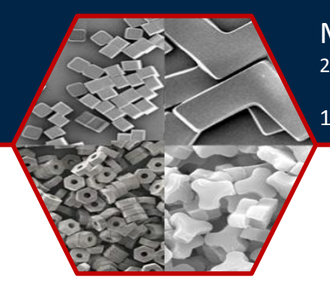
# PRINT nanoparticle-based delivery of immunostimulants and the MAGE-A3 antigen induces high IgG, CD4 +, & CD8+ T cell responses in swine



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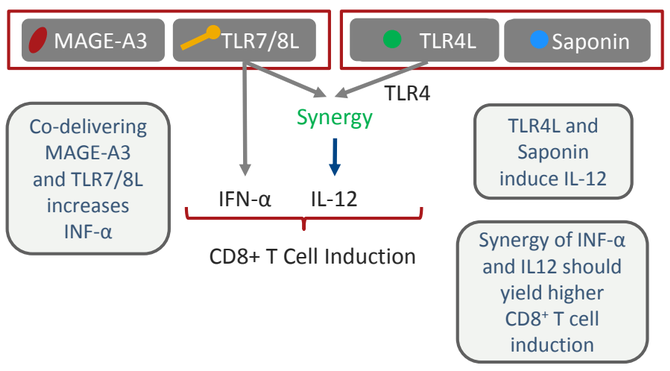
## Introduction

The *MAGE-A3* gene is expressed in a wide variety of tumors.[1] It is presented to specific T cells by HLA molecules at the cell surface as a tumor-specific antigen.[2] *MAGE-A3* is not expressed on most adult tissues and the few that do express it do not bear HLA molecules; therefore, it is a suitable selective target for a tumor-specific active immunotherapy.

Historical studies have shown that recombinant MAGE-A3 protein used as an immunotherapy had antitumor activity in patients with metastatic melanoma[3,4] (see article by Kruit et al) or bladder cancer,[3] in which a few, but significant, long-term clinical responses with good tolerability were documented. Recent studies targeted to non–small cell lung cancer and melanoma using MAGE-A3 with various adjuvant systems, however, did not demonstrate improved patient outcomes. [6,7] Therefore it is valuable to explore additional novel formulations that would enable a more robust CD8 + T cell response. [5]




Utilizing Liquidia's PRINT technology, TLR7/8L agonist was co-particulated with the MAGE-A3 protein and dosed with the Liposomal TLR4L/saponin adjuvant system into Londrace pigs. Co-encapsulation and delivery of TLR7/8L and MAGE-A3 showed significantly enhanced CD8+ T cell responses, as well as CD4 + T cell and B cell when compared to the Benchmark control.

## Why PRINT Particles?

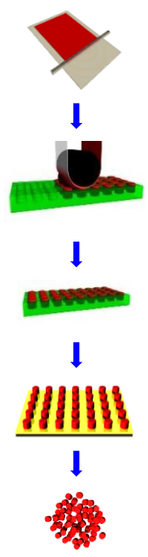
- Co-encapsulation of multiple compounds with very different solubility profiles within each particle possible to improve cellular uptake and immune responses.
- Precise control over particle shape and size at nano-scale.
- Sterile filtration capabilities.
- Particulate delivery shown to enhance cellular uptake and antigen processing.
- Flexibility to combine particles with soluble antigen or adjuvant for final formulation with no observed interference.

## The PRINT Process

**The core process involves four basic steps**:

1. Create a film of the desired composition on a delivery sheet.
2. Laminate the film with a mold where the material fills the mold cavities.
3. Remove particles from the mold.
4. Collect particles to create a particle suspension or dry powder.

*There are several variables that can be leveraged to create particles of a wide range of shapes, sizes, and chemical and physical composition.*



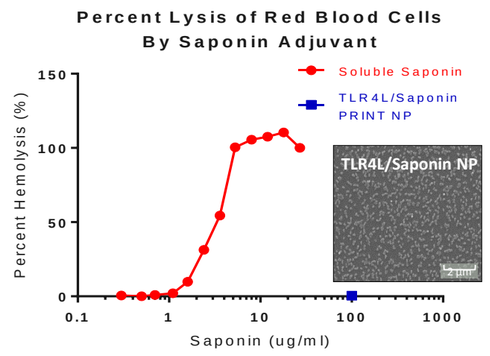
## Particle Characterization

**M3/TLR7-8L-NP**

Purified MAGE-3A and TLR8L were co-encapsulated within 80 nm x 80 nm x 320 nm rod-shaped PLGA particles.

