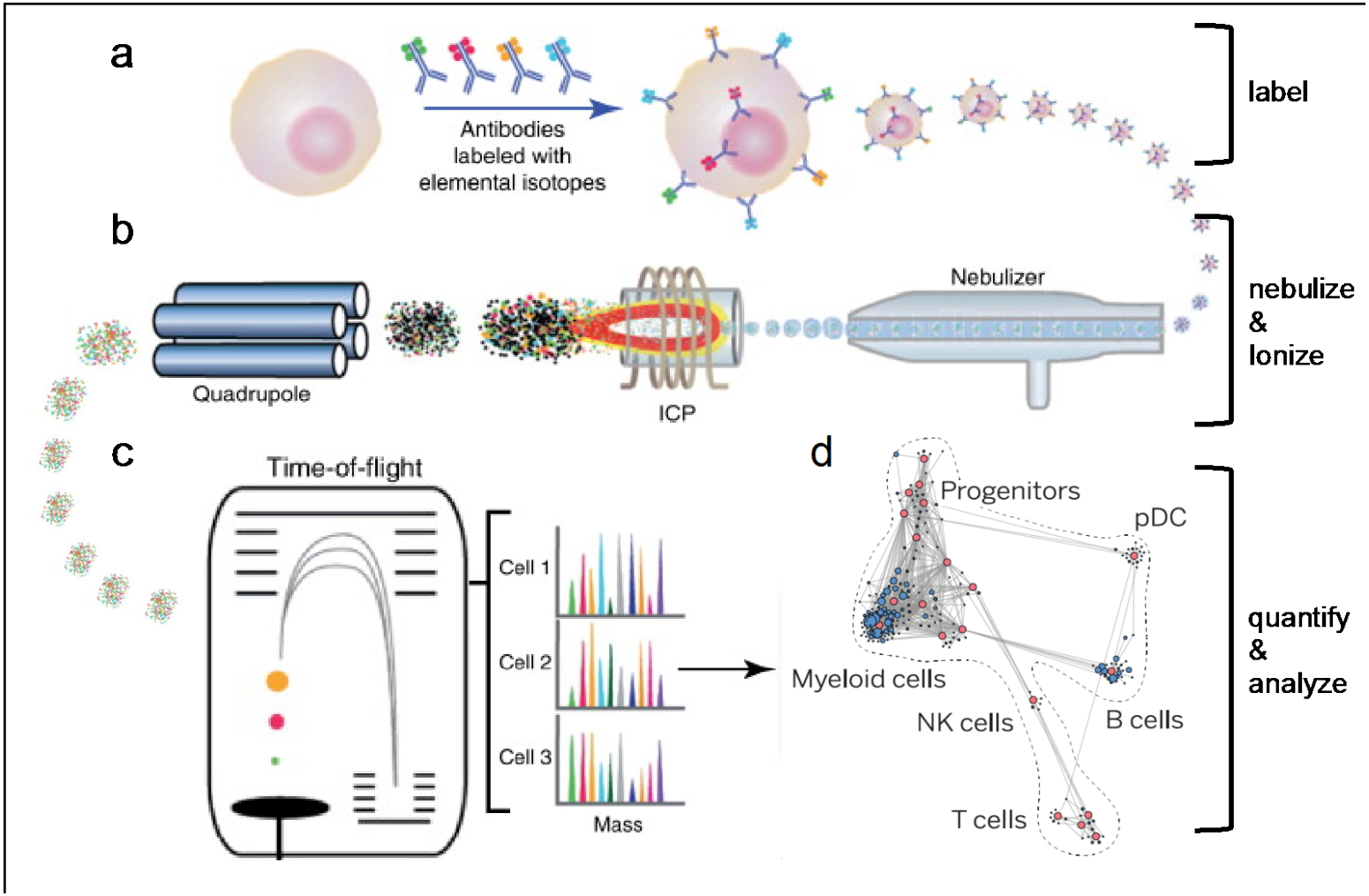
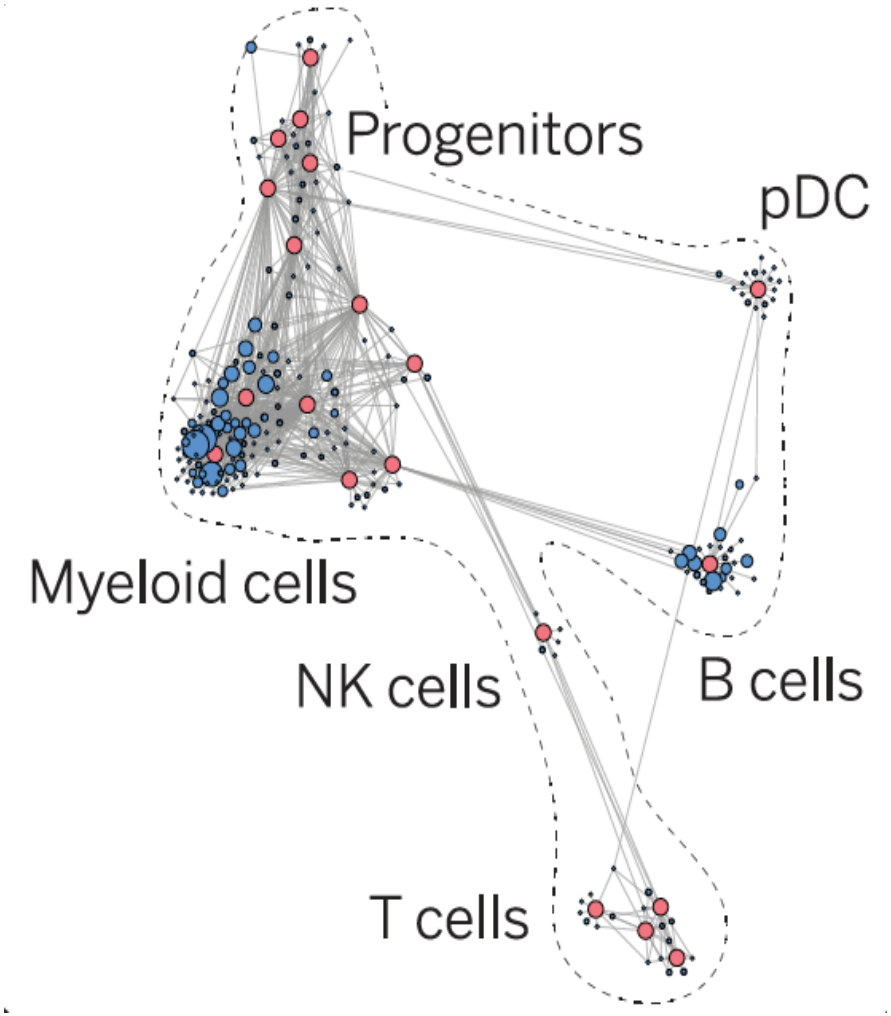


CyTOF



Modified from Bendall, S.C., et al., *A deep profiler's guide to cytometry.* Trends Immunol, 2012. **33**(7): p. 323-32.

SCAFFoLD



- Single-cell analysis by fixed force and landmark-directed maps
- exploits knowledge of normal hematopoiesis
- nodes (red) are normal landmarks
- clusters (blue) are projected into normal map

Modified from Bendall, S.C., et al., *A deep profiler's guide to cytometry.* Trends Immunol, 2012. **33**(7): p. 323-32.

Aim 1. Characterize CD200 receptor and ligand distribution on AML blasts, LSCs, and immune cell subsets in primary human AML samples using CyTOF

- *Motivation*
  1. build an extension to the SCAFFoLD platform to identify outlier cell subsets
  2. characterize and compare expression of CD200 on the surface of LSCs, blasts, and bone marrow resident immune cell subsets at the single-cell level
- **Hypothesis**
  1. Euclidean distance scoring allow for identification of outliers
  2. CD200 will be most highly expressed in abnormal cells populations enriched for known stem cell markers (CD34, CD123, or TIM-3)

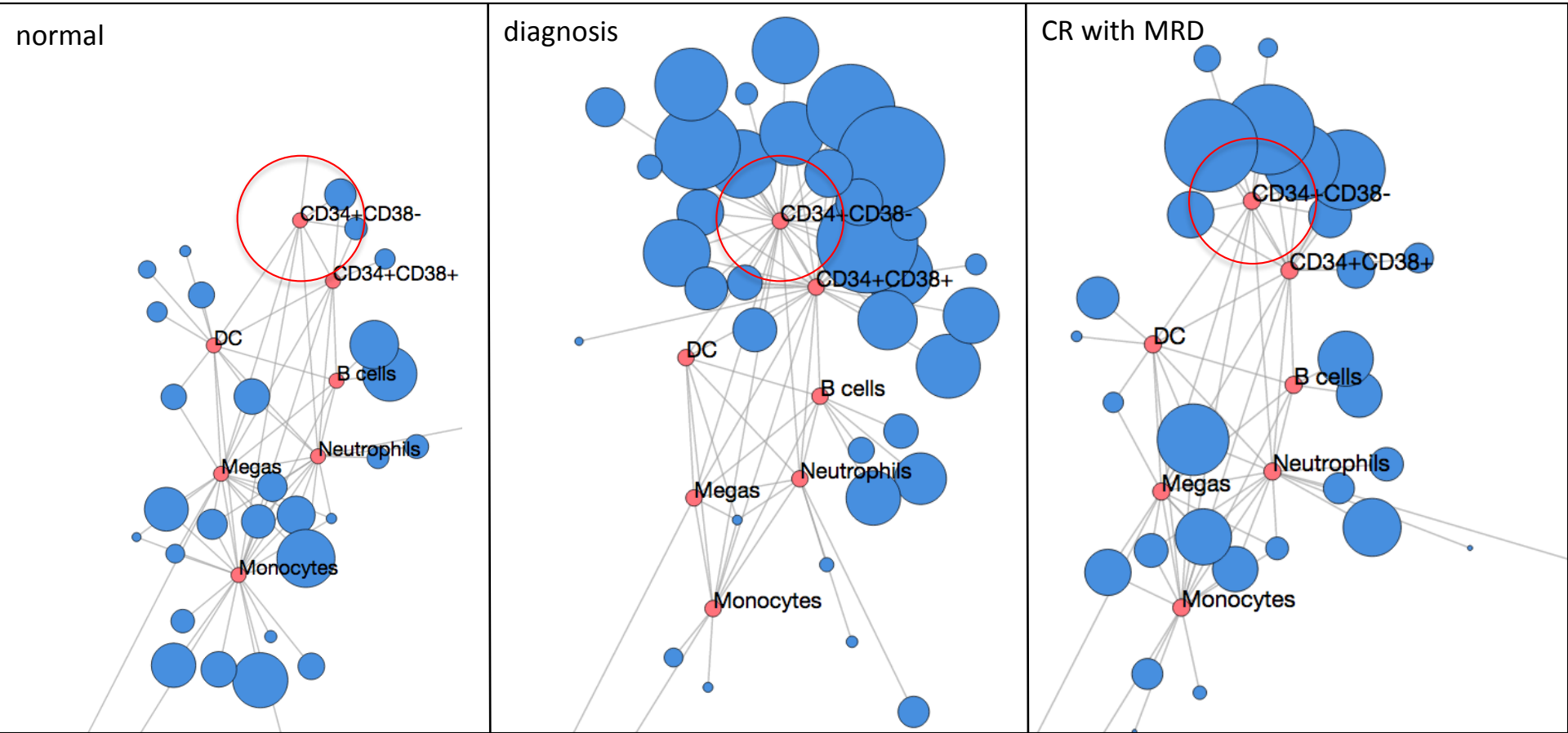
Aim 1. Characterize CD200 receptor and ligand distribution on AML blasts, LSCs, and immune cell subsets in primary human AML samples using CyTOF

