agenT-797, a native allogeneic “off-the-shelf” iNKT cell therapy product shows anti-tumor activity

Poster 205

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Background

iNKT cells target tumor cells and reshape the TME

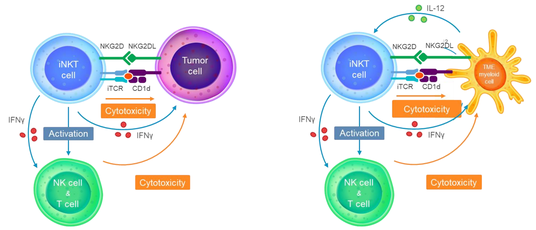
iNKT cells directly target tumor cells through:

- The invariant T cell receptor (iTCR), which detects glycolipids presented by CD1d
- NKG2D, which detects stress ligands expressed on tumor cells

iNKT cells indirectly target tumors by:

- Recruiting and trans-activating Natural Killer (NK) cells and T cells
- Targeting myeloid cells in the tumor to repolarize the immunosuppressive Tumor Microenvironment (TME)

Direct Tumor Targeting **Reshaping of the TME**



iNKT cells repolarize the TME via cell-to-cell contacts and soluble mediators. These interactions:

- Promote polarization of Tumor-associated macrophages (TAMs) to a M1 proinflammatory phenotype
- Deplete Tumor-associated neutrophils
- Reduce activity of myeloid-derived suppressor cells (MDSCs)
- Convert Dendritic cells (DCs) from an immature immunosuppressive state into mature DCs
- Induce an IL-12 mediated positive feedback loop which boosts the activity of other tumor-resident immune effector cells, including T cells and NK cells

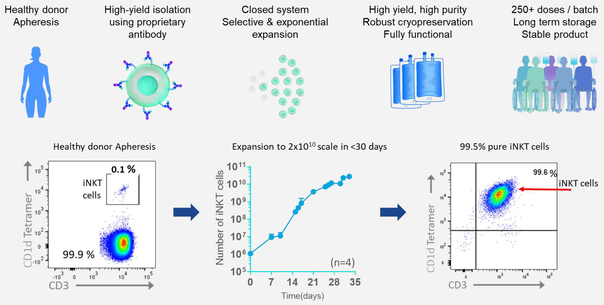
iNKT cell-based allogeneic cell therapy offers increased benefits over other cell formats

		T cells	NK Cells	iNKT Cells
Potent Cancer Killing	Special population of T cells with NK properties	✗	✗	✓
	Potential for durable anti-tumor immunity	✓	✗	✓
	Orchestrate innate and adaptive immune responses and modulate suppressive myeloid compartment	✗	✗	✓
Enhanced Tolerability	No gene engineering needed for allogeneic application	✗	✓	✓
	Naturally suppresses GvHD/supports engraftment	✗	✗	✓
	Ability to multi-dose	✗	✓	✓
	Administered without lymphodepletion	✗	✗	✓
Possibly Most Scalable and Stable Off-The-Shelf Approach	Ready-made, scalable, off-the-shelf approach with proprietary process for ~99% purity and scaling beyond 10,000 doses/yr	✗	?	✓

*GVHD: Graft vs. host disease

** iNKTs express a molecule known as invariant TCR (iTCR) at their cell surface. iTCRs are highly specific to iNKTs and are not expressed by normal tissue. In the theoretical event that iNKTs trigger severe adverse events in a patient, iTCR can be targeted with a specific antibody to kill iNKTs without killing healthy immune cells. MiNK has IP rights over such an antibody

