Emergence of Novel Long-acting Mono- and Bi-specific IL-2/IL-13 Superkines as Potent Immune Modulators

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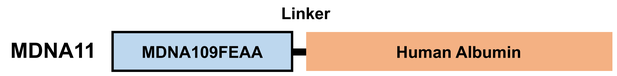
BACKGROUND:

Use of IL-2 (Proleukin) to treat renal carcinoma and metastatic melanoma achieved durable response in ~15% of cases, but its application is limited due to its short half-life, toxicity and undesirable activation of Tregs, the latter due to binding to IL-2Rαβγ.

MDNA11 is a long-acting IL-2 Superkine with enhanced affinity to IL-2Rβγ (expressed by CD8⁺ T & NK cells) and diminished binding to IL-2Rαβγ (expressed by Tregs)

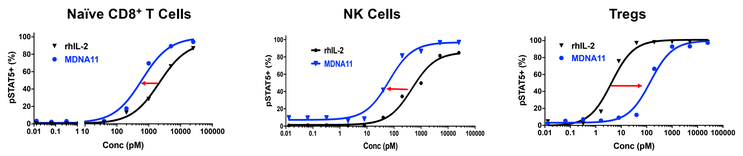


MDNA109 = IL-2 superkine with enhanced affinity for CD122 (IL-2Rβ).
FEAA = mutations to abolish binding to CD25 (IL-2Rα)

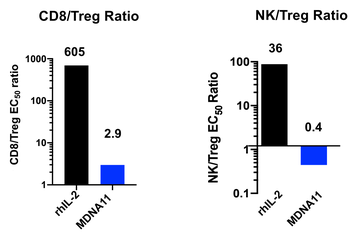
Increases MW to reduce clearance
Facilitates FcRn recycling
Known to allow accumulation at tumor sites

K_D by BLI Octet	CD122 (IL-2Rβ)	CD25 (IL-2Rα)
rhIL-2	210 nM	24 nM
MDNA11	6.6 nM	No binding

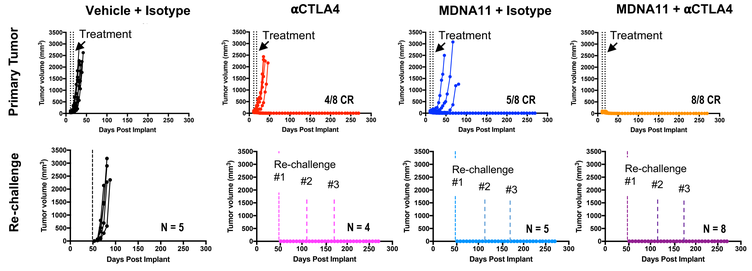
MDNA11 exhibits enhanced potency on naïve CD8⁺ T and NK cells & diminished activity on Tregs



Human PBMC P-STAT5 (EC_{50}, pM)	rhIL-2	MDNA11
Naïve CD8⁺ T cells	3390	460
NK cells	201.5	68.9
Tregs	5.6	160

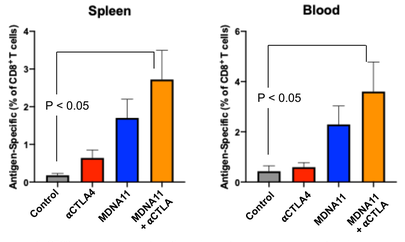


MDNA11 inhibits tumor growth & induces a strong memory response as monotherapy & in combination with αCTLA4 in CT26 tumor model



CT26 tumor (~60 mm³) bearing Balb/c mice were treated with MDNA11 (5 mg/kg 1x/week, 2 weeks) or Anti-CTLA4 (200 μg 2x/week, 2 weeks) by IP injection.
Re-challenge experiment performed by implanting 2 x 10^6 CT26 cells in opposite flank (Day 49, Day 116 and Day 165), without further treatment. Control mice showed robust tumor growth at each re-challenge experiment (representative data for control shown)

