Nammisomes: Preclinical validation of a platform for selective and synchronized delivery of novel immunotherapy prodrug combinations to tumors

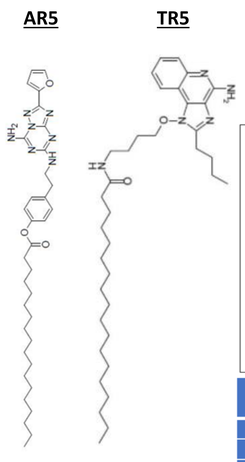
1581

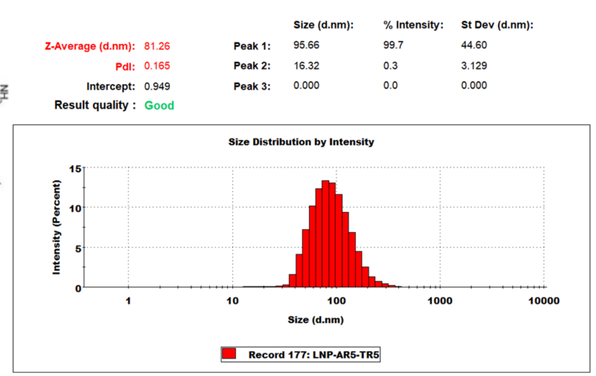
Dhruba Bharali, Tahmineh Safaie, Bruce A. Hay, Rajesh K Sharma, Scott Ayers, David R. Stover

Nammi Therapeutics, Inc., Los Angeles, CA (WWW.NammiRX.com)

Abstract:

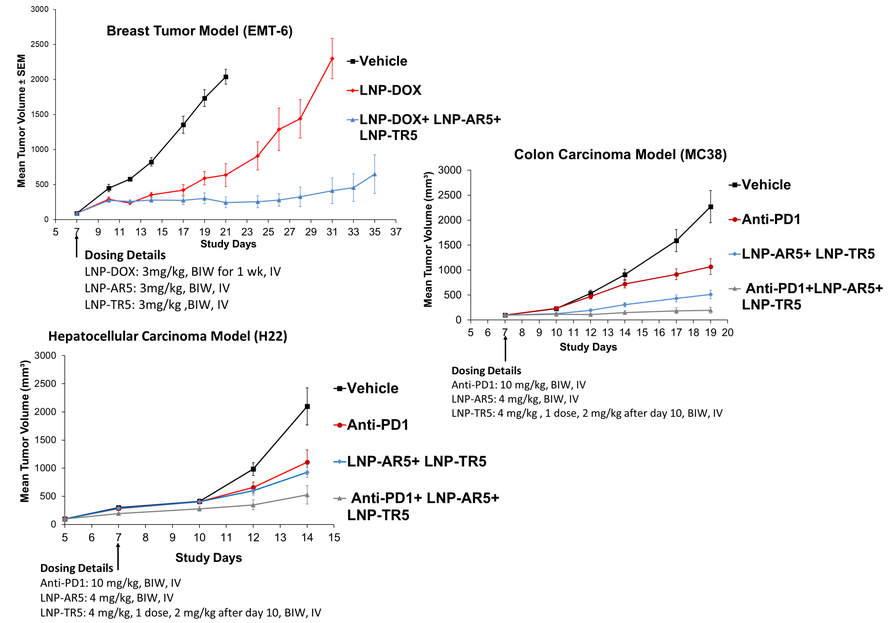
The approval of checkpoint inhibitors, CAR-T cell therapies, and other immunotherapies has led to a significant improvement in cancer patient survival rates over the past 7 years. However, the pace of improvement has slowed lately as the field moves beyond the minority of patients with immune primed or accessible cancers. It is generally expected that rational combinations of immunotherapies that provide mechanisms to both induce as well as maintain the immune responses in the face of tumor-derived immune checkpoint mediators, will be required to effectively treat these patients. Systemic exposure to such combinations often can give rise to severe toxicity profiles that limit their therapeutic efficacy. Nammisomes are an innovative and unique platform designed to deliver novel immune modulating prodrugs more selectively and synchronously to the tumor site. The immune active agents are conjugated to lipids to create prodrugs that self-assemble into a liposomal bilayer. By mixing multiple prodrugs at the desired ratio, co-formulations are produced with the optimum stoichiometry. These Nammisomes are iv injected and selectively enter tumors through the leaky vasculature within tumors, while remaining in circulation in normal tissues with intact blood vessels, The enriched phagocytic activity in tumor environments causes the release of the active agents within tumors relative to normal tissues limiting systemic exposure and toxicities. Preclinical efficacy and safety data of Nammisomes delivering combinations of a TLR agonist and a checkpoint inhibitor showing anti-tumor efficacy and tolerability in syngeneic mouse models will be presented. In summary, Nammisomes will be highlighted as a combination therapeutic modality to demonstrate the potential of this approach in anti-cancer therapy..