- **1.1** Develop a novel method for identifying unique subsets of AML and of the corresponding immune microenvironment

- **1.2** Characterize CD200 expression across AML blasts, LSCs, and immune cell microenvironment using CyTOF

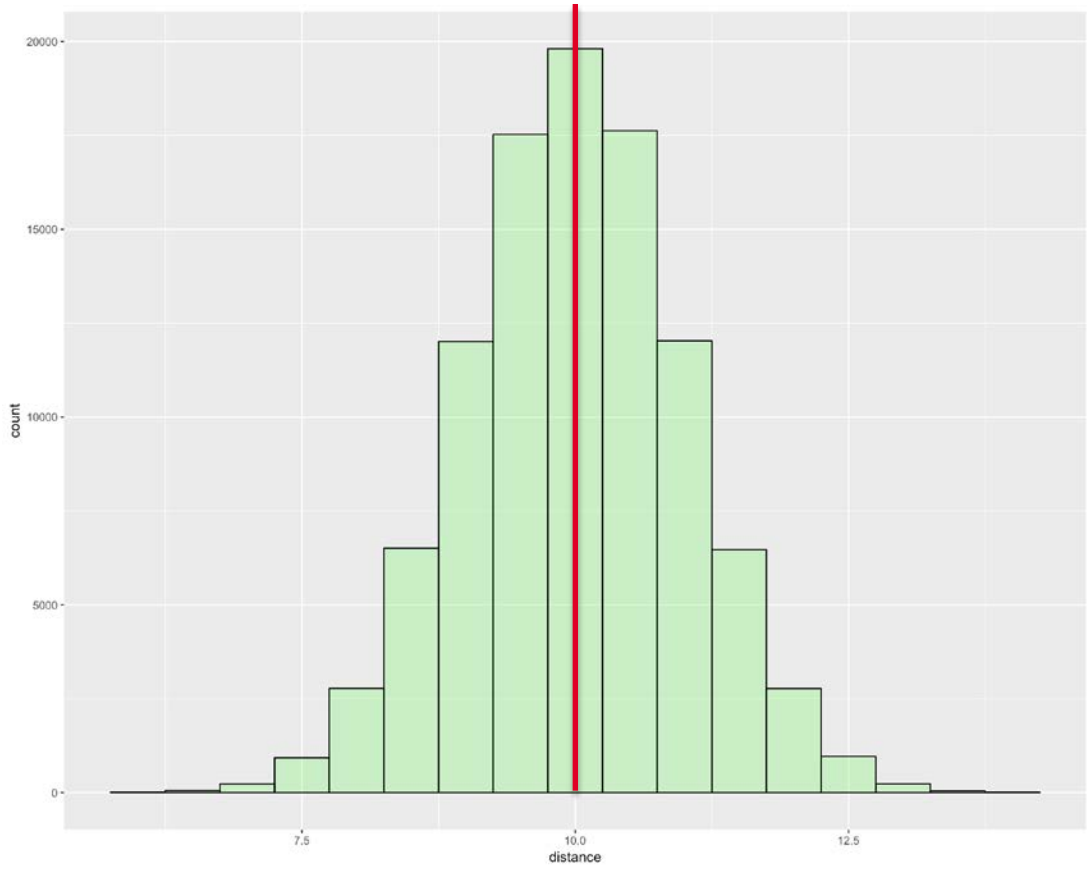
1.1 Develop a novel method for identifying unique subsets of AML and the corresponding immune microenvironment

- Input: existing clustered SCAFFoLD data for 10 normal and 10 AML BM samples
- Approach: 1) use Euclidean distance from nearest neighbor to define a statistic



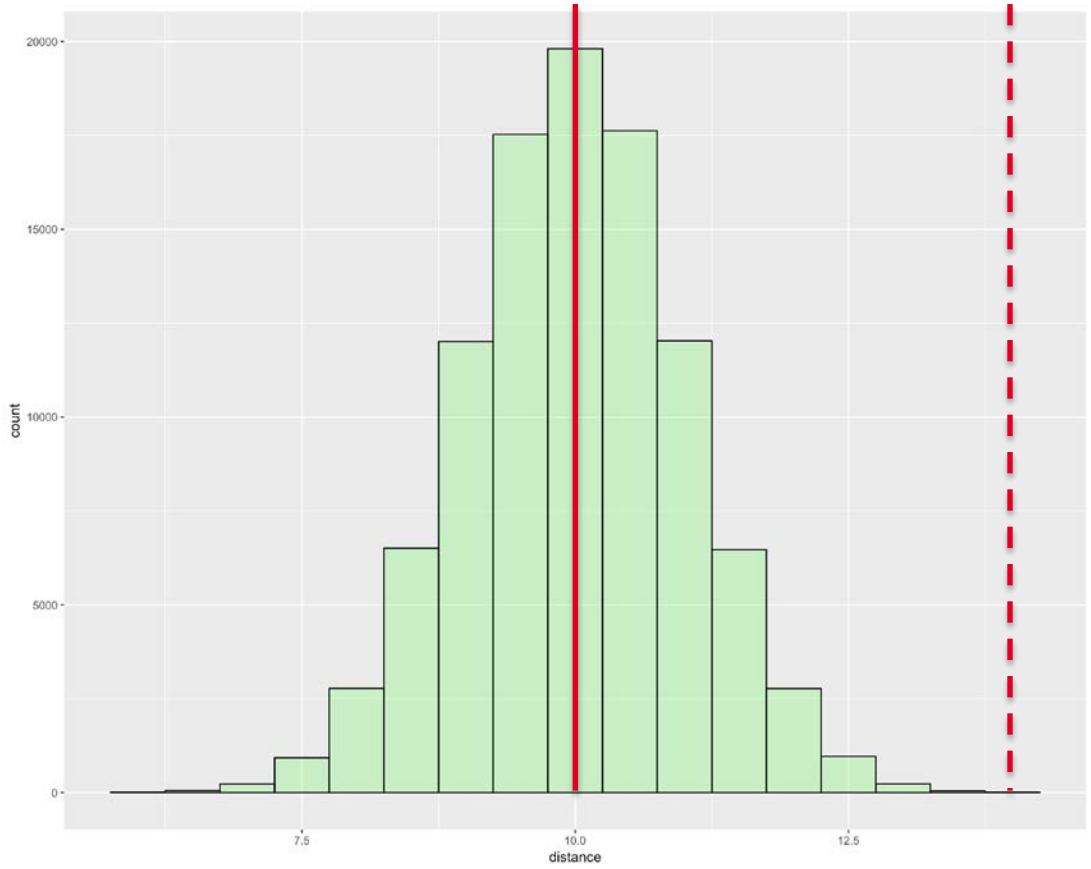
1.1 Develop a novel method for identifying unique subsets of AML and the corresponding immune microenvironment

- Approach: 2) use resampling techniques from the 5 normal BM biopsies to define the null distribution of "normal" distance measurements per landmark.



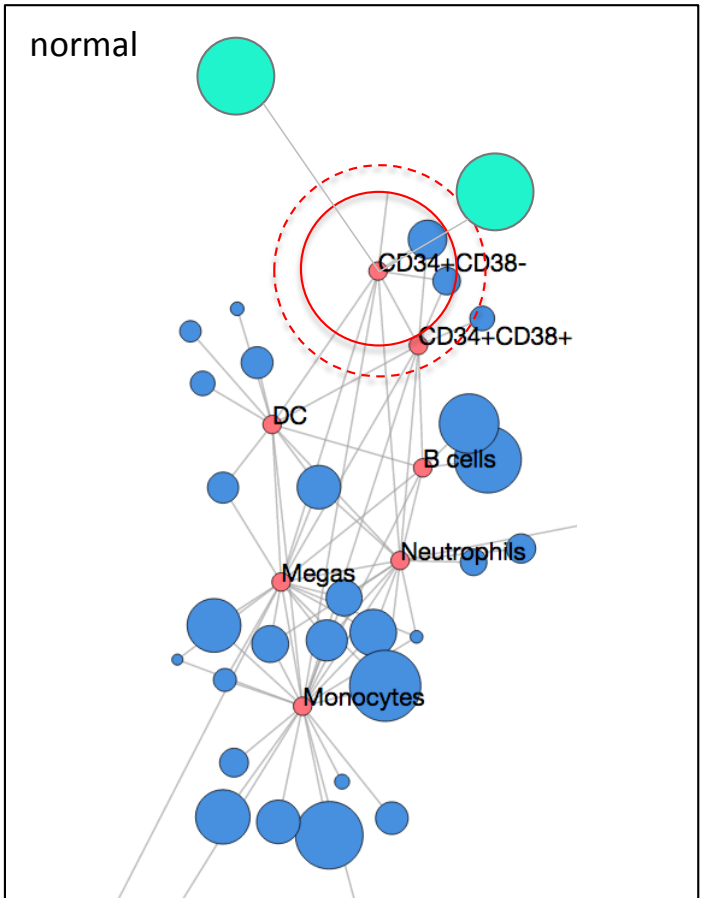
1.1 Develop a novel method for identifying unique subsets of AML and the corresponding immune microenvironment

- Approach: 3) set a threshold for detecting outliers (4 standard deviations from the mean, to start)



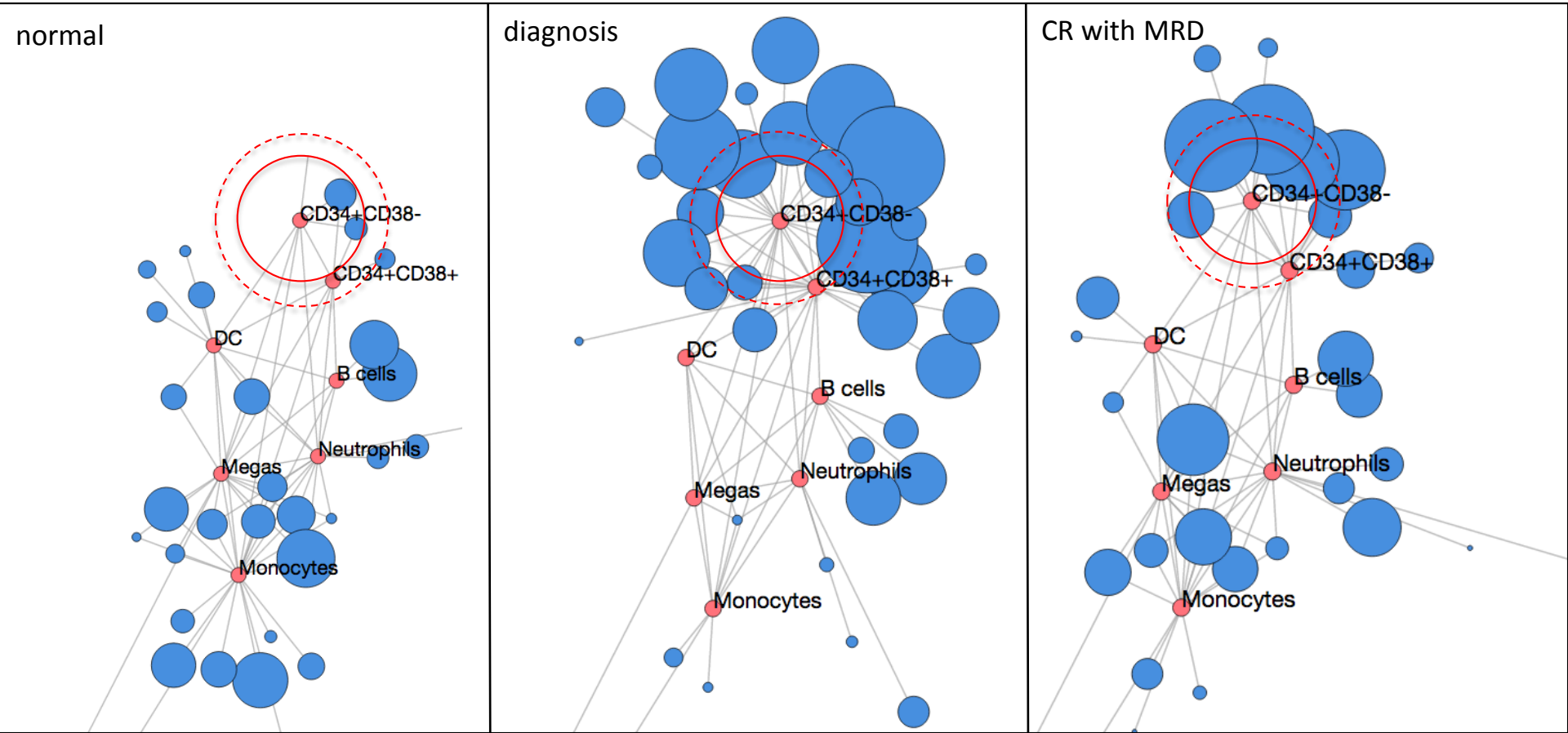
1.1 Develop a novel method for identifying unique subsets of AML and of the corresponding immune microenvironment