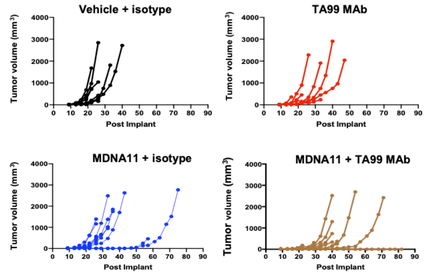
Development of long-term (gp70) specific CD8 T cells protected mice against CT26 tumors

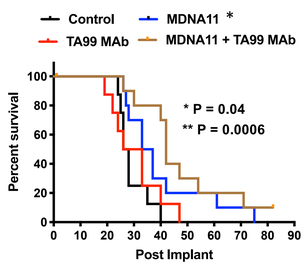


Analysis of antigen-specific CD8 T cells at study day 270, more than 8 months after last treatment.

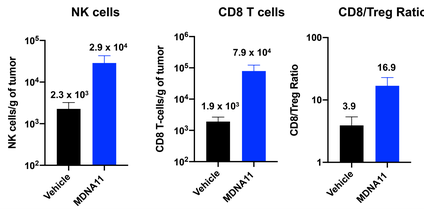
Mice challenged with CT26 cells 5 days prior to euthanasia; control mice were age matched.
Analysis by flow cytometry using APC anti-mouse CD3ε, anti-CD8 FITC clone KT15 and H-2Ld MuLV gp70 Tetramer-PE

MDNA11 delays the growth of B16F10 melanomas as monotherapy & in combination with TA99 MAb by promoting Tumor Infiltrating Lymphocytes (TILs)





TIL analysis at 6 days post MDNA11 treatment



For efficacy study:
- Treatment initiated 9-days post implant; average tumor size ~15 mm³
- MDNA11 (5 mg/kg, IP, Q.W); TA99 MAb (150 μg, IP, B.I.W.) for 3 cycles

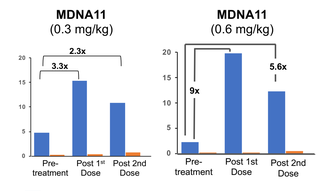
For TILs analysis:
- Average tumor size ~50 mm³ when mice received a single dosing with MDNA11 (5 mg/kg; IP).
- TILs analyzed by flow cytometry:
  - CD8 T cells (CD45⁺CD8⁺)
  - NK cells (CD45⁺NK1.1⁺)
  - Tregs (CD4⁺CD25⁺FoxP3⁺)

MDNA11 displays extended half-life in mice and NHP

	$T_{1/2}$ in Mice (h)	$T_{1/2}$ in NHP (h)	Cmax in NHP (ng/mL)	AUC in NHP (h.ng/mL)
MDNA11	6.83	12.8 – 24.7 [a]	3,446 [b]	76,297 [b]

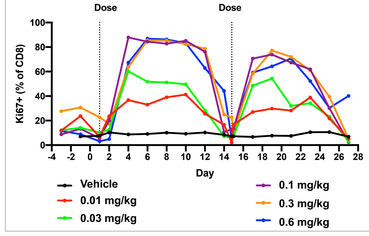
(a) Based on a population PK model containing both linear and non-linear elimination pathways using Monolix 5.1.1; Data cover the dose range of 0.01 - 0.6 mg/kg after first and second dosing.
(b) Based on analysis of data of first dosing for the 0.3 mg/kg dose level.

MDNA11 induces lymphocyte but not eosinophil expansion in NHP

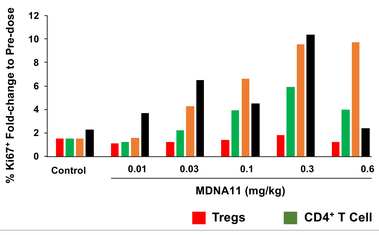


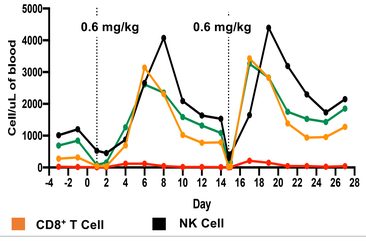
Cynomolgus monkeys dosed 14 days apart by slow IV bolus; N = 1 per group.

