Persistence and tissue distribution of Agent-797 – a native allogeneic iNKT cell-based cell therapy drug product

Poster 400

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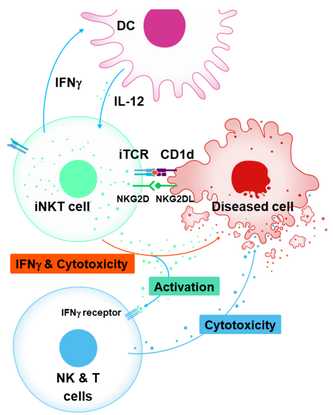
Background

iNKT cells combat disease through direct and indirect mechanisms

Invariant Natural Killer T (iNKT) cells are key effectors and regulators of immune responses, making them an ideal immunotherapy.

Figure 1 Functions of iNKT cells in disease



iNKT cells directly target diseased cells through:

- The invariant T cell receptor (iTCR), which detects glycolipids presented by the non-polymorphic MHC-I like molecule CD1d
- NKG2D, which detects stress ligands expressed on tumor cells

iNKT cells indirectly target diseased cells by:

- Recruiting and trans-activating Natural Killer (NK) cells and T cells
- Acting as master regulators of immune responses through interaction with myeloid cells via cell-to-cell contacts and soluble mediators

In cancer, iNKT cells reshape the tumor microenvironment. Specifically, they:
- Promote polarization of tumor-associated macrophages to a M1 phenotype
- Deplete tumor-associated neutrophils
- Reduce activity of myeloid-derived suppressor cells (MSDCs)
- Induce maturation of immature Dendritic cells (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

In viral lung disease, iNKT cells:
- Induce maturation of immature DCs
- Recruit NK cells and cytotoxic T cells
- Control secondary bacterial outgrowth through cytokine secretion
- Reduce activity of myeloid-derived suppressor cells
- Kill inflammatory monocytes
- Protect airway epithelial cells from damage

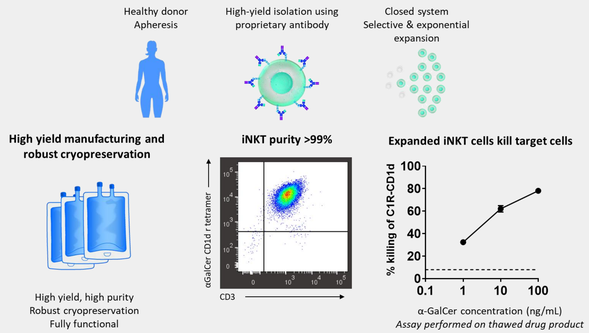
Table 1 iNKT cell-based allogeneic cell therapy offers increased benefits over other cell formats

		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/year	✗	?	✓

MiNK Manufactured iNKT cells

MiNK Therapeutics' iNKT cell-based allogeneic drug product agent-797
- Proprietary critical reagents and process enable manufacture of >99% pure iNKT cells with retained potency before and after cryopreservation
- Off-the-shelf, scalable, on-site when the patient needs them
- Early phase manufacturing capacity for in-house production of >10,000 doses / year

Figure 2 MiNK Manufactured iNKT cells Scale with Full Functionality



Agent-797 (iNKT)-treated intubated ICU patients with ARDS (COVID-19) show 77% survival (NCT04582201)

- Phase 1 study to evaluate the safety and efficacy of agenT-797, an unmodified, allogeneic iNKT cell therapy, in patients with acute respiratory distress syndrome (ARDS) secondary to COVID-19, requiring mechanical ventilation
- Agent-797 can be administered tolerably to 1000 x 10^6 cells/dose without neurotoxicity or cytokine release syndrome

Table 2 77% Overall Survival in intubated ICU Patients with ARDS (COVID-19)

Dose level	Agent-797 dose (No. cells)	No. patients	No. ongoing	No. discharged	No. deaths
1	100 x 10^6	3	-	2	1
2	300 x 10^6	4	-	3	1
3	1000 x 10^6	6	2	3	1

Data presented from phase 1 study in COVID-19 ARDS
No neurotoxicity or cytokine release syndrome observed

Trial objectives and design: Evaluating the safety, tolerability, dose limiting toxicity (DLT), and preliminary clinical activity of agent-797 in patients with ARDS in COVID19 (NCT04582201). Part 1 will employ a standard 3+3 dose escalation design of agenT 797 to determine MTD. Part 2 will enroll eligible patients in an Expansion Cohort.

Trial population: Male and female, ≥ 18 years of age, confirmed diagnosis of SARS-CoV-2 infection by PCR test, evidence of SARS-CoV-2 infection with the diagnosis of moderate to severe ARDS, requires intubation with mechanical ventilation. Up to 28 patients.

Treatment schedule: A single dose of agent-797 is administered i.v. The study period is 12 weeks following dose infusion.

Clinical status: Survival rate of 77% (10 of 13) compared to National average of approx. 40 % (range 24-53%) for intubated or mechanically ventilated patients during time of enrollment (Source CDC; https://www.cdc.gov/nchs/covid19/nhcs/hospital-mortality-by-week.htm).