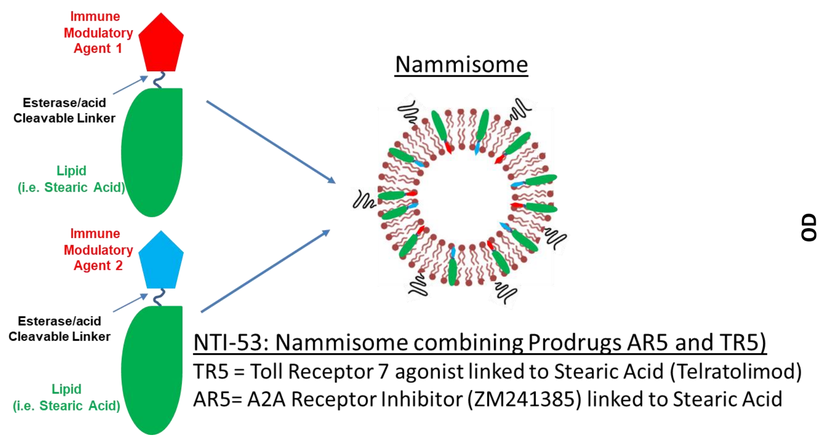


LNP-AR5-TR5(different Batches)	Size in nm diameter	PDI	Zeta potential (mV)
Batch 81120-1	81.26	0.165	-13.2
Batch 71120-2	79.23	0.146	-13.4
Batch 20121-4	78.07	0.143	-11.9

A lipid mixture of HSPC, CHOL, AR5, TR5 and DSPE-PEG at a molar ratio of 52:31:9:3:5 was prepared in ethanol and mixed with an aqueous solution of PBS using a NanoAssemblr Bench top microfluidics instrument (Precision Nanosytems). Size distribution and Zeta potential of LNP-AR5-TR5 was determined using a Malvern Zetasizer (Malvern Instrumentation Co., Westborough, MA, USA). The process provides consistent batch-to-batch formulation specifications.



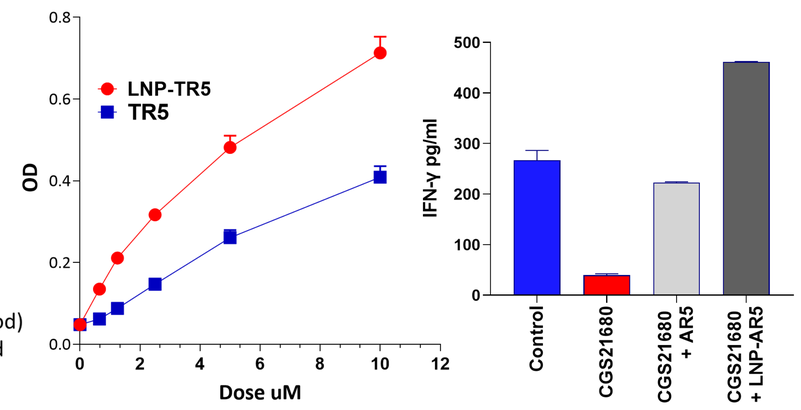
- NTI-53 efficacy in breast (EMT-6), Colon (MC-38), and Hepatocellular (H22) Carcinomas in combination with Doxil or anti-PD-1 antibody.



NTI-53: Nammisome combining Prodrugs AR5 and TR5)

TR5 = Toll Receptor 7 agonist linked to Stearic Acid (Telratolimod)

AR5= A2A Receptor Inhibitor (ZM241385) linked to Stearic Acid



- TLR7 agonist activity of Liposomal TR5 vs free 852A in RAW-Blue™ Cells (In vitrogen)
- A2A receptor inhibition by AR5 vs free ZM241385 in C57BL/6 mouse splenocytes activated with CD3/28 beads in the presence of A2A receptor agonist CGS21680.
- Both agents demonstrate enhanced cell activity as liposomal prodrugs than as free agents

Conclusions:

Formulation: Lipid particle with A2A receptor inhibitor prodrug (AR5) and Toll Receptor 7 agonist prodrug (TR5) incorporated. Robust and consistent production process.