- Validate: the method will be tested using immunophenotypically abnormal spike-in data



1.1 Develop a novel method for identifying unique subsets of AML and of the corresponding immune microenvironment

- Measure:
  - identification and quantification of “abnormal” cells
  - abundance and characteristics of “normal” cells



Aim 1.2 Characterize CD200 expression across AML blasts, LSCs, and immune cell subsets with CyTOF

- Input: normal and AML bone marrow biopsies
- Approach: CyTOF with optimized antibody panel
- Measure:
  - normal vs outlier cell abundance
  - protein expression by cell type

Immuno-phenotyping	Immune modulators
CD3	CD80 (CTLA-4L)
CD4	CD86 (CTLA-4L)
CD8a	CD137 (41BB)
CD25	CD152 (CTLA-4)
CD27	CD200
CD28	CD200R1
CD44	CD223 (LAG-3)
FOXP3	CD272 (BTLA)
HLA-DR	CD273 (PD-L2)
CD33	CD274 (PD-L1)
CD34	CD278 (ICOS)
CD38	CD279 (PD-1)
CD45	CD357 (GITR)
CD90	OX40
CD117	TIGIT
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AML/LSC
other

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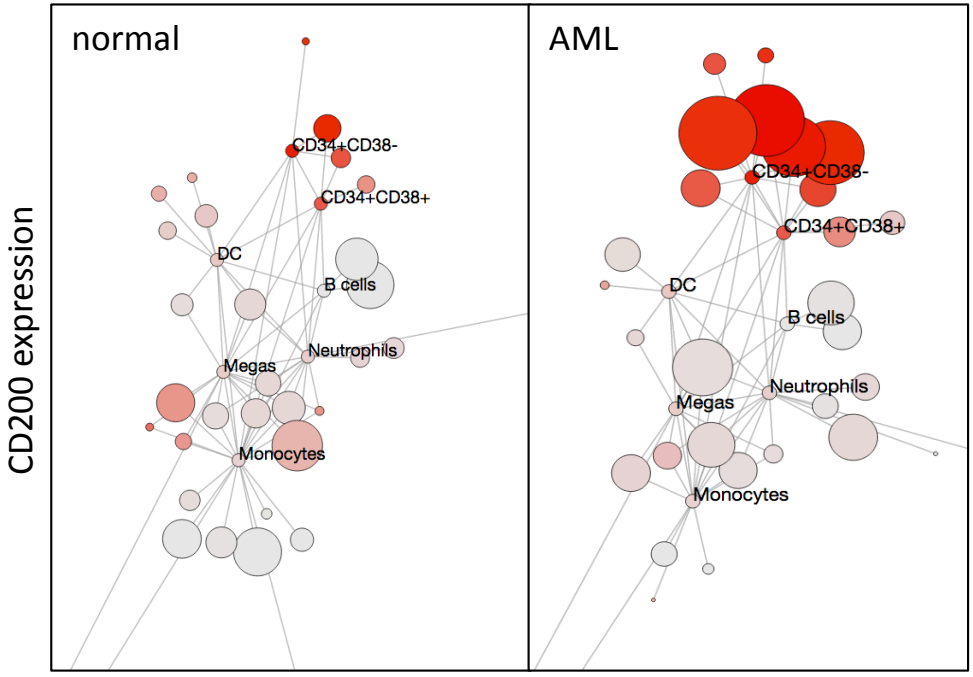

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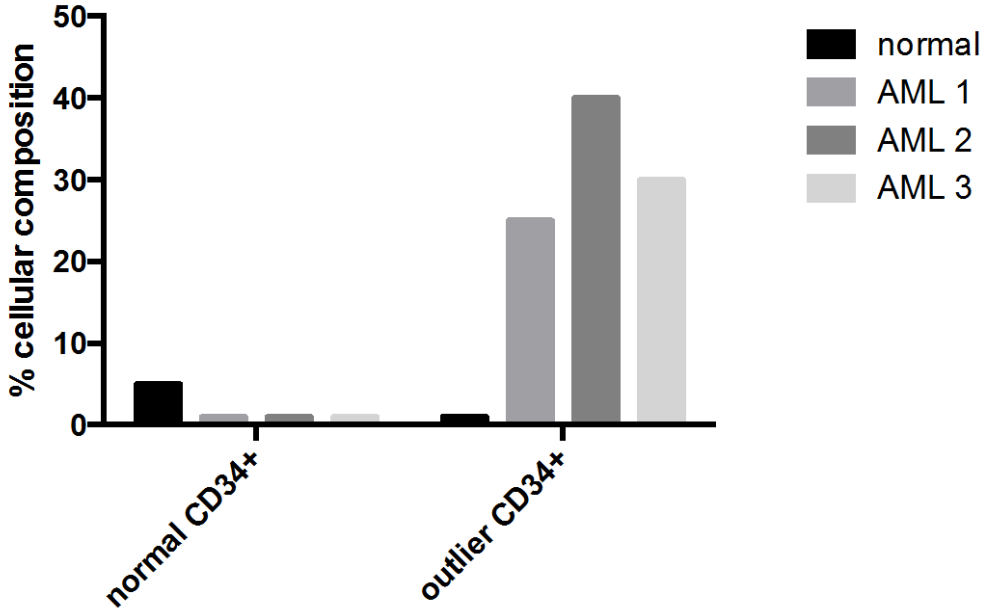

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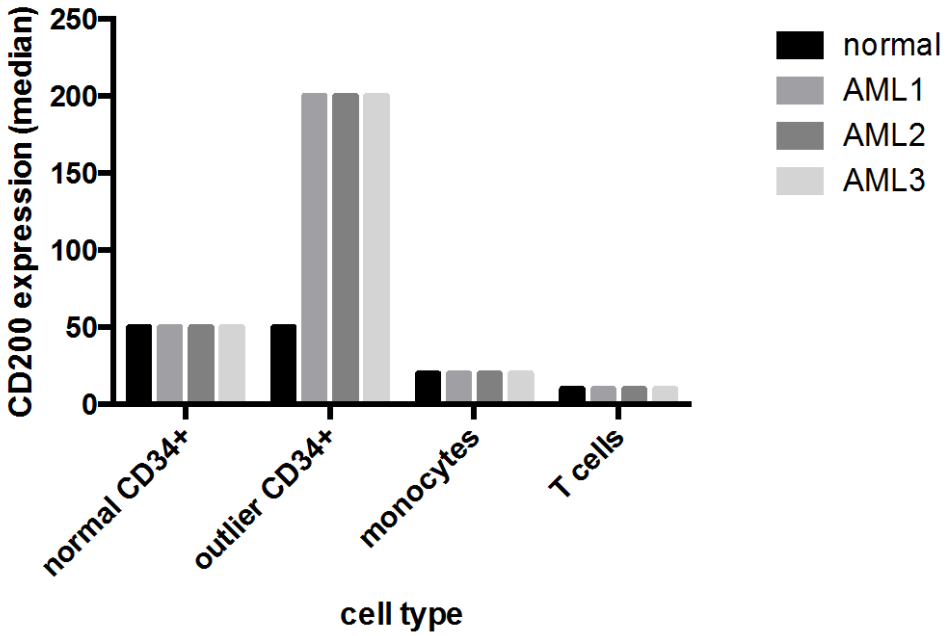

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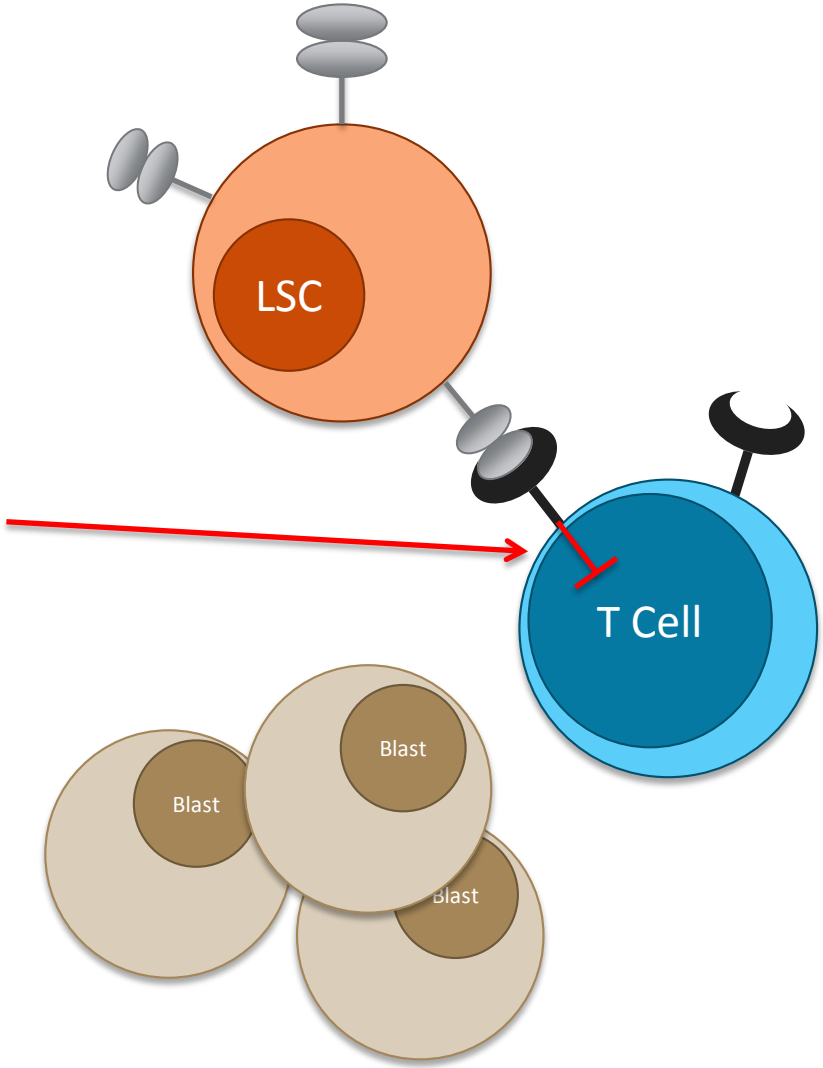


Aim 2. Determine the role of LSC-expressing CD200 on the cytotoxic function of CD8+ T cells

- *Motivation:* determine the effect of CD200 on T cell mediated cell death and on CD8+ effector cell function in AML