**TLR4L/Saponin-NP**

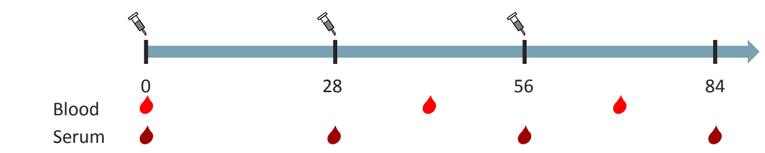
TLR4L and saponin were co-encapsulated within 80 nm x 80 nm x 180 nm rod-shaped PLGA + Cholesterol particles.




Hemolytic activity of the saponin adjuvant was measured using chicken red blood cells. Lysed cells were detected in a plate based absorbance assay at 540 nm. Percent lysis was calculated from 100% lysis controls.

## Study Design

N=12 Londrace pigs dosed D0, D28, D56. Blood drawn for T cell response D0, D14P2, D14P3. Sera collected for IgG titers D0, D28, D56, D84.



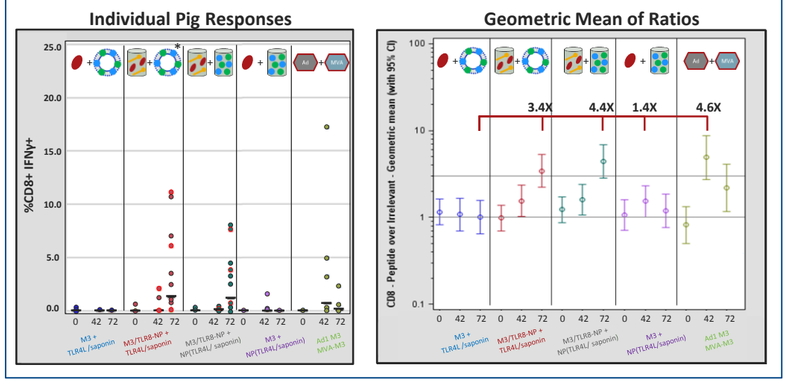

*Treatment Groups:*

| Group | Formulation | M3 Dose | TLR7/8L Dose | TLR4L Dose | Saponin Dose |
|---|---|---|---|---|---|
| 1 | Benchmark: Soluble MAGE-A3 (M3) + Liposomal TLR4L/Saponin (Adjuvant System) | 300 µg | N/A | 50 µg | 50 µg |
| 2 | PRINT M3/TLR8L (M3/TLR8-NP) + Liposomal TLR4L/Saponin (Adjuvant System) | 300 µg* | 919 µg* | 50 µg | 50 µg |
| 3 | PRINT M3/TLR8L (M3/TLR8-NP) + PRINT TLR4L/Saponin (NP-Adjuvant System) | 300 µg* | 919 µg* | 52 µg | 53 µg |
| 4 | Soluble MAGE-A3 (M3) + PRINT TLR4L/Saponin (NP-Adjuvant System) | 300 µg | N/A | 52 µg | 53 µg |
| 5 | Control: Ad (Adenovirus MAGE-3A) + MVA (Vaccinia virus MAGE-3A) | N/A | N/A | N/A | N/A |

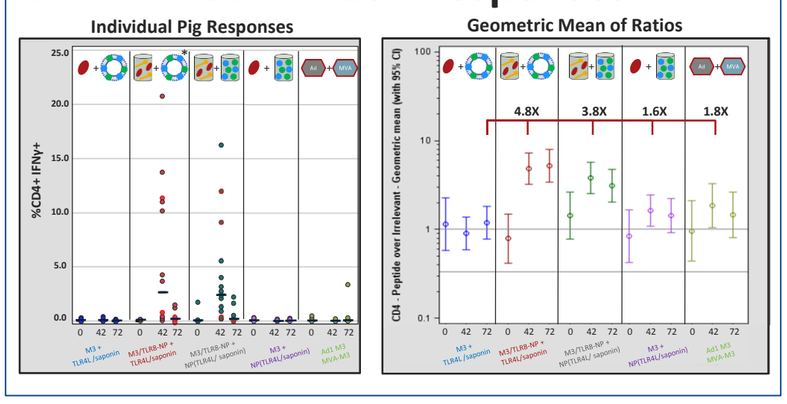
* Some animals received smaller doses.
Group 2: 2 animals received [823.5 µg TLR7/8L + 270 µg M3], 1 received [730 µg TLR7/8L + 240 µg M3]
Group 3: 2 animals received [823.5 µg TLR7/8L + 270 µg M3], 1 received [686 µg TLR7/8L + 225 µg M3]