AR5 rationale: A2A receptor has been clinically validated to be an important immune checkpoint. Inhibition of this receptor by the active component of AR5 and other inhibitors have demonstrated anti-tumor activity including in human patients

TR5 rationale: Toll receptor 7 agonists are clinically validated to have anti-tumor activity but are generally too potent to give systemically as free drugs. Incorporation of the prodrug in a Nammisome targets the activity to tumors while reducing systemic activity allowing for safe delivery of effective doses.

Target Indications: While the immune mechanisms targeted by AR5/TR5 are independent of cancer type, the mechanism of selective tumor delivery of Nammisomes is dependent on presence of a tumor mass (as opposed to dispersed cells). Therefore, solid tumors would be the potential market for this product. Initial development will be done in multiple indications to demonstrate efficacy in a large indication such as breast cancer and in an indication with Orphan Drug Potential such as bladder, pancreatic, and renal cancers

For further information please contact us at Info@Nammirx.com

NAMMI Therapeutics

1726

Preclinical evaluation of a novel tumor selectively targeted and activated immunocytokine platform

Kham Ryan Trinh, Alex Vasuthasawat, George Ayoub, David R. Stover, Sherie L. Morrison

Qwixel Therapeutics, LLC (Now merged with Nammi Therapeutics, Inc., Los Angeles, CA; WWW.NammiRX.com)

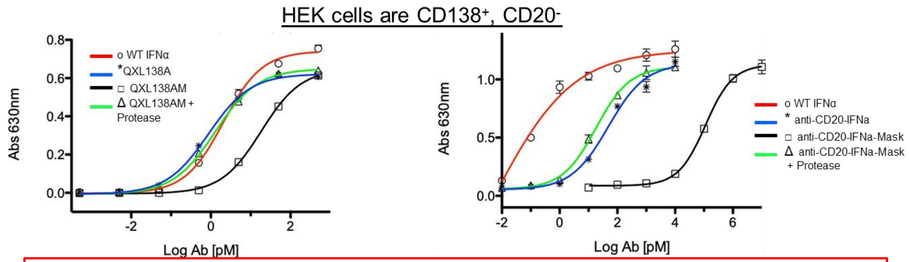
Abstract:

Interferon alpha (IFNα) is a potent cytokine with receptors expressed universally on virtually all cell types. Recombinant versions of IFNα have demonstrated efficacy in multiple tumor indications and have been FDA approved for treatment of various cancers. However, the full anti-tumor potential of IFNα is not realized with these therapies due to dose limiting toxicity, primarily represented by severe flu-like symptoms resulting from systemic activation of immune cells. Antibody-cytokine fusion proteins have been evaluated as an approach to enhance tumor delivery of the cytokine and thereby increase the therapeutic window. While this approach has merit and can increase the efficacy of such constructs, it typically does not reduce the systemic toxicity since the cytokine is still free to interact with its receptor in circulation. We have designed a novel fusion protein in which the IFNα is active only after it has entered the tumor environment, thereby preventing toxicity resulting from systemic activation of IFNα receptors. Direct antitumor efficacy of an anti-CD138-IFNα2 fusion protein with a tumor selectively cleaved peptide mask was seen in models of multiple myeloma and ovarian cancer. The mask was also shown to prevent IFN mediated activation of peripheral blood cells.

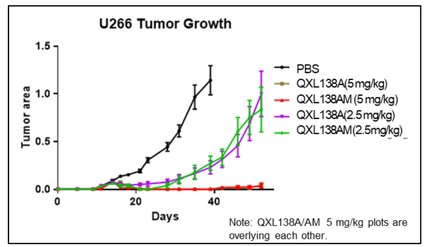
Interferons have direct anti-tumor activity as well as being master regulators of immune response

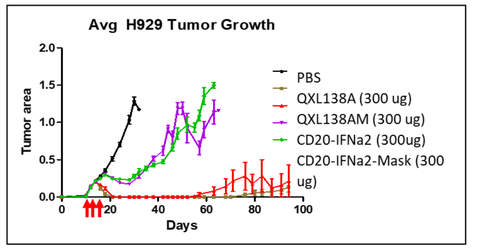
Cytokine	Inhibition of Tumor Cell Growth	Inhibition of Angiogenesis	Enhanced ADCC	Maturation of APCs (Innate Immunity)	Stimulation of T-cells (Adaptive Immunity)	Established Clinical Experience
IL-2	-	-	+	-	+	+
IL-7	-	-	-	-	+	-
IL-12	-	+/-	+	-	+	+/-
IL-15	-	-	+	-	+	-
IL-18	-	+/-	+	-	+	-
IL-21	+/-	-	+	-	+	+/-
GM-CSF	-	-	+	+	+	+
IFN α /β	**+**	**+**	**+**	**+**	**+**	**+**
IFN γ	**+**	**+**	**+**	**+**	**+**	**+/-**