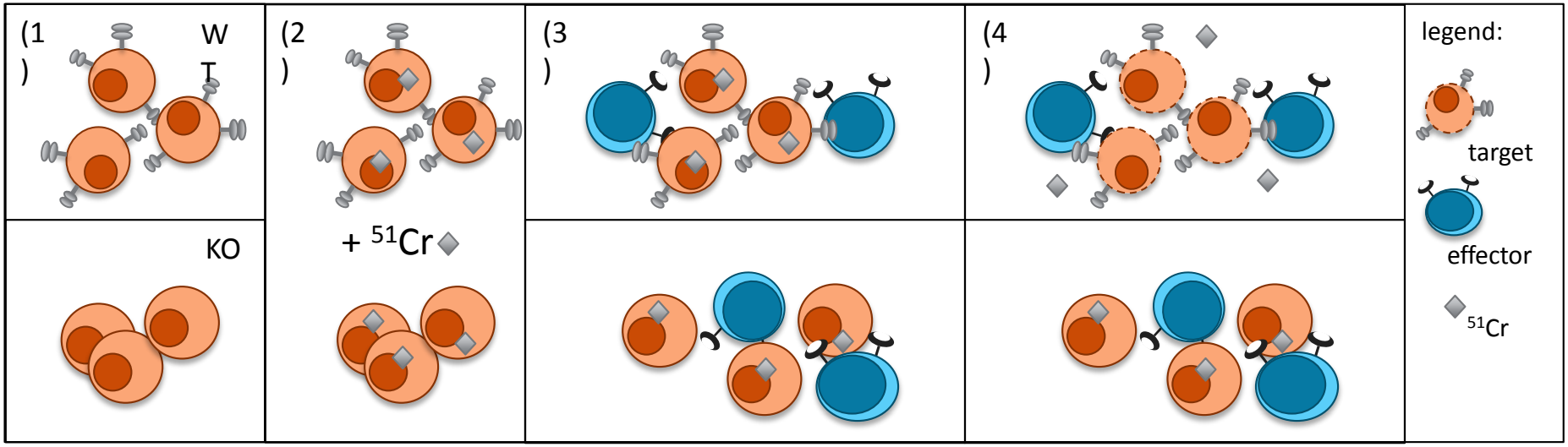
- **Hypothesis:** CD200 expression on AML LSCs suppresses T cell dependent cytotoxicity by inhibiting the production of necessary cytolytic enzymes.

Aim 2. Determine the role of LSC-expressing CD200 on the cytotoxic function of CD8+ T cells

- **2.1** Determine if CD200 surface expression inhibits cytotoxic T cell killing
- **2.2** Determine whether CD200 antibody blockade is sufficient for T cell mediated cytotoxicity
- **2.3** Test whether CD200 has a functional affect on the cytokine production of effector CD8+ T cells from AML patient samples

2.1 Determine if CD200 surface expression inhibits cytotoxic T cell killing

- Approach: MLR



- Measure: chromium release

$$\%\ cell\ lysis = \frac{(Experimental\ release - spontaneous\ release)}{(Maximum\ release - spontaneous\ release)} \times 100\%$$

- controls:
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  - maximum release: target cells cultured with detergent (Triton-X)

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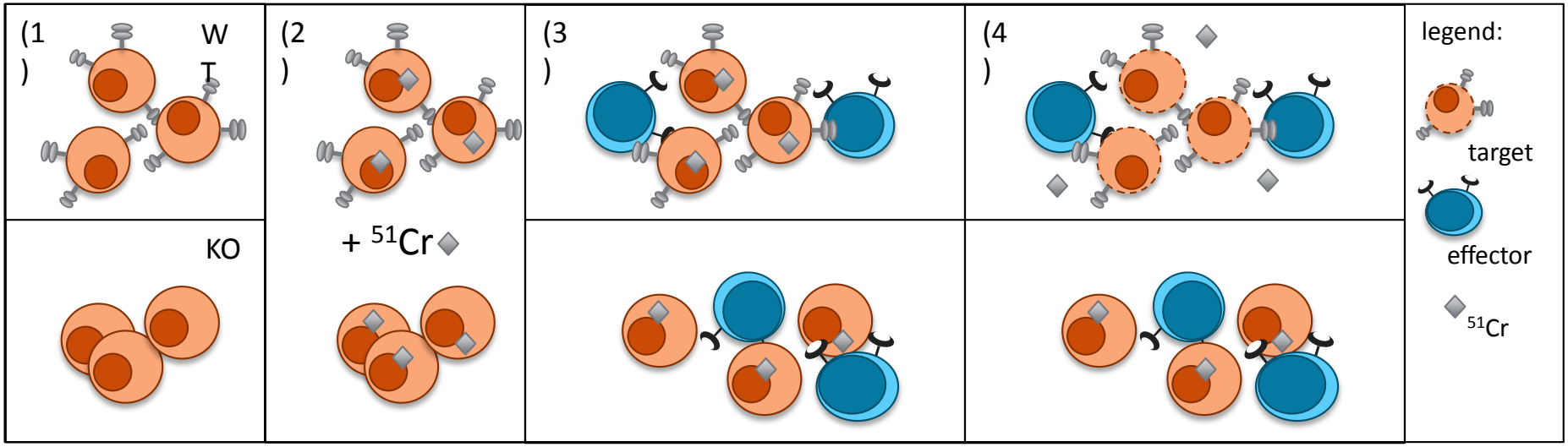

Target Cells:

- conditions
  - WT
  - CD200ko (CRISPR)
- cell lines
  - Kasumi1
  - KG1

Effector Cells:

- Normal PBMCs
  - CD8+ (positive selection with magnetic beads)
  - CD8 depleted (negative selection)
  - whole PBMCs

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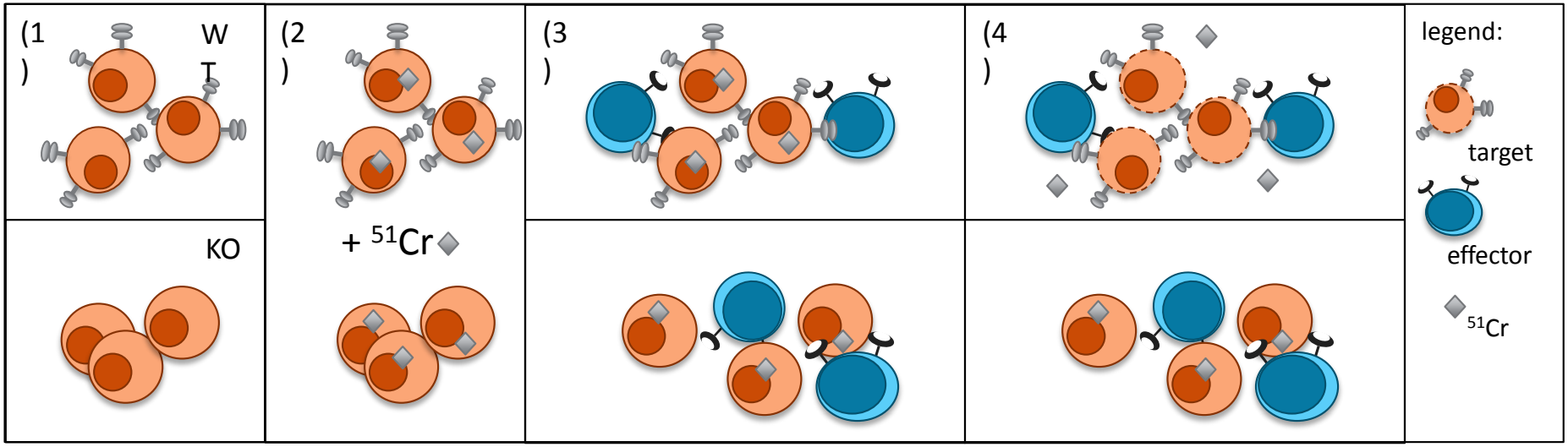
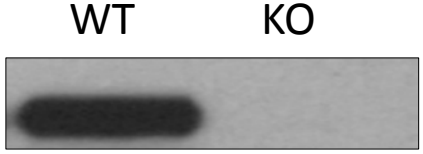
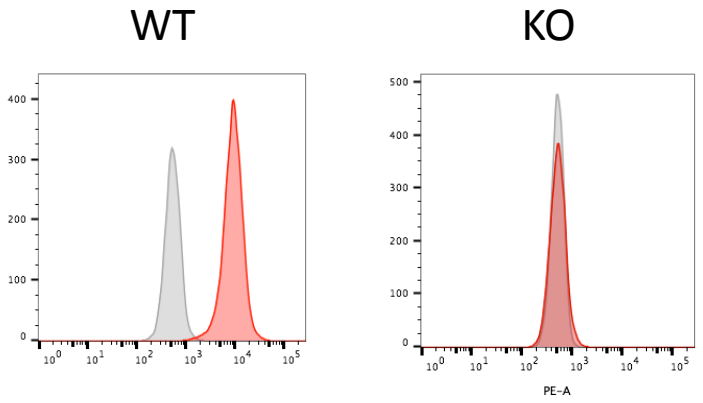


- Measure: confirm knockout

Total protein



surface protein



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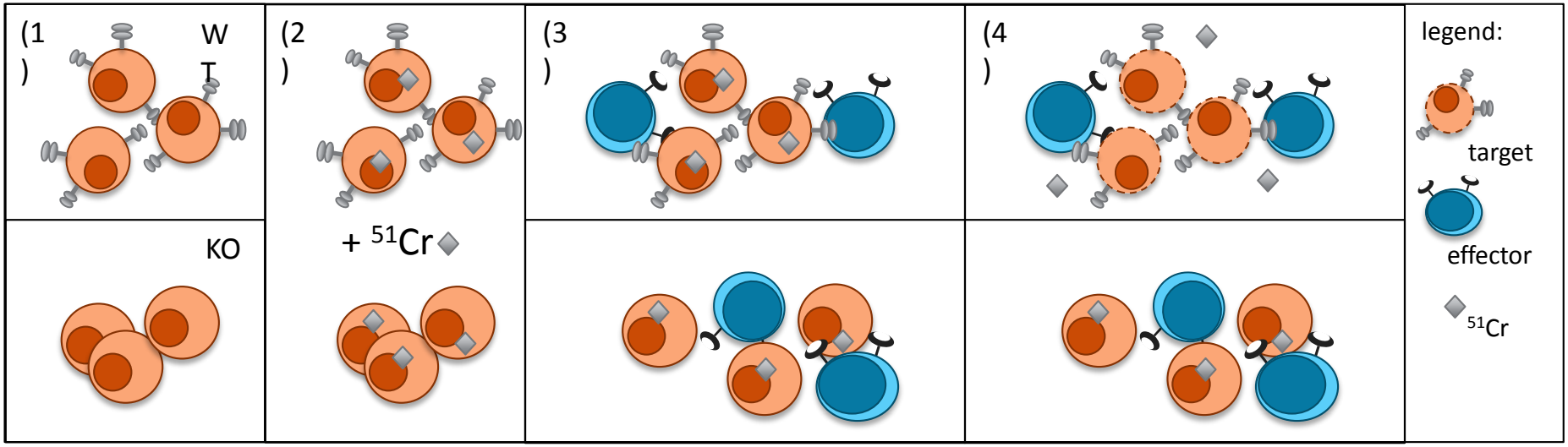
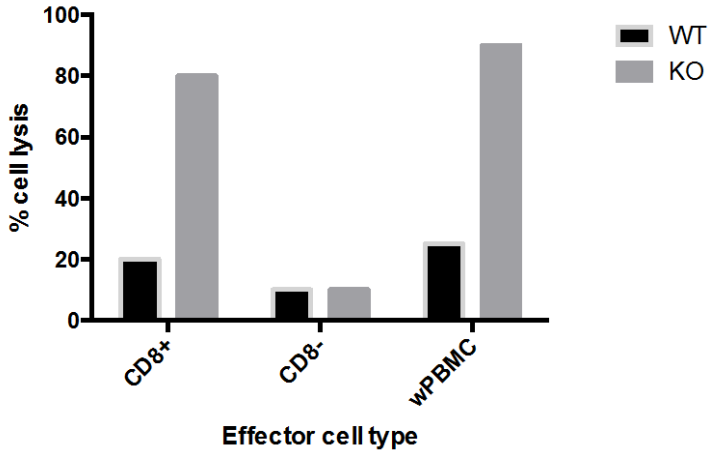
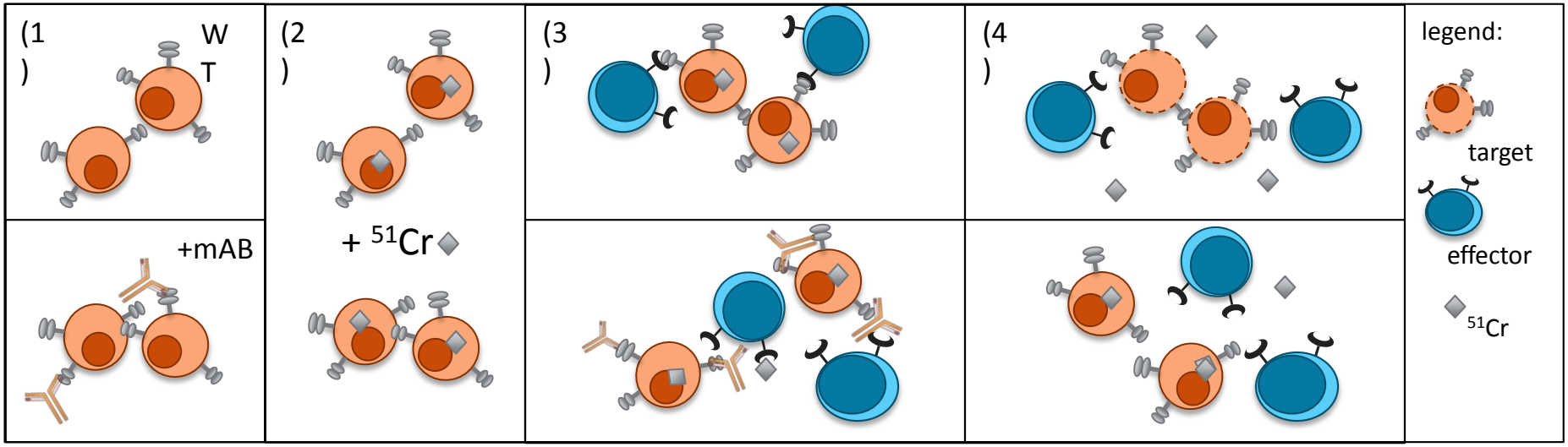


