ISCOMATRIX™ adjuvant links adaptive and innate immune responses

VTIII June 2010
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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)

ISCOM, ISCOMATRIX and ISCOPREP are trademarks of CSL, AbISCO is a trademark of Isconova, Posintro is a trademark of 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>
<td></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>
</tr>
<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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<tr>
<td>AbISCO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POSintro</td>
<td>Nordic</td>
<td>QuilA</td>
<td>-</td>
<td>-</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
<table>
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<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>
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<tr>
<td>AS02 (III)</td>
<td>GSK</td>
<td>QS21, MPL</td>
<td>Oil-in-water</td>
</tr>
<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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</table>
Saponin complexes induce balanced immune responses

- **CD4⁺ and CD8⁺ T cell responses**: Mice, monkeys and humans
  - Th1 and Th2 cytokines
    - IFN-γ
    - IL-5
    - IL-2
    - IL-10
  - IgG₁ and IgG₂a Antibodies in mice
  - Broad antibodies in 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

Antigen Delivery
- Antigen Presenting Cells (APCs)
- Migration to sites of immune induction
- MHC Class I & II Presentation
- Processing
- Endosomal Escape

Integrated Adjuvant

Immunomodulation
- Immune Cell Activation
- Chemokines (e.g., KC, MIP1, RANTES)
- Cytokines (e.g., IL-2, IL-6, IL-1)
- DC Maturation
- Recruitment
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

Control

Concanamycin B

Chloroquine

Time (min)

Con B (ng/ml)

Chloroquine (µg/ml)

Translocation (in %)
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
ISCOMATRIX™ 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?

Radio-resistant Langerhans cells

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

Diphtheria toxin treatment 2-3days

Radiosensitive bone marrow-derived Dendritic cells
( diphtheria toxin (DT) receptor positive)
Radio-resistant Langerhans cells are not required

CD8⁺ T cell responses induced by OVA-ISCOMATRIX™ vaccine in mice depleted of DCs
Which DC Population(s) Cross-present ISCOMATRIX™ Vaccines?
Migratory and CD8α+ DC cross present Ag in response to ISCOMATRIX™ vaccines *in vivo*
Ag presentation: Two waves of ISCOMATRIX™ vaccine presentation

- ISCOMATRIX™ adjuvant + Ag
- Ag alone

Graph showing CD8+ T cell proliferation for different conditions.
Does ISCOMATRIX™ adjuvant facilitate cross-presentation by human dendritic cells?
Cross presentation is rapid and persistent

Enhanced and prolonged presentation

*100-fold @ 12hrs


NY-ESO-1/ISCOMATRIX™ Peptide

Class I MHC

Percent IFN-γ+ CD8+ T cells

Time after Ag-pulsing (h)

Prolonged presentation

MHC class I presentation in the draining Lymph node

- Antigen + ISCOMATRIX™
- Antigen alone
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

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

NK cells (CXCL10)

Neutrophils (CXCL1)

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

\textbf{NF-kB 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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</table>

\textbf{NF-kB reporter}

- THP-1: human or mouse cell lines \textit{in vitro}

35
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

% CD8+ IFNγ+ T cells

Wild-type  IL-18-/−   Naive

% CD8+ IFNγ+ T cells

Wild-type  MyD88-/−   Naive
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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<tr>
<th>CSL</th>
<th>LICR (Austin)</th>
<th>Univ of Munich</th>
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