## Improved Cellular & Humoral Immunity

### A. CD8+ T Cell Responses




### B. CD4+ T Cell Responses




### C. Anti MAGE-A3 IgG Responses

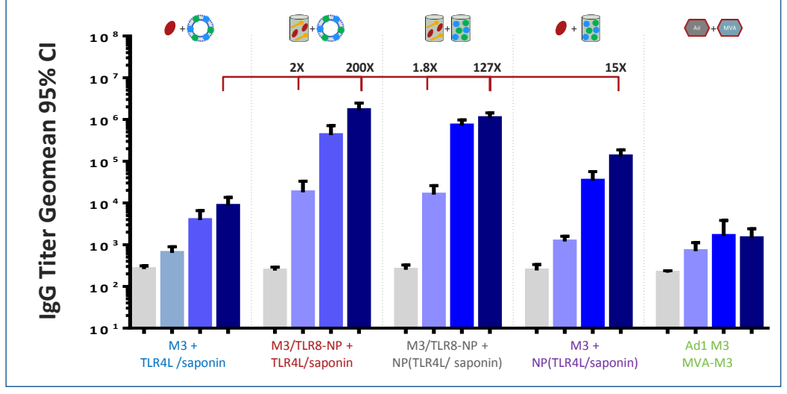



**Figure 1. Significant Improvements in Both Cellular and Humoral Immunity**.

**T cell response:** CD8+ T cell (A) CD4+ T cell (B) IFNγ responses were observed in the PRINT M3/TLR8-NP groups compared with the benchmark regardless of the form of the adjuvant systems. Soluble M3-Adj System did not elicit a robust CD8+ T cell response, demonstrating the positive impact of the PRINT-TLR8 agonist. In the left panel, data points outlined in orange represent pigs who received lower doses of M3 or TLR7/8L according to dosing table. An undefined trend was for increased local reactogenicity observed in some groups (*) that will need further investigation (data not shown).

*Methods: For both CD8 + and CD4+ T cell detection, PBMCs were amplified for 2 weeks in paired wells with either MAGE-A3 peptides or with irrelevant control peptides. The T cell response is expressed as the geomean of ratios between MAGE-A3-specific wells and their control paired wells (right panel) or as individual pig responses expressed as the geomean per pig of MAGE-A3 specific response deduced with background response (left panel)*

**Humoral response:** (C) A highly significant improvement in anti-MAGE-A3 IgG was observed for PRINT NP formulations compared with benchmark and viral control groups. Co-encapsulation within PRINT NPs provided equivalent titers following first dose and superior titers following second and third doses as compared with 3 doses of the benchmark. Anti MAGE-A3 antibodies were detected using an ELISA assay.

- *PRINT protein/adjuvant formulations induced superior immune responses across multiple arms of the immune system that were superior or equivalent to benchmark and viral controls.*

## References

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## Conclusions

- The PRINT platform offers a high degree of flexibility and can be utilized to co-encapsulate multiple target molecules (antigens and adjuvants) to enhance immune responses.
- Antibody titers from a single dose of PRINT (M3- TLR8L) nanoparticles yields equivalent IgG titers to 3 doses of benchmark.
- Observed a 15x improvement in IgG titers when soluble MAGE-A3 was dosed with PRINT (TLR4L + saponin) as compared to benchmark.
- A 5x improvement in CD4 T cell response observed with PRINT (M3-TLR8L) dosed groups.
- Particulate delivery of MAGE-A3 and TLR7/8L leads to significant enhancement of CD8+ T cell responses that are on par with viral vector controls.
- Future studies would include adjuvant dose sparing to optimize vaccine formulation.

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