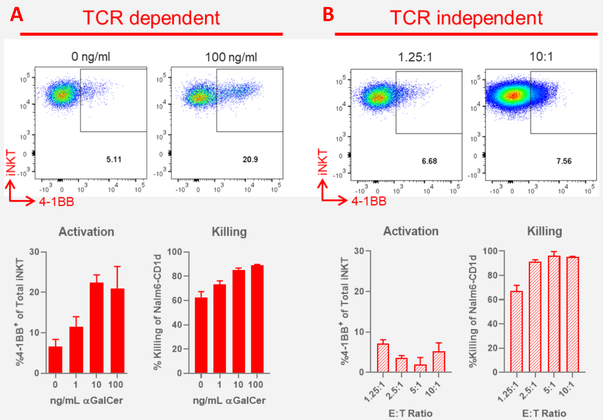
iNKT cell manufacture is scalable



- iNKT cells can be manufactured from healthy donors
- Scaling up for in-house production of >10,000 doses / year
- MiNK’s iNKT cells (agent-797) are off-the-shelf, scalable, potent before/after cryopreservation and efficiently transported and stored

Results

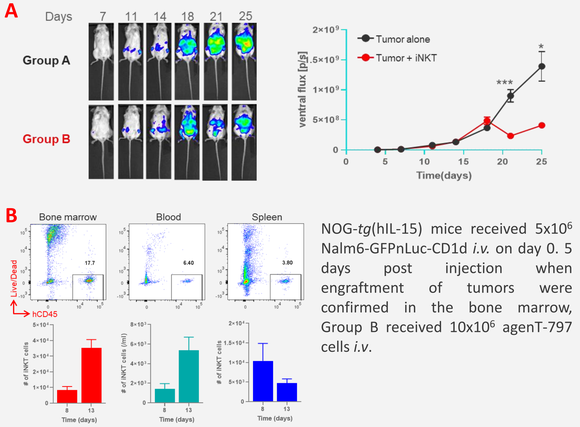
iNKT cells efficacy against the Nalm6-CD1d cells were tested *in vitro*.



A. Nalm6-CD1d cells were pulsed with various doses of α-GalCer (1,10,100 ng/ml) for 2 hours and incubated with iNKT cells at 1:1 ratio for ~20 hours. Dose dependent increase in 4-1BB expression on iNKT cells is observed. Increase in killing based on dose is also seen. Even without any α-GalCer iNKT cells kill at ~60% (role for NK cell receptors). B. Nalm6-CD1d target cells were co-cultured with varying E:T ratios (1.25, 2.5, 5 and 10 iNKT to 1 target) of iNKT cells in absence of lipid for ~20 hours. No TCR mediated activation observed, as measured by 4-1BB expression. Increased E:T ratio, induced further killing of target cells. iNKT cells injected are functional against the tumor cells injected in NOG-hIL15 mice.

*E:T = Effector:Target

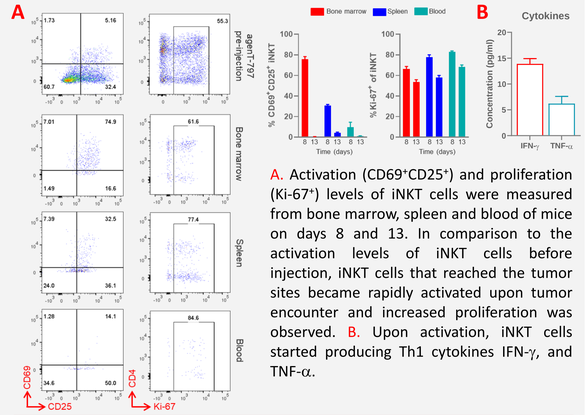
agenT-797 show Tumor Killing potential in a liquid tumor xenograft model



NOG-*tg*(hIL-15) mice received 5x10^6 Nalm6-GFPnLuc-CD1d *i.v.* on day 0. 5 days post injection when engraftment of tumors were confirmed in the bone marrow, Group B received 10x10^6 agenT-797 cells *i.v.*

A. Tumor burden and expansion was monitored via IVIS imaging. Mice injected with tumor only showed progressive tumor growth with survival of 25 days. B. On days 8 and 13, 3 mice from each group were euthanized for analysis. iNKT cells were detectable in bone marrow, blood and spleen of mice, with increasing numbers in blood and bone marrow over time.