MDNA11 induces durable proliferation of CD8⁺ T cells in NHP



MDNA11 induces proliferation & expansion of CD4⁺ T, CD8⁺ T and NK cells but not Tregs in NHP





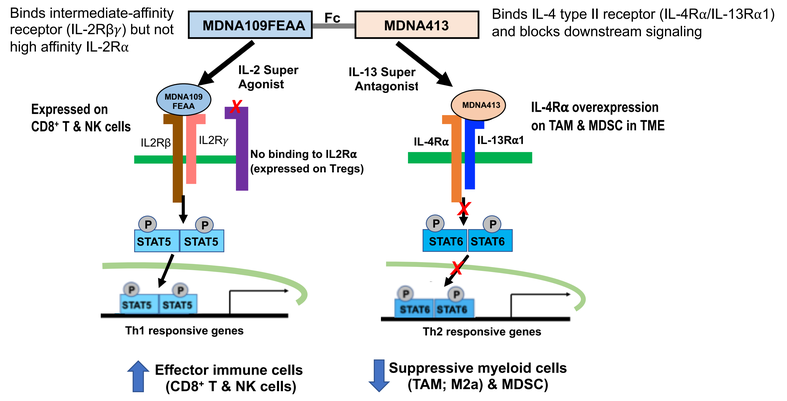
CONCLUSIONS

- MDNA11 demonstrated potent therapeutic efficacy in mouse tumor models as monotherapy and in combination with immune checkpoint inhibitor.
- In NHP, MDNA11 exhibited prolonged half-life and induced durable proliferation and expansion of CD8 T cells, CD4 T helper cells, NK cells but not Tregs.
- MDNA11 did not cause vascular leak syndrome (i.e pulmonary edema), hypotension, cytokine storm and anti-drug antibody response.
- The versatility of IL-2 and IL-13 superkine platforms enables engineering of long-acting bi-specific constructs to simultaneously activate IL-2 signaling (i.e. pro-inflammatory) and suppress IL-4/IL-13 function (i.e. anti-inflammatory) to target immunologically 'cold' tumors.

Targeting immunologic 'cold tumors' by modulation of TME with IL-2/IL-13 Bi-specific Superkines

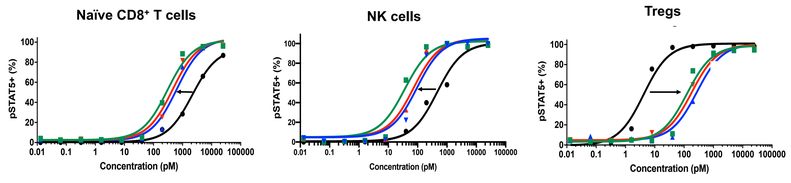
'Cold' tumors are not responsive to check-point inhibitors because of a pro-tumoral TME:
- Low CD8⁺ & NK cell counts; high Treg counts
- High number of immune-suppressive myeloid cells (i.e. TAM & MDSC)

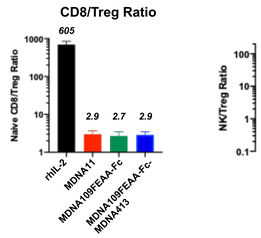
IL-2/IL-13 Bi-specific Superkine

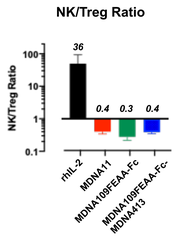


Bi-specific MDNA109FEAA-Fc-MDNA413 Superkine induces Th1 and reduces Th2 immune responses

Enhanced potency on CD8+ T and NK cells; diminished activity on Tregs
- Strong Th1 response -







MDNA109FEAA retains similar potency on immune cells as mono-specific (MDNA11; MDNA109FEAA-Fc) and bi-specific (MDNA109FEAA-Fc-MDNA413) superkines

MDNA109FEAA-Fc-MDNA413 inhibits hIL-4/hIL-13 induced signaling in HEK-Blue reporter cells
- Suppression of Th2 response -