Abstract No. 1734

Modulation of Immune Responses to Cancer With Bi-specific IL-2/IL-13 Superkines

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MEDICENNA

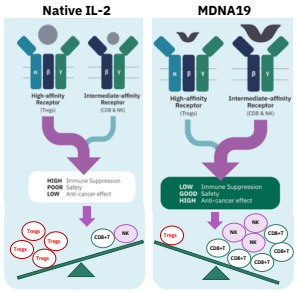
1. Background

Directed Evolution + Yeast Display = Tunable Superkines

Leveraging IL-2, IL-4, and IL-13 Superkines to develop next generation immunotherapies

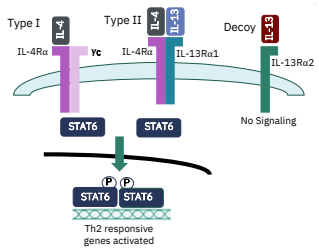
Levin et al., Nature 2012
Moraga et., Science Signaling 2015

2. MDNA19 is a '*Beta-only*' IL-2 Super Agonist



- rhIL-2 (Proleukin) is an approved immunotherapy for renal cell carcinoma and metastatic melanoma
- Its use is limited by toxicities, short half-life and preferential activation of T_{regs} due to strong binding to CD25
- MDNA19 (MDNA109FEAA-Fc; sIL2^{M}) is a long-acting '*beta-only*' IL-2 super-agonist with enhanced affinity for CD122 (expressed on CD8$^+$ T and NK cells) and does not bind CD25

3. IL-4 & IL-13 Receptors Play an Important Role in Cancer



MDNA413 (sIL13^{M1}) is a dominant-negative inhibitor and selectively binds to type II IL-4 receptor and blocks downstream signaling

- **Suppresses Th2 response** (blocks M2a polarization of TAM)
- **Potential effect directly on tumors**

MDNA132 (sIL13^{M2}) selectively binds the decoy receptor IL13Rα2 over-expressed in a broad range of tumors.

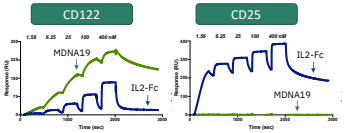
- **Highly selective tumor localization**

4. Long Acting Bi-functional Superkine Immunotherapies (BiSKITs™)

Long-acting **BiSKITs** engineered with the following objectives:

- Modulate TME by stimulating Th1 response (IL-2 agonism) and suppressing Th2 response (IL-4/IL-13 antagonism) to enhance therapeutic efficacy including immunologically 'cold tumors'
  - **MDNA19-MDNA413 (sIL2^{M}-Fc-sIL13^{M1})**
- Enhance therapeutic efficacy of check-point inhibitors and T cell engagers by localizing to IL13Rα2 over-expressing tumors
  - **Anti-PD1-MDNA132 (Anti-PD1-sIL13^{M2})**
  - **Anti-CD3-MDNA132 (Anti-CD3-sIL13^{M2})**

5. MDNA19 (MDNA109FEAA-Fc) Exhibits Preferential Binding to CD122

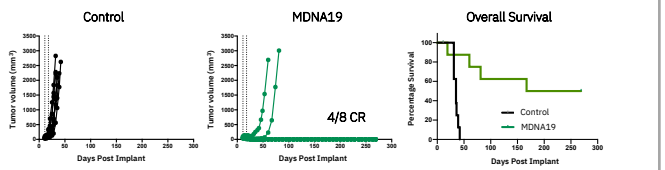


K_D (nM)	IL2-Fc	MDNA19
CD122 (IL-2Rβ)	135	2.1
CD25 (IL-2Rα)	26	No binding

SPR performed on immobilized ligands with constructs as flow analytes

- MDNA19 shows enhanced affinity to CD122 compared to IL2-Fc
- MDNA19 does not bind CD25

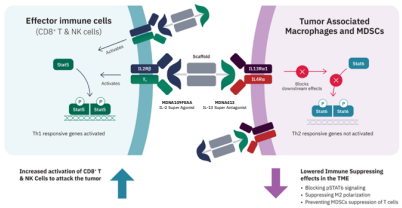
6. MDNA19 Inhibits CT26 Tumor Growth and Extends Survival



MDNA19: 5 mg/kg IP; QWx2
Avg tumor size at initiation of dosing ~60 mm³
CR = complete regression (tumor volume = 0)

7. MDNA19-MDNA413 is a Bi-functional Superkine

Targeting immunologic 'cold tumors' by modulation of TME

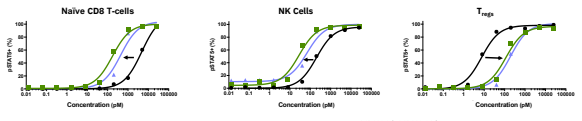


'Cold' tumors are not responsive to immunotherapies because of a Th2 (pro-tumoral) microenvironment:

- Low CD8$^+$ & NK cell counts; high T_{reg} counts
- High number of immune-suppressive myeloid cells (TAM & MDSC)
- TAMs & MDSCs overexpress the Type II IL-4 receptor