- Measure:
  - chromium release
  - calculate % cell lysis



2.2 Determine whether CD200 antibody blockade is sufficient for T cell mediated cytotoxicity

- Approach: MLR



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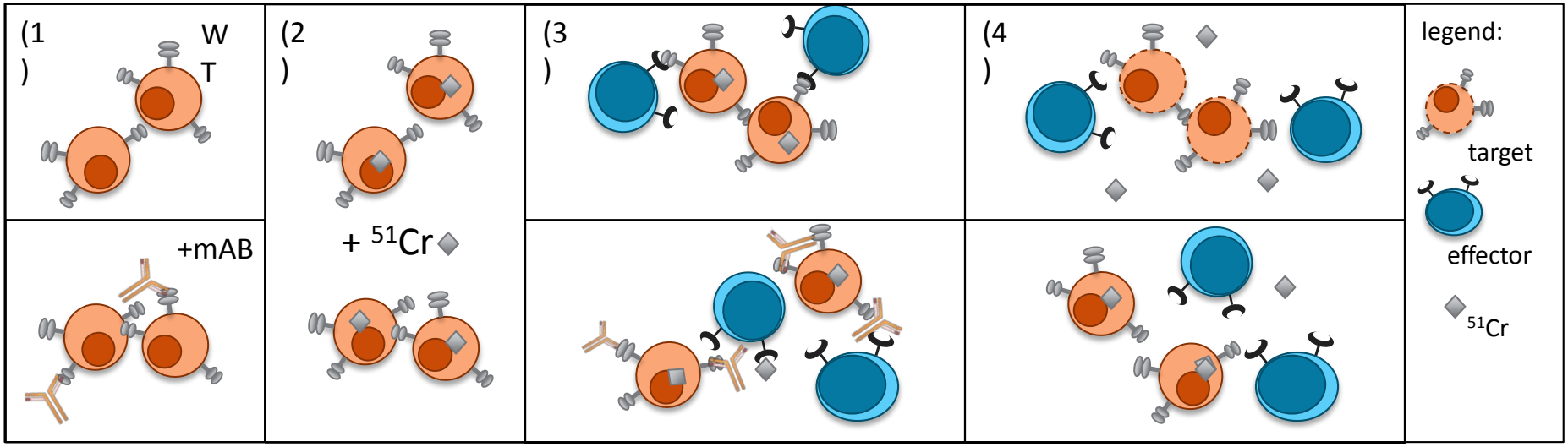


Target Cells:

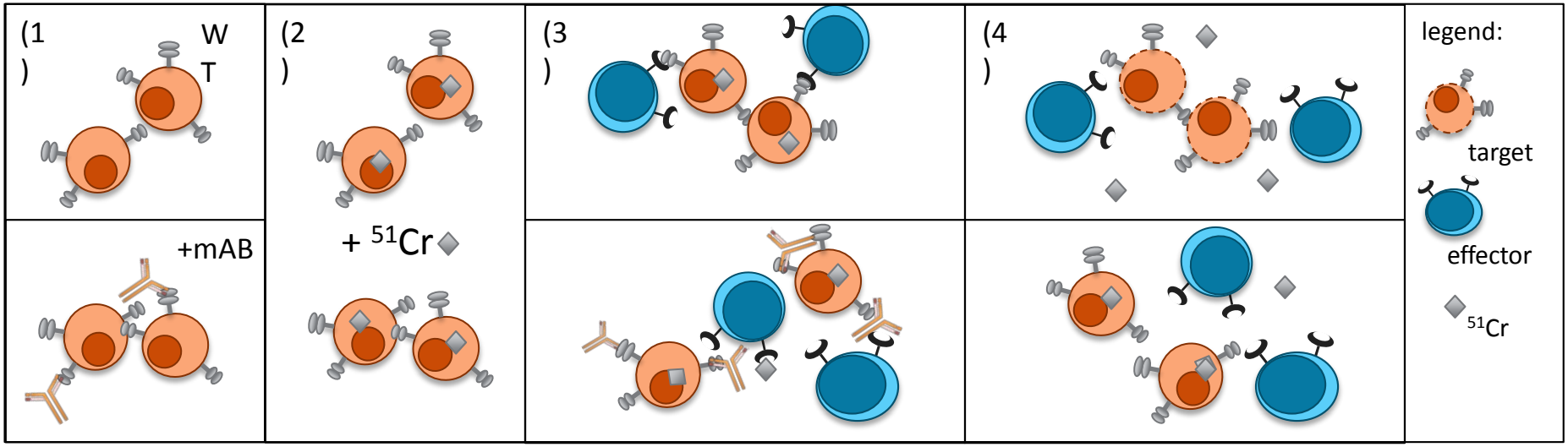
- conditions
  - IgG control
  - low dose CD200 mAB
  - high dose CD200 mAB
- samples
  - cell lines
  - patient samples

Effector Cells:

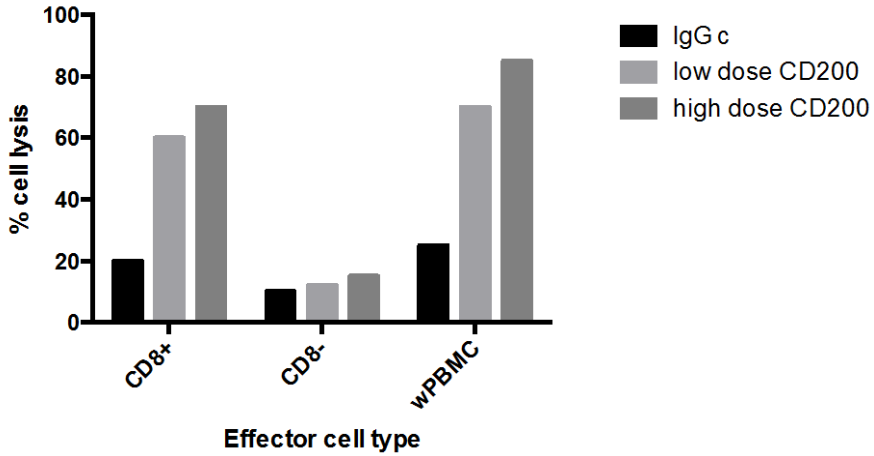
- Normal PBMCs
  - CD8+ (positive selection with magnetic beads)
  - CD8 depleted (negative selection)
  - whole PBMCs

2.2 Determine whether CD200 antibody blockade is sufficient for T cell mediated cytotoxicity

- Approach: MLR

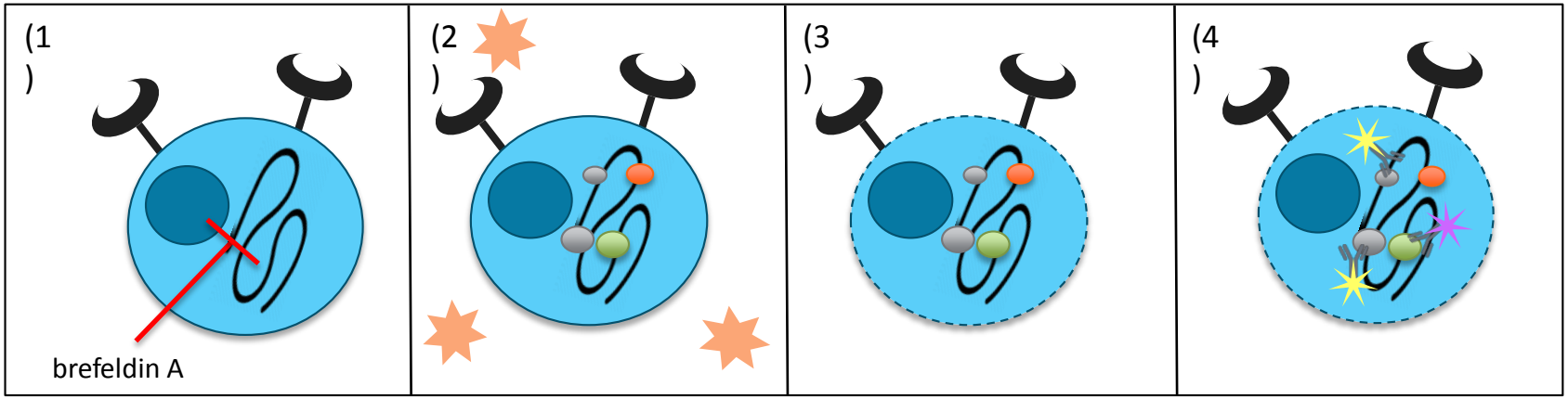


- Measure:
  - chromium release
  - calculate % cell lysis



2.3 Determine the functional affect of CD200 on the cytokine production of effector CD8+ T cells from AML patient samples

- Approach: Intracellular cytokine flow cytometry



Stimuli:

- $KG1^{WT}$ or $KG1^{CD200ko}$
- KG1 cell line +CD200 mAB or IgG control

Controls:

- unstimulated (negative)
- CD3/CD28 stimulating beads (positive)

