ISCOMATRIX™ adjuvant links adaptive and innate immune responses

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CSL Limited
Contents

• Introduction to saponins

• Overview of ISCOMATRIX® adjuvant

• Mechanism of action
Saponins as adjuvants
Structure of quillaia saponin

High-molecular weight glycosides consisting of a sugar moieties linked to a triterpene
Quillaia saponins

- *Quillaia saponaria* tree
  - Indigenous to Chile and Peru
- Crude Quillaia
  - Agriculture
  - Cosmetics
  - Food and beverages
  - Mining
  - Vaccines
History of saponin as a vaccine adjuvant

- 1926 – Crude saponins - adjuvant activity reported (Ramon)
- 1951 – veterinary vaccine (Espinet)
- 1970s – Quil A (Dalsgaard)
  - Complex and toxic
- 1982 – ISCOM (Morein)
- 1987 – QS21 (Kensil)
- 1989 – ISCOMATRIX (Morein)
- 1995 – ISCOPREP 703 (CSL)
- 2000’s – AS series (GSK), ISCOPREP saponin (CSL), GPI0100 (HB), AbISCO (Isconova), Posintro (Nordic)
## Summary of saponin based adjuvants

<table>
<thead>
<tr>
<th>Adjuvant Name</th>
<th>Company</th>
<th>Saponin</th>
<th>Human Studies</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISCOM</td>
<td>CSL</td>
<td>ISCOPREP</td>
<td>Flu, HCV, HPV E6E7, cancer, others</td>
<td>Licensed to Merck, Pfizer, Abbott</td>
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<tr>
<td>ISCOMATRIX</td>
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<tr>
<td>QS21</td>
<td>Antigenics</td>
<td>QS21</td>
<td>Alzheimers, cancer</td>
<td>Licensed to GSK (AS series below), Pfizer, Progenics</td>
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<tr>
<td>AS01, AS02, AS15</td>
<td>GSK</td>
<td>QS21</td>
<td>Malaria, cancer, others</td>
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<tr>
<td>GPI0100</td>
<td>Hawaii Biotech</td>
<td>GPI0100</td>
<td>Cancer</td>
<td>Licensed to Pfizer (veterinary), Endocyte</td>
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<tr>
<td>Matrix-M</td>
<td>ISCONOVA</td>
<td>QuilA QHA, QHC</td>
<td>-</td>
<td>Multiple veterinary Licenses Crucell (flu)</td>
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<td>AbISCO</td>
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<tr>
<td>POSintro</td>
<td>Nordic</td>
<td>QuilA</td>
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Issues with saponin based adjuvants

- Saponin alone
  - Haemolytic activity → reactogenicity
  - Alkaline breakdown → unstable

- Solution → complex with cholesterol (+ lipid)
  - AS01, AS15 (AS02 lipid o/w)
  - ISCOM
  - ISCOMATRIX
  - Matrix-M
  - AbISCO
Saponin adjuvants in advanced clinical development

<table>
<thead>
<tr>
<th>Adjuvant Name (Phase)</th>
<th>Company</th>
<th>Immune modulator/s</th>
<th>Complex</th>
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<tr>
<td>QS21 (II)</td>
<td>Pfizer</td>
<td>QS21</td>
<td>-</td>
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<tr>
<td>AS01 (III)</td>
<td>GSK</td>
<td>QS21, MPL</td>
<td>Liposome</td>
</tr>
<tr>
<td>AS02 (III)</td>
<td>GSK</td>
<td>QS21, MPL</td>
<td>Oil-in-water</td>
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<tr>
<td>AS15 (III)</td>
<td>GSK</td>
<td>QS21, MPL, CpG</td>
<td>Liposome</td>
</tr>
<tr>
<td>ISCOMATRIX (II)</td>
<td>CSL</td>
<td>ISCOPREP Saponin</td>
<td>Cholesterol and lipid</td>
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Saponin complexes induce balanced immune responses

Th1 and Th2 cytokines
Mice and humans
IFN-γ  IL-5
IL-2    IL-10

IgG_1 and IgG_2α
Antibodies in mice
Broad antibodies in humans

CD4^+ and CD8^+ T cell responses
Mice, monkeys and humans
Summary of quillaia saponins

• Long history as immunomodulators in vaccines (veterinary)
• Purified fractions required for human use
• Need to be complexed with cholesterol

• Balanced immune responses
ISCOMATRIX™ Adjuvant

Overview
ISCOMATRIX™ adjuvant

- A saponin-based adjuvant capable of inducing significant humoral and cellular immune responses in humans

- Contains ISCOPREP™ saponin (critical immunomodulatory component), cholesterol and phospholipid
Key features of ISCOMATRIX™ adjuvant technology

- Broad immune responses
  - Antibody and cellular (including CTL)

- Safe and well tolerated in humans
- Clinical experience with range of antigens
- Non-clinical safety/toxicology package
- Industrial scale manufacturing