- Immunocytokines with IFNs fused to Abs are potent anti- cancer therapeutics, but can result in systemic IFN receptor binding that can reduce the half-life of the therapeutic and/or cause toxicity
- To prevent systemic IFN receptor binding, while maintaining anti-tumor activity, Qwixel has designed peptide masks that are expressed on the C-terminal end of the IFN and block its ability to bind to its receptor.
- The masks are attached through a peptide sequence that is sensitive to cleavage by tumor-selective proteases; thereby allowing activation of the IFN selectively within the tumor environment

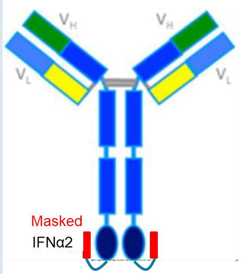


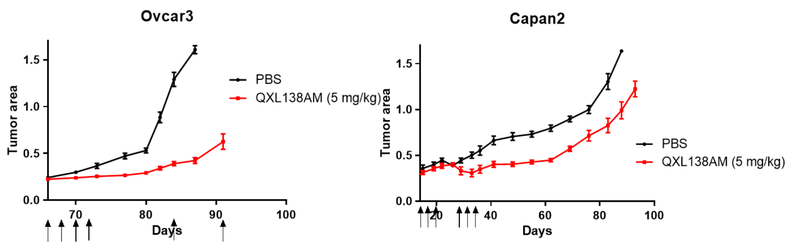
- **Ab targeted IFNα is as potent as wild type IFNα**
- **Non-targeted Ab-IFNα is ~100x less potent than Wt IFNα**
- **The mask is very effective at blocking non-targeted IFNα**
- **The mask reduces IFNα activity even when targeted to transformed (but non-tumorigenic) HEK cells expressing CD138**



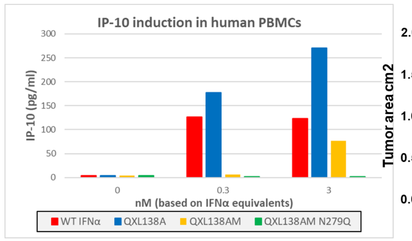


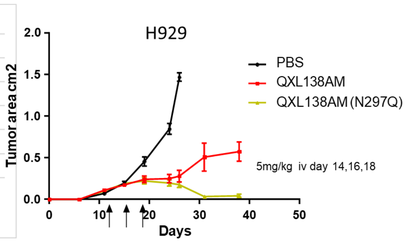
- Efficacy in immune compromised mice, demonstrates direct anti-tumor activity
- QXL138AM (masked) is as potent as QXL138A (unmasked)
- Optimal QXL138A/AM potency requires specific tumor targeting
- Single Agent QXL138A/AM is capable of achieving durable CRs that last for months after treatment stops





- Efficacy in CD138+ solid tumors including Ovarian (OVCAR3) and Pancreatic (Capan2)





- IP-10 (CXCL10) is an inflammatory cytokine that activates and recruits leukocytes such as T-cells, NK cells, and macrophage
- IFNα induces PBMCs to release IP-10, Ab-IFNα fusion proteins are also able to induce IP-10
- Masking the IFNα reduces (>10x) the potency of IP-10 induction
- Masking and mutating the Ab glycosylation site (thus reducing FcR binding) further reduces PBMC activation (>100x)
- Removing the glycosylation site prevents interaction with Fc receptors that mediate ADCC, but does not reduce QXL138AM efficacy

Conclusions:

QXL138AM is a novel Ab-IFNα2 fusion protein with a cleavable peptide mask that blocks the activity of IFN until the mask is released in the tumor environment. Potent efficacy in myeloma, ovarian, and pancreatic cancers have been observed in immune-deficient mice. Studies in syngeneic tumor models with immune competent mice are underway to evaluate the added efficacy that immune activation can provide.

This design provides 3 levels of tumor selectivity

1. Tumor antigen targeting enhances tumor retention and localizes the fused cytokine to the target cell surface

2. Mechanism of IFNα
   a. Synergy of anti-TAA ADCC with IFNα activation of NK cells
   b. Tumor cells are more susceptible to IFNα induced apoptosis than normal epithelial cells
   c. Activation of an immune response is triggered by IFNα, but requires tumor antigens for sustained cytolytic activity
3. Release of the mask is enhanced in tumors by upregulation of matriptase expression and activity within most tumors

CD138AM preclinical development is underway with an IND scheduled for mid-2022.

NAMMI Therapeutics

For further information please contact us at Info@Nammirx.com