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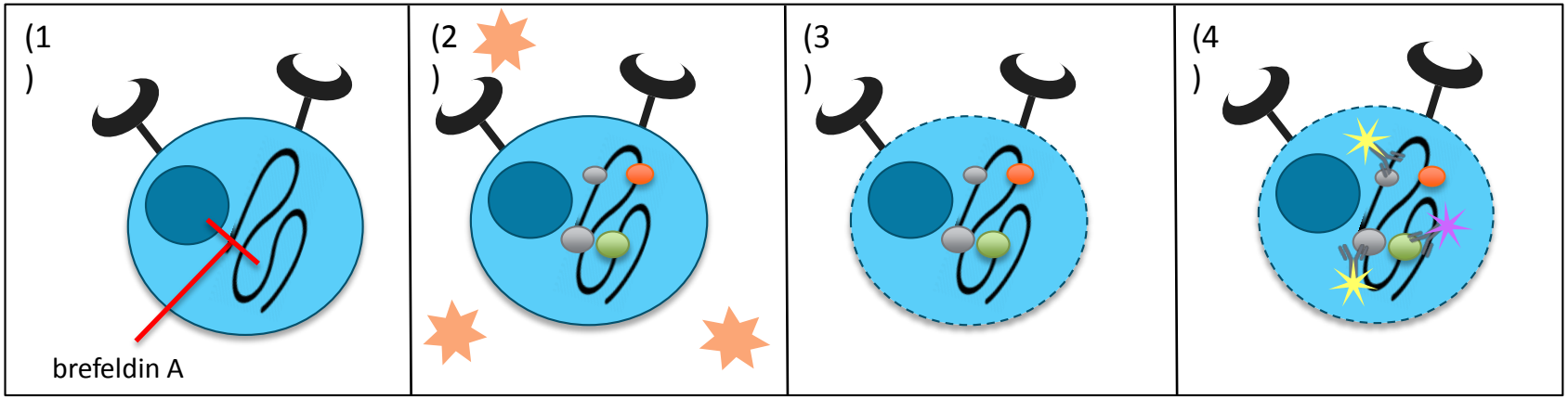

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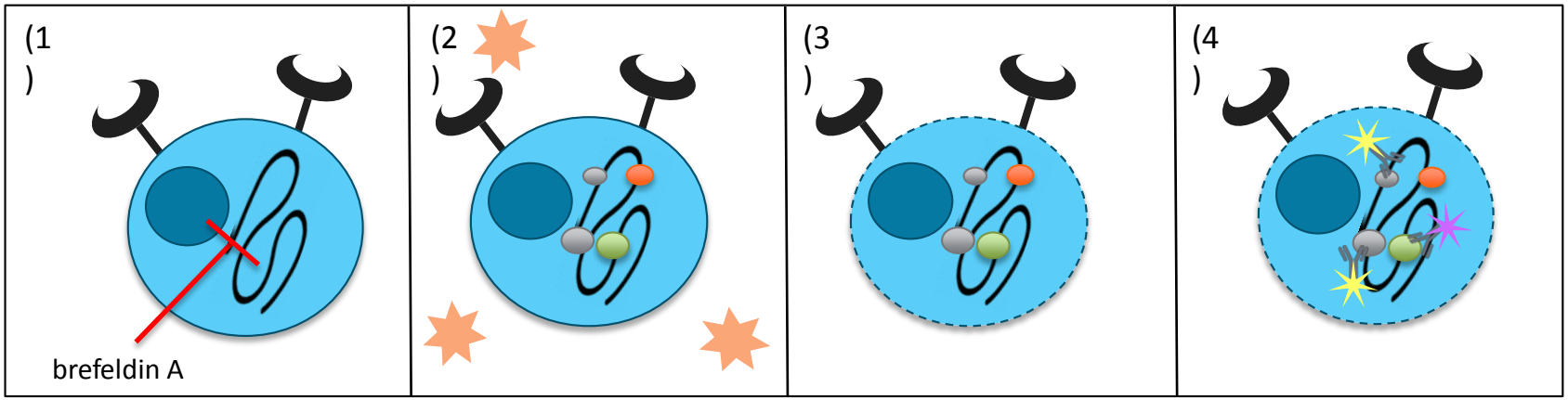
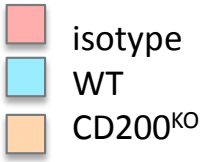
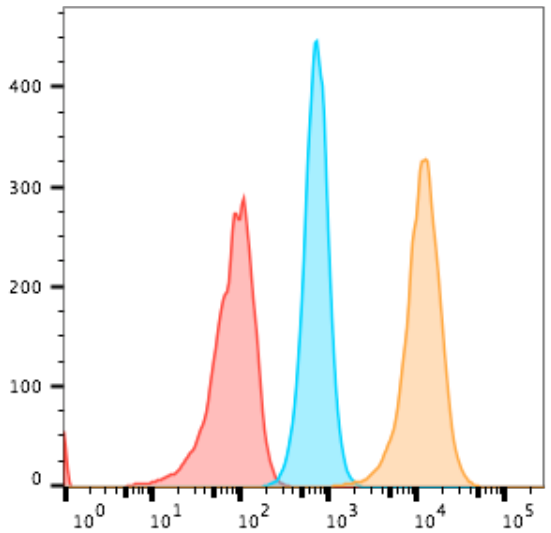


- Stimuli
  - $KG1^{WT}$
  - $KG1^{CD200ko}$
- Measure:
  - IFN-γ
  - granzyme B
  - and TNF-α





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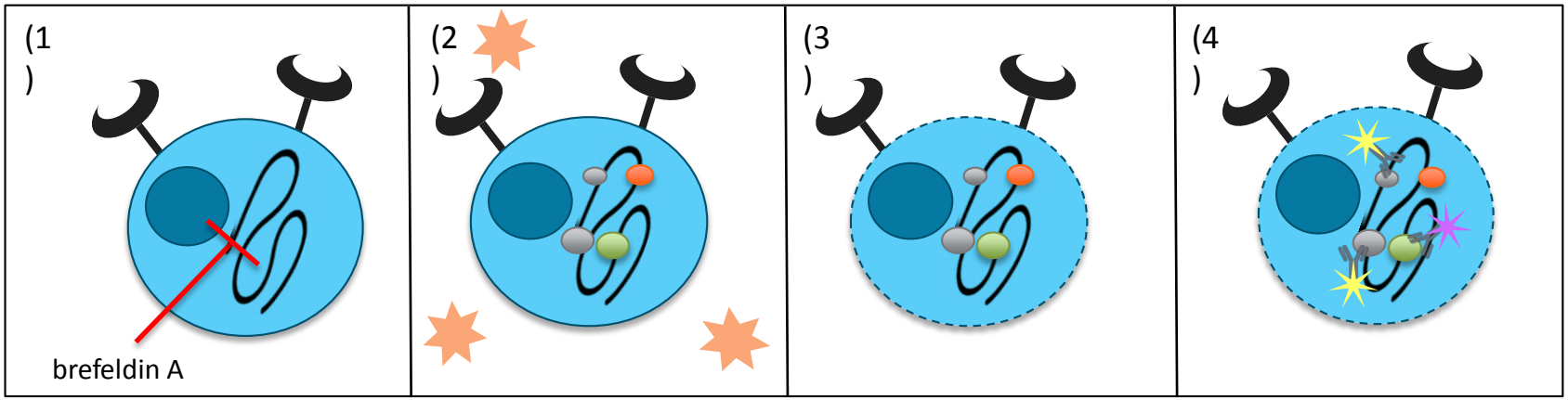
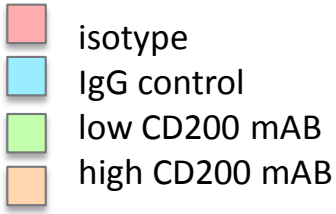
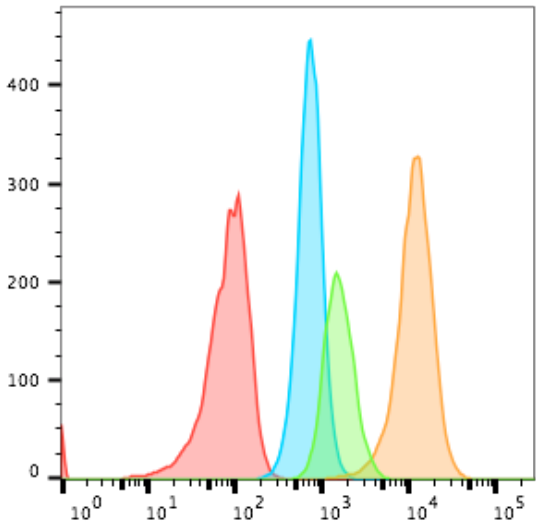


- Stimuli
  - KG1 + IgG
  - KG1 + low CD200 mAB
  - KG1+ high CD200 mAB
- Measure:
  - IFN-γ
  - granzyme B
  - and TNF-α



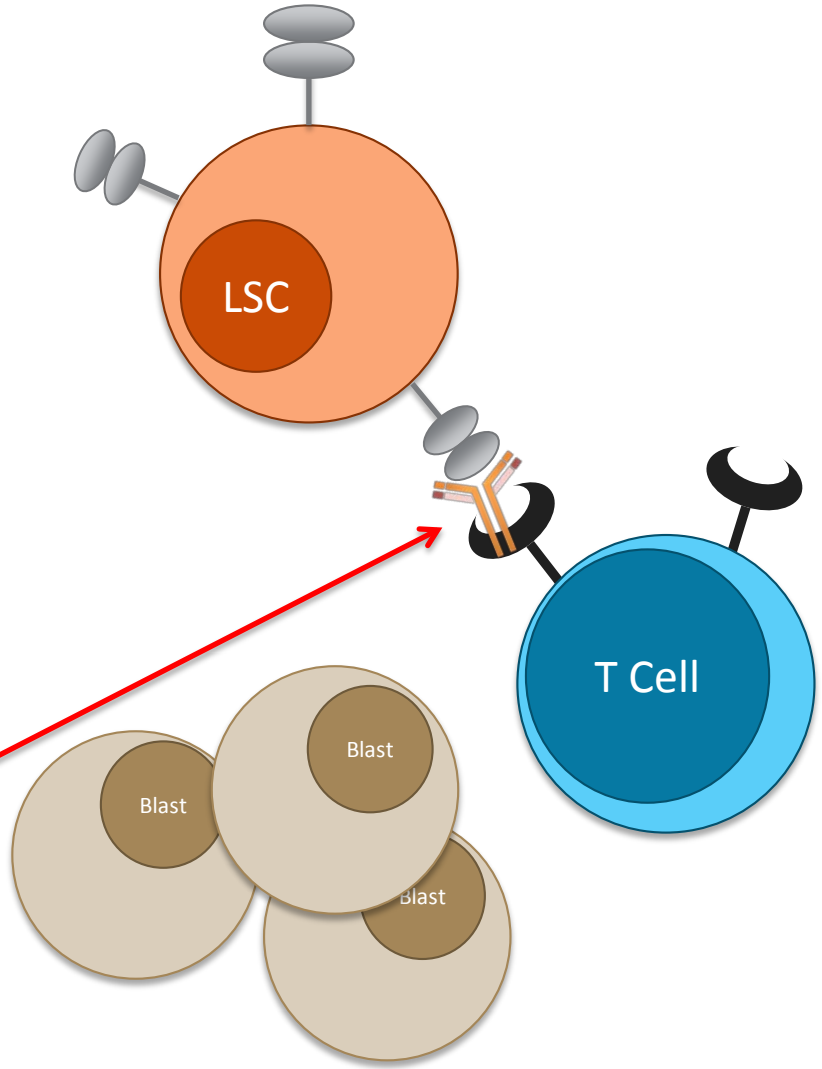


Specific Aims

Aim 1. Characterize CD200 receptor and ligand distribution on AML LSCs, blasts, and immune cell subsets in primary human AML samples using CyTOF

