Alveolar Macrophage Changes Induced by Inhaled Oligonucleotides in Preclinical Toxicology Studies

APSGB-HESI Workshop on Inhaled Drug Induced Alveolar Macrophage Responses, Stevenage (30th-31st October 2012)

Joel Parry, GlaxoSmithKline R&D

Representing the Inhaled Oligonucleotide Subcommittee of the OSWG
Overview

• Introduction
  – OSWG (Oligonucleotide Safety Working Group)
  – Oligo modalities and the inhaled oligo pipeline

• Preclinical toxicology of inhaled oligos

• The \textit{in vitro} effects of oligos on macrophage-derived cells

• Summary and outstanding issues
OSWG - Safety Working Group

- Formed in early 2008 – DIA Oligo Symposium
- Discuss nonclinical safety issues and challenges associated with therapeutic oligonucleotides
- Industry, academia, consultants and regulators

EP
- Draft white paper
- Use of rodent surrogates

Inhaled Oligos
- Key issues white paper
- Monitoring lung tox. in clinic (NAR manuscript)

Immunostim.
- Key issues
- Complement and TLR white papers

Safety Pharm.
- Relevance of hERG
- Best practices for in vivo SP

OSWG
- Monthly TCs
- >120 members
- Group positions on regulatory issues

Repro/Carc.
- Key issues

Genetic Tox.
- Historical database
- Oligo-specific risks
- Triplex research

Off-target RNAi
- ‘Best practice’ consensus
- Nat Biotech manuscript

EP = exaggerated pharmacology
OSWG: Inhaled oligo subcommittee

• Chair
  – Nicolay Ferrari (ferrarinicolay@hotmail.com)

• Members
  – **Consultants:** Joy Cavagnaro, Paula Imbro, Doug Kornbrust, Jennifer Lockridge, Tim McGovern, Steve Shrewsbury, Jeff Tepper
  – **FDA:** Luqi Pei, Shwu-Luan Lei
  – **Industry:** Alnylam Pharma, AstraZeneca, Cubist Pharma, GlaxoSmithKline, Idera Pharma, Novartis, Pfizer, Topigen, part of the Pharmaxis Group

• Activities
  – Regular (monthly) TCs
  – “Key issues white paper”, reported at DIA Oligonucleotide Conference, March 2010
  – Manuscript published in Nucleic Acid Therapeutics, August 2012, along with an editorial introducing the remit and membership of the wider OSWG.
    “Clinical expert panel on monitoring potential lung toxicity of inhaled oligonucleotides: Consensus points and recommendations”
The diversity of therapeutic oligonucleotides

Generally considered to be “large small molecules” and not biopharmaceuticals

- **Ribozymes**
  - RNA enzyme
  - HCV & oncology

- **Steric Blockers**