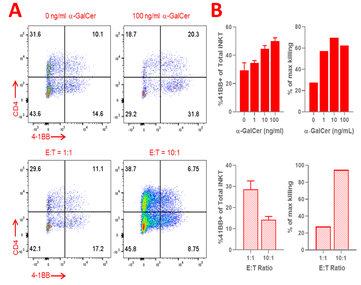
agenT-797 cells are reaching tumor sites and are functional rapidly post infusion



A. Activation (CD69$^+$CD25$^+$) and proliferation (Ki-67$^+$) levels of iNKT cells were measured from bone marrow, spleen and blood of mice on days 8 and 13. In comparison to the activation levels of iNKT cells before injection, iNKT cells that reached the tumor sites became rapidly activated upon tumor encounter and increased proliferation was observed. B. Upon activation, iNKT cells started producing Th1 cytokines IFN-γ, and TNF-α.

iNKT trafficking, homing, and functional characteristics underscore data from an ongoing clinical trial in r/r MM (NCT04754100). Early data of AgenT-797 with IV administration with no lymphodepletion in a heavily pre-treated population refractory to anti-BCMA therapy reveals suppression of M spike protein, tumor cells in the bone marrow, and durable disease stabilization >6m.

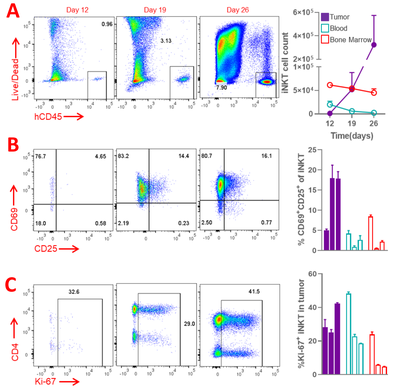
agenT-797 shows *in vitro* cytotoxic activity toward the melanoma solid tumor A375



A. A375-CD1d cells were pulsed with various doses of α-GalCer (1,10,100 ng/ml) for 2 hours and incubated with iNKT cells at 1:1 ratio for ~20 hours. B. A375-CD1d target cells were co-cultured with varying E:T ratios (1 and 10 iNKT to 1 target) of iNKT cells in absence of lipid for ~20 hours. Activation was measured by flow cytometry, whereas killing was measured by incucyte. iNKT cells became activated in response to α-GalCer and induced dose dependent killing of target cells. Similarly, increased E:T ratio resulted in enhanced killing.

$$Killing\ (\%Max) = \frac{AUC\ test}{AUC\ staurosporine} \times 100$$

agenT-797 cells are active *in vivo* in a melanoma solid tumor model A375



A. In the subcutaneous model of A375-CD1d, upon injection of iNKT cells, cells infiltrated their natural homing site, bone marrow, whereas some of the iNKT cells made it in the tumor. Over time, iNKT cells residing in blood and bone marrow of mice did not expand, whereas the iNKT cells in the tumors expanded over time. B. Only the iNKT cells that reached the tumor became activated in response to their environment but not the cells in the blood or bone marrow. C. Whereas the iNKT cells in the blood or bone marrow cannot maintain their proliferative capacity, proliferation of iNKTs in the tumor increases. (Representative plots from tumor samples)

Conclusions

- MiNK Therapeutics is a clinical stage biopharmaceutical company pioneering the discovery, development, and commercialization of allogeneic, off-the-shelf, invariant natural killer T (iNKT) cell therapies to treat cancer and other immune-mediated diseases.
- MiNK Therapeutics delivered 3 INDs for lead product candidate (agenT-797) targeting heme malignancies (multiple myeloma), solid tumors and ARDS secondary to COVID-19 infection.
- We developed murine xenograft models to address the impact of agenT-797 in liquid and solid tumors, demonstrating trafficking, activation and expansion of these cells in response to different tumors.

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