Aim 2. Determine the role of LSC-expressing CD200 on the cytotoxic function of CD8+ T cells

Aim 3. Evaluate the utility of CD200 inhibition as a mechanism for eliminating AML LSCs *in vivo*



Aim 3. Evaluate the utility of CD200 inhibition as a mechanism for eliminating AML LSCs *in vivo*

- *Motivation:* translate CD200 mAB therapy to specifically target residual CD200+ LSCs in remission

- **Hypothesis**: specifically blocking CD200 in remission will strip LSCs of their immune privilege and make them vulnerable to clearance by the immune system

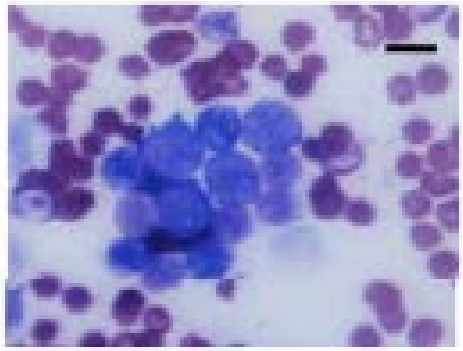
Aim 3. Evaluate the utility of CD200 inhibition as a mechanism for eliminating AML LSCs *in vivo*

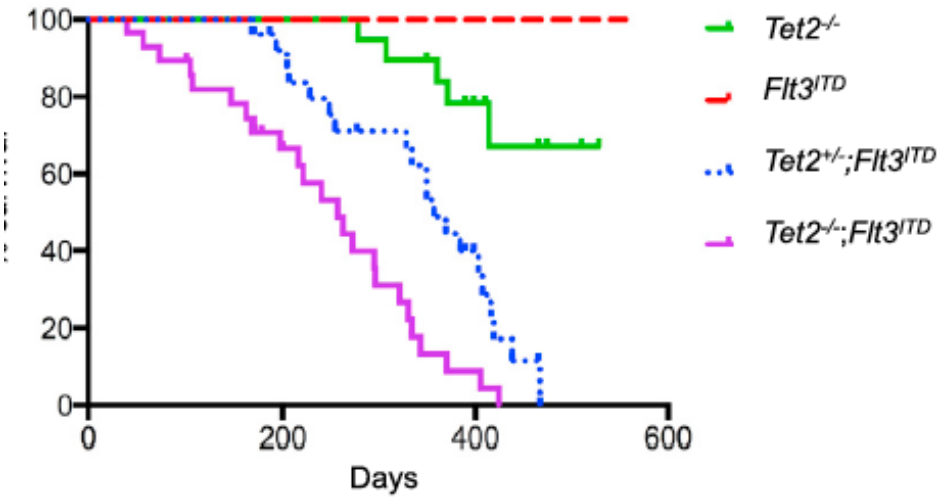
- **3.1** Test CD200 inhibition as a mechanism for eliminating residual leukemia during remission

- **3.2** Determine AML LSC reduction with CD200 therapy

Aim 3. model selection

- *Vav-cre*$^{+}$*Tet2*$^{fl/fl}$ (*VTet2*$^{-/-}$) x constitutive knockin *Flt3*^{ITD}
- Why?
  - 100% lethal, AML penetrance
  - well-defined, transplantable leukemic stem cells (CD48+CD150-)
  - refractory to 7+3 chemo
  - mimics human CD200 expression patterns





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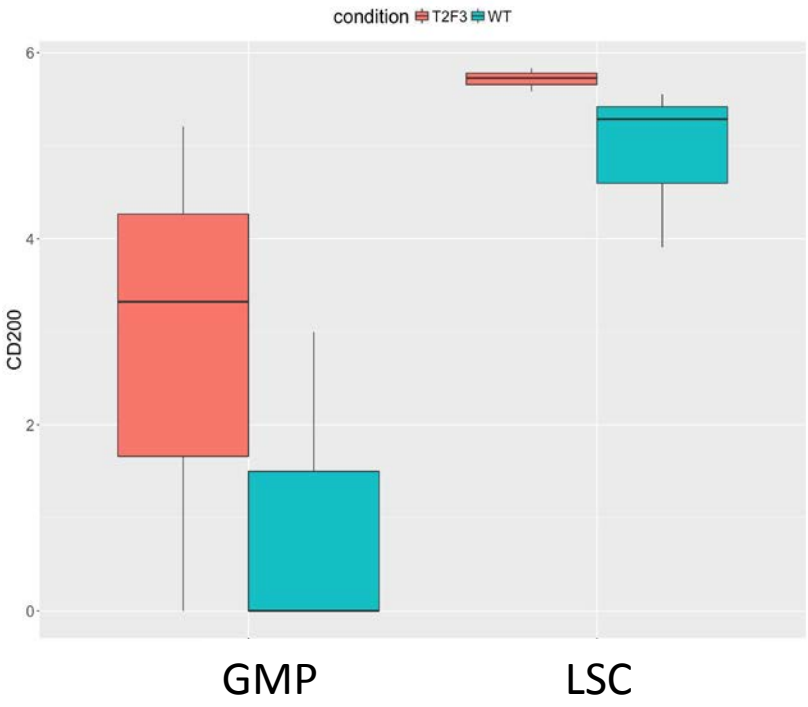
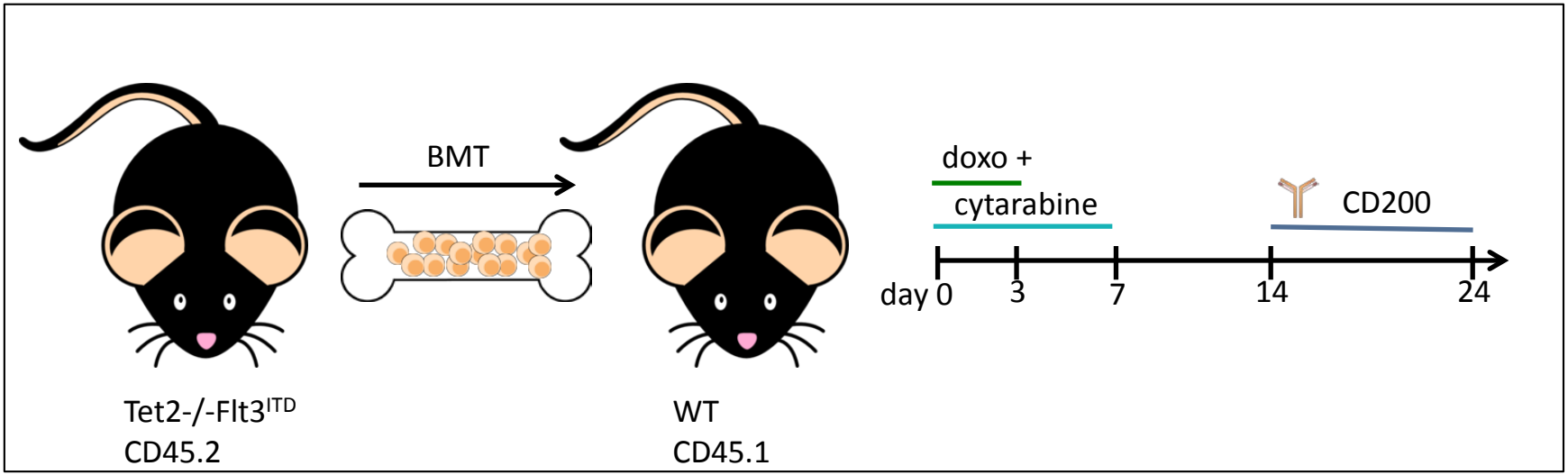


3.1 Test CD200 inhibition as a mechanism for eliminating residual leukemia during remission

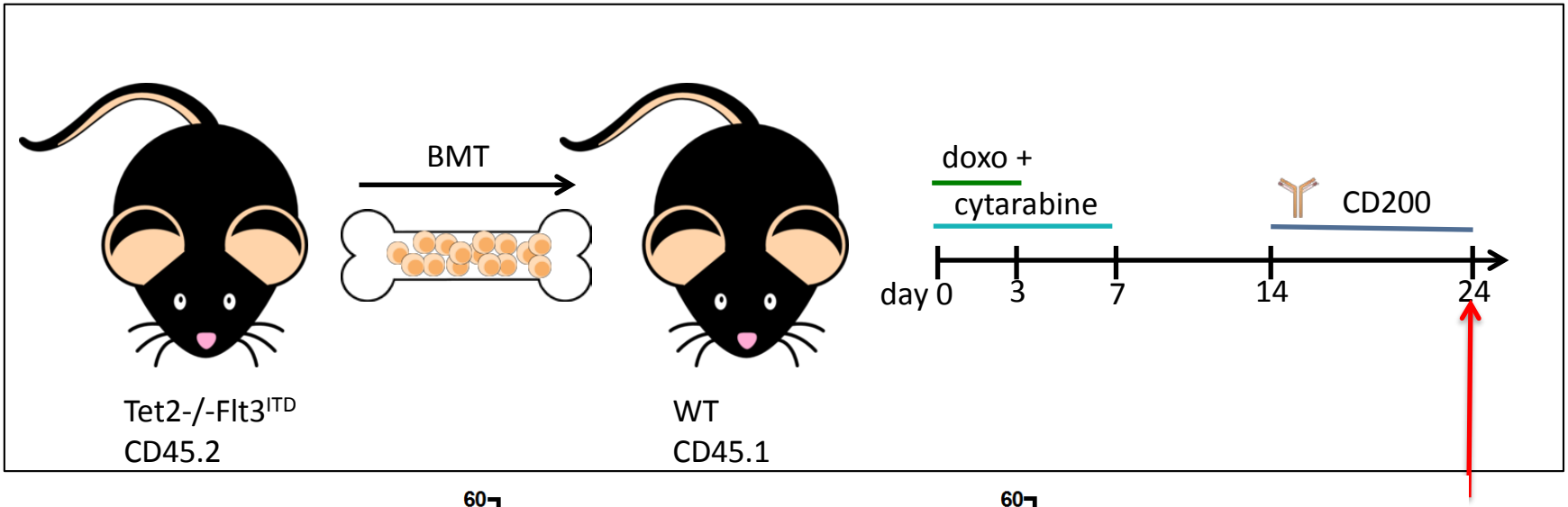
- Approach:



- Measure:
  - WBC
  - %CD45.2
  - overall survival

3.1 Test CD200 inhibition as a mechanism for eliminating residual leukemia after chemotherapy

- Approach:



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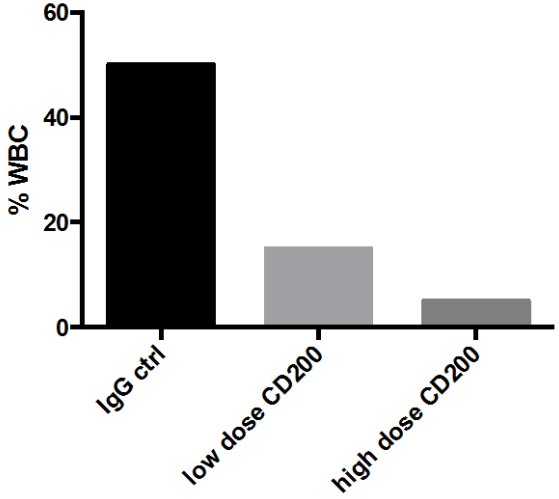