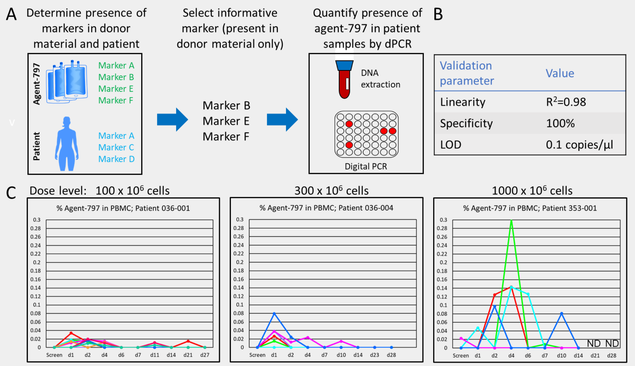
Table 3 Baseline demographics

Characteristic	All patients (n=13)
Age	
Median (range)	69 (49-77)
Sex, *n (%)*	
Male	3 (23)
Female	10 (77)
Co-medications, *n*	
Remdesivir Steroids	5
Remdesivir Steroids Tocilizumab	5
Other	3

MiNK Therapeutics has also initiated studies of agent-797 in the treatment of patients with relapsed/refractory multiple myeloma (NCT04754100) and solid tumor cancers (FDA IND-cleared).

Peripheral persistence of agent-797

Figure 3 Agent-797 cells rapidly translocate from peripheral blood into tissue

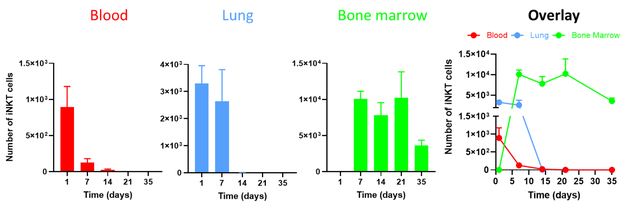


An assay to detect allogenic iNKT cells in the PBMC of patients treated with agent797 was developed and validated. (A) The assay is based on digital PCR technology to detect genetic chimerism in patient samples. 30+ genetic markers (In/Del) are utilized to differentiate donor (agent-797) from patient material. For each patient/donor pair a unique set of informative markers is determined. Informative markers are used to determine % chimerism by digital PCR. (B) Assay validation data. (C) Persistence of allogeneic iNKT cells (agent-797) in peripheral blood was determined over 28 days. Representative data of agent-797 persistence at different dose levels from trial NCT04582201. Each line indicates measurement of a separate informative marker.

Key observations:
- Initial spike of agent-797 cells in peripheral blood observed post-infusion
- Detection of agent-797 in peripheral blood up to day 6 of treatment
- Detected level scales with dose level
- Data consistent with the expected rapid translocation of iNKT cells from blood into tissue
- Method can be validated for any tissue

MiNK iNKT cells are persistent and home to critical organs

Figure 4 iNKT persistence and tissue homing



We developed a murine xenograft model for preclinical studies on agent-797, based on NOG mice expressing human IL-15 (NOG-hIL15) for the maintenance of human T lymphocytes. Mice were injected with 10 million agent-797 cells and revealed near-immediate trafficking to bone marrow, lung, liver, spleen. Persistence of agent-797 in different tissues was determined by flow cytometry through staining for cells expressing human CD45. Graphs show absolute numbers of human iNKT cells detected.

Key observations
- iNKT cells rapidly leave the circulation and infiltrate critical organs, including lung, liver, and bone marrow
- iNKT cells persist in the bone marrow for beyond the 35 day measuring timeline

Our NOG-hIL15 mice provide a suitable model for preclinical studies of agent-797. In this model agent-797 demonstrates both tissue trafficking and persistence, thus allowing the study of biological effect. **Application of this model in preclinical studies of agent-797 for targeting cancer is presented in MiNK poster #205 and the clinical impact of iNKT homing for viral and tumor clearance are described herein**.

Conclusions

- **AgenT-797 (IV)** demonstrates a pronounced survival rate of 77% in mechanically ventilated elderly patients with COVID-ARDS and can be administered to 1x10^9 cells/dose with no DLTs, no neurotoxicity, and no cytokine release syndrome
- **MiNK** has established a proprietary manufacturing process and critical reagents to facilitate rapid iNKT cell production and global distribution at scale; cells are potent before and after cryopreservation and available when needed
- **Clinical trials** in patients with solid tumors (+/- CPIs), multiple myeloma, and viral ARDS are underway. Engineered CAR-iNKTs and iNKT engagers will advance to IND starting in 2022
- **MiNK** developed a digital PCR-based method to characterize agent-797 in patients; analyses of tissue distribution, persistence, and functional capacity of agent-797 are underway
- **MiNK** established a xenograft model for the study of agent-797 to recapitulate human iNKT cell distribution and evaluate efficacy in tumor models **[poster #205]**

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