- Regulatory acceptance
  - Well defined and characterised
  - Master File

- Strong IP portfolio
ISCOMATRIX™ adjuvant: Ag delivery and immunomodulatory capabilities
ISCOMATRIX™ adjuvant integrates innate and adaptive immunity for CTL induction

- Antigen delivery
  - Enhances cross-presentation
    - Exogenous Ag into MHC Class I pathway (CD8⁺ T cells)
    - Prolonged presentation in draining lymph node

- Immunomodulation
  - Cytokine and chemokine induction
    - Potent DC activation in vivo
ISCOMATRIX™ Adjuvant: Antigen Delivery

Intracellular location
Immature MoDC pulsed with Alexa488-OVA + ISCOMATRIX™ adjuvant

Ag alone

Ag + ISCOMATRIX® adjuvant

T=10min
Cytosolic translocation is pH-dependent
Conclusion

• ISCOMATRIX™ adjuvant:
  • Rapidly traffics protein via early (EEA-1+) and late (LAMP-1+) endosomes
  • Trans-locates protein into cytosol
  • Translocation is pH-dependent

• Results in efficient presentation onto class I and class II MHC
ISCOMATIC® Adjuvant: Antigen Delivery

Dendritic cells
ISCOMATRIX™ vaccines recruit DC into DLN and prolong MHC I presentation

Immigration kinetics

MHC class I cross-presentation

*100-fold @ 12hrs

Prolonged presentation
Are DCs are required for induction of CD8$^+$ T cell responses?

CD11c promoter
Diphtheria toxin (DT) receptor (GFP+) chimeric mice

Radiosensitive bone marrow-derived Dendritic cells
(diphtheria toxin (DT) receptor positive)

Radio-resistant
Langerhans cells

Diphtheria toxin treatment 2-3 days

Host-derived
32% 

Donor-GFP+

Untreated

Host-derived
3% 

Donor-GFP+

Treated
Radio-resistant Langerhans cells are not required

CD8$^+$ T cell responses induced by OVA-ISCOMATRIX™ vaccine in mice depleted of DCs

Radio-resistant Langerhans cells are not required
Which DC Population(s) Cross-present ISCOMATRIX™ Vaccines?

Lopez-Bravo and Ardavin, Immunity, 2009
Migratory and CD8α+ DC cross present Ag in response to ISCOMATRIX™ vaccines in vivo
Ag presentation: Two waves of ISCOMATRIX™ vaccine presentation

- Migratory DC
- Classical crosspresenting DC

Graph showing the proliferation of CD8+ T cells over time for different groups:
- Black line: ISCOMATRIX™ adjuvant + Ag
- Gray line: Ag alone

Days: 0, 1, 2, 3, 4, 5, 6, 7
Does ISCOMATRIX™ adjuvant facilitate cross-presentation by human dendritic cells?
Cross presentation is rapid and persistent

Enhanced and prolonged presentation

ISCOMATRIX™ Adjuvant: Antigen Delivery

- ISCOMATRIX™ adjuvant:
  - Accesses several human DCs populations
  - Rapidly delivers Ag into cytosol for access to MHC class I pathway (in addition to class II)
  - Allows processing by proteasome dependent and independent mechanisms
  - Results in efficient and prolonged presentation
  - Intracellular depot??
ISCOMATRIX™ Adjuvant: Immunomodulation
Rapid and transient induction of cytokines in draining lymph node

Sheep cannulation study

Widon RG et al, Vaccine 19 (2001) 572-578
**ISCOMATRIX™ Adjuvant induces rapid and transient influx of innate cells to the DLN**

**NK cells (CXCL10)**

**NK T cells**

**Neutrophils (CXCL1)**

**Macrophages (CCL2, CCL3)**
ISCOMATRIX™ Adjuvant: Intracellular signalling
ISCOMATRIX™ Adjuvant does not activate TLR signaling pathways in vitro

NF-κB gel shift assay

<table>
<thead>
<tr>
<th>ISCOMATRIX® adjuvant (µg/ml)</th>
<th>Positive Control (µg/ml)</th>
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<tr>
<td>0  0.5  1  5  10  20</td>
<td>0  0.5  1  5  10  20</td>
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THP-1

NF-κB reporter

human or mouse cell lines in vitro
MyD88 and TRIF mediated TLR signalling cascades

ISCOMATRIX™ vaccine induced CD8+ T cell responses are MyD88 dependent.
DC activation and presentation is normal in MyD88 deficient mice

IMX=ISCOMATRIX™ adjuvant
MyD88 is also required for IL-1 and IL-18 receptor signaling

Nakanishi K et al., 2001
ISCOMATRIX™ vaccines require IL-18 for CTL induction

![Bar chart comparison of CD8+ IFNγ+ T cells in different genetic backgrounds](chart.png)
CONCLUSIONS

- ISCOMATRIX™ adjuvant:
  - Targets and conditions multiple DC populations in vivo
  - Delivers proteins into cytosol for class I MHC presentation (intracellular depot??)
  - Induction of T cell responses involves MyD88/IL-18-dependent pathway (non-TLR)
## Acknowledgements

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<th>CSL</th>
<th>LICR (Austin)</th>
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