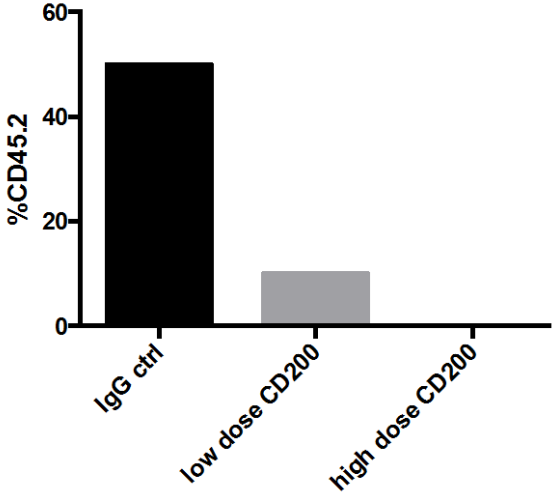

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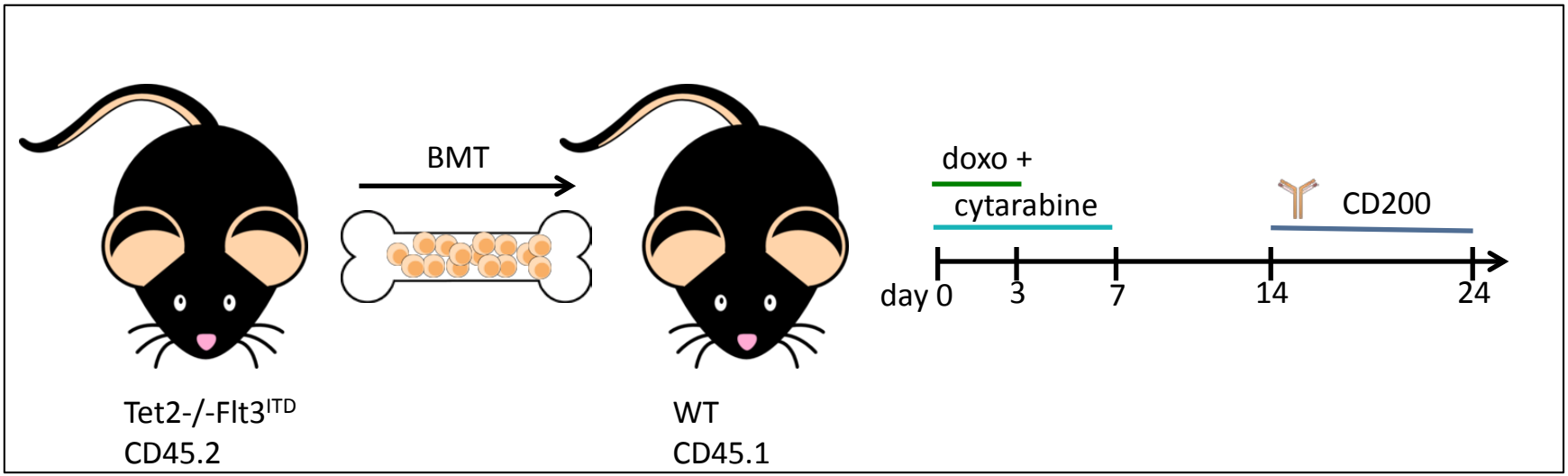
- Approach:



- Measure:
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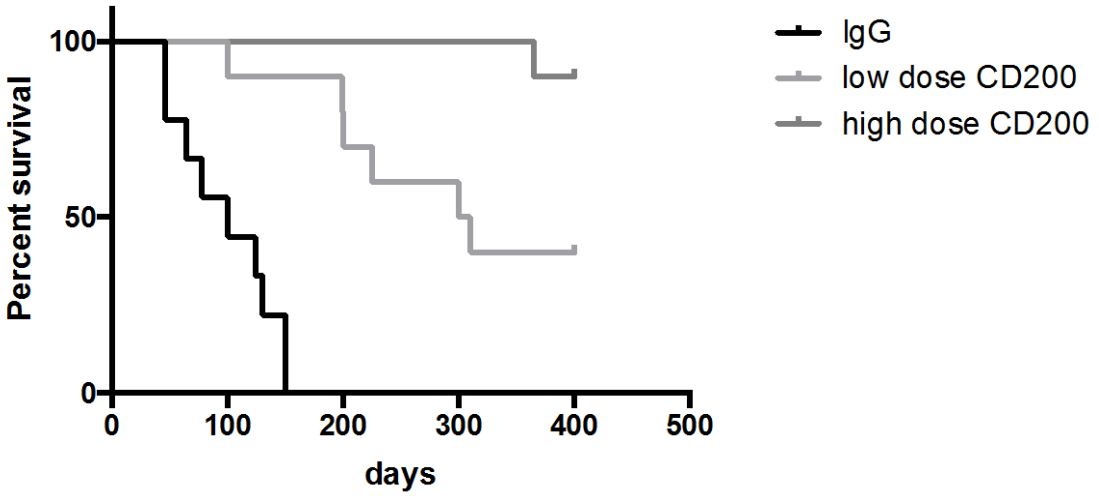
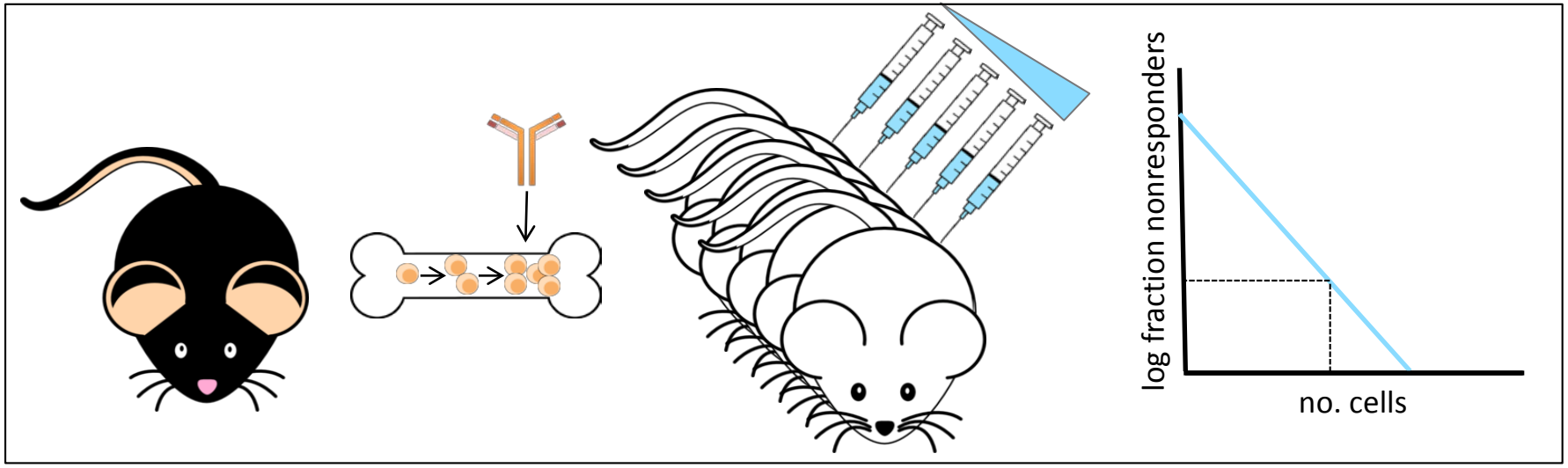


3.2 Determine AML LSC reduction with CD200 therapy

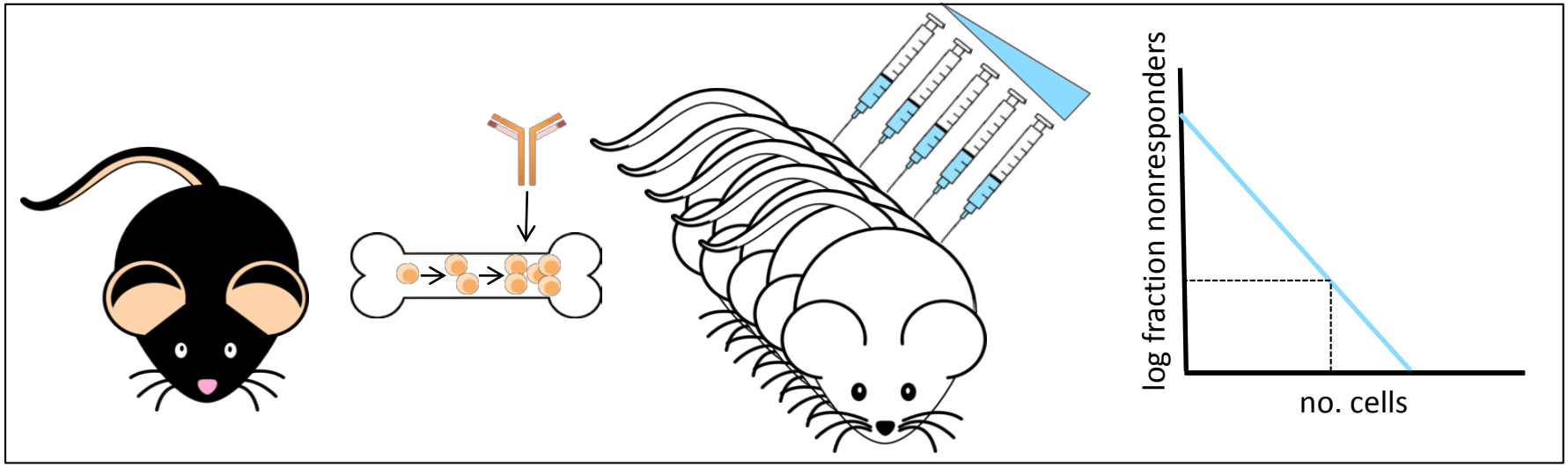
- Approach: limiting dilution assay



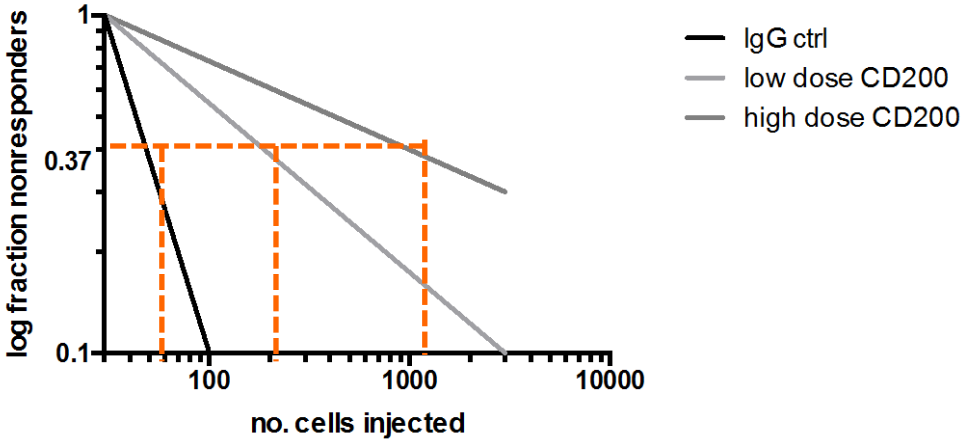
- 9 AML+ Tet2-/-Flt3^{ITD} mice will be treated with either high dose CD200 (3) , low dose CD200 (3), or an IgG control (3)
- mice are sacrificed and BM cells are harvested after 10 days treatment
- 4 dilutions of pooled cells will each be injected into 5 sub-lethally irradiated NSG secondary recipients
  - 5 mice/dilution x 4 dilutions x 3 treatment groups
- mice are sacrificed and engraftment is determined after 4 weeks

3.2 Determine AML LSC reduction with CD200 therapy

- Approach: limiting dilution assay



- Measure:
  - fraction of mice (per dilution group) CD45.2+
  - approximate stem cell number



Proposal Summary

- Can SCAFFoLD data be systematically analyzed using Euclidean distance?
- Is CD200 protein expression enriched in AML LSCs at the single-cell level?
- Does CD200 reduce CD8+ mediated cell death? Specifically, by reducing the production of cytotoxic enzymes?
- Does CD200 therapy at remission eliminate LSCs and increase overall survival?