Novel single chain antibodies to inhibit CCR7 mediated-entry of pediatric T-cell acute lymphoblastic leukemia into the CNS

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Disclosures

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Background

• Chemokine-receptor 7 (CCR7): Naïve T-cells, B-cells, NK T cells, dendritic cells
• Regulates T-cell egress from tissues into lymph nodes, induced by EBV
• CCR7 - Up-regulated on malignant cells:
  – Hematologic: pediatric T-cell ALL, CLL, diffuse large B-cell lymphoma
  – GI: esophageal, pancreatic, CRC, gastric cancer
  – Breast cancer
  – Melanoma
  – Head & Neck Cancer
  – NSCLC
  – Lymph node metastasis
CCL 21
Adheres to lymphatics and HEV
Promotes migration of naïve T-cells to lymph nodes
Internalizes 20% of CCR7*

CCL19
Expressed by activated dendritic cells
Promotes migration of activated dendritic cells to lymph nodes
Mediates adhesion to antigen presenting cells**
Internalizes 80% of CCR7*
Regulates T-cell differentiation**

CCR7 signaling as an essential regulator of CNS infiltration in T-cell leukemia

- NOTCH 1 activation – ↑ CCR7 expression
- *Rag2*−/− *IL2rg*−/− mice – injected with human T-ALL cell line (CEM/CCR7+)
  - ↑ CNS infiltration = ↓ Overall survival
- CCL19 expression required for CNS infiltration
- CCR7 sufficient for infiltration of CD3+ leukemic cells into CNS

R707 Mutants #27-30 Inhibition of CCL19-Induced Ca-Flux (MaxPoint)

- R707 Mutant #28 ~ 70% inhibition of CCL19-induced Ca-Flux
- R707 Mutants #27, 29, 30 → ~ full inhibition of CCL19-induced Ca-Flux
R707-29 (R707-LKM) IgG1 Inhibition of CCL19-Induced Ca-Flux on CCR7+ Cells

IC50 ~ 2.3nM
MSM707 Blocks Migration of the CEM cell line to 200nM CCL19 on Fibronectin

![Graph showing the effect of MSM707 on cell migration](image-url)

- **MSM707 + PBS**: Minimal cell migration
- **PBS**: Minimal cell migration
- **MSM707 + CCL19**: Minimal cell migration
- **CCL19**: Significant cell migration

P-value: p<0.05
MSM antibody 707 blocks migration of pediatric primary T-ALL via \( \alpha_v\beta_3 \)
CCR7 sufficient for infiltration of T-ALL cells into the CNS

RAG−/IL2γR−/ (T-B-NK−)

Inject 200,000 CEM(CCR7(+)) or DND41 (CCR7(−)) cells

Peripheral blood counts to confirm Leukemia (>20,000/μl)

Euthanize

450 rads

Day 0

Day 1

Day 28

Day 30–35

CEM/(CCR7+)

DND41/(CCR7−)
Conclusions

• Single chain-antibody (MSM 707) blocks migration of pediatric T-cell ALL cells (both CEM/CCR7+ and primary cells) in vitro
• CCR7 sufficient for infiltration of T-cell ALL into the CNS in murine model
• Goal of single-chain CCR7 antibody therapy - minimize systemic toxicity from chemotherapy/radiation
• CCR7 antibody - broad therapeutic potential across several malignancies
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