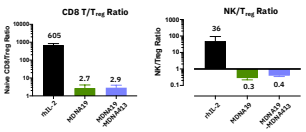
8. MDNA19-MDNA413 Induces a Potent Th1 Response

Enhanced signaling in CD8$^+$ T and NK cells; diminished signaling in T_{regs}
IL-2 agonism maintained in bi-functional superkine

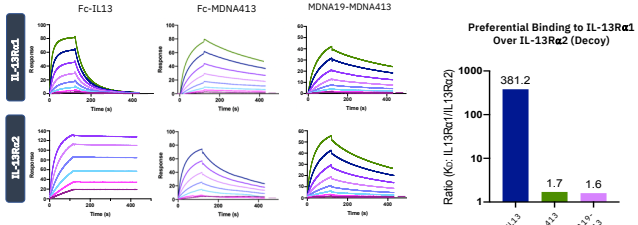


P-STAT5 (EC_{50}, pM)	rhIL-2	MDNA19	MDNA19-MDNA413
Naïve CD8$^+$ T cells	3389.5	370.6	575.8
NK cells	201.5	71.0	80.1
T_{regs}	5.6	135.5	210.3

Studies in human PBMC



9. MDNA19-MDNA413 Demonstrates Selectivity Towards IL-13Rα1 and Reduced Affinity to IL-13Rα2

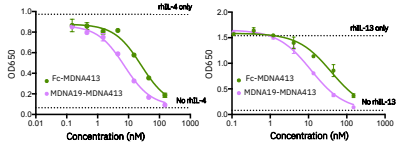


K_D (nM)	Fc-IL13	Fc-MDNA413	MDNA19-MDNA413
IL-13Rα1	263	50.9	73
IL-13Rα2	0.69	30	46.1

SPR performed on immobilized ligands with receptors as flow analytes using MCK

10. MDNA19-MDNA413 Inhibits IL-4 & IL-13 Induced Signaling, TF-1 Proliferation and M2a Polarization

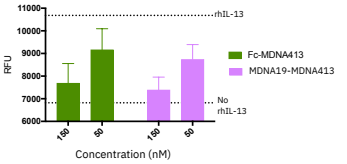
- Inhibition of IL-4 (left panel) and IL-13 (right panel) Induced Signaling



IC_{50} (nM)	Fc-MDNA413	MDNA19-MDNA413
IL-4 Signaling	26.4	8.0
IL-13 Signaling	39	12.3

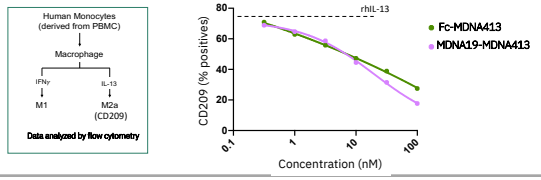
Assay performed in HEK Blue IL-4/IL-13 reporter cells (InvivoGen); Measurement of pSTAT6 activity

- Inhibition of IL-13 Induced TF-1 Proliferation

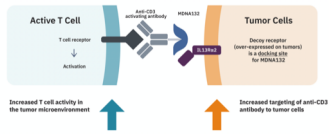


TF-1 is a human erythro-leukemia cell line that is highly dependent on IL-4/IL-13 for proliferation

- Inhibition of IL-13 Induced M2a Polarization of Macrophages (responsible for a pro-tumoral environment)

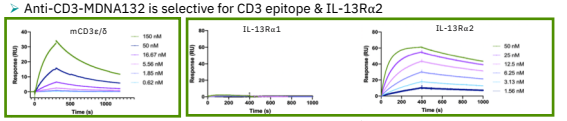


11. Targeting IL-13Rα2 Decoy Receptor (a tumor associated antigen)

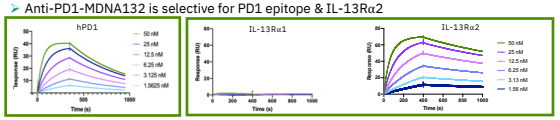


- IL-13Rα2 is over-expressed on tumors:
  - Triple negative breast cancer
  - Pancreatic cancer
  - Colorectal cancer
  - Renal cancer
  - Lung Cancer
  - Glioblastoma
  - Prostate cancer
  - Ovarian cancer
  - Mesothelioma

- Anti-CD3-MDNA132 is selective for CD3 epitope & IL-13Rα2



- Anti-PD1-MDNA132 is selective for PD1 epitope & IL-13Rα2



12. Summary and Future Directions

- Bi-functional MDNA19-MDNA413 superkine shows:
  - Enhanced stimulation of Th1 immune effector cells (CD8 T and NK cells) while exhibiting diminished activity on T_{reg}
  - Selectivity towards IL-13Rα1 while blocking IL-4 and IL-13 mediated activities (antagonizes pSTAT6 signaling, TF-1 proliferation and M2a polarization of macrophages)
- Anti-PD1-MDNA132 and anti-CD3-MDNA132 show:
  - Strong receptor selectivity for decoy receptor IL-13Rα2 known to be over-expressed in many tumor types, while retaining affinity towards immune epitope
- Utilize library of IL-2, IL-4 and IL-13 Superkines to engineer novel long-acting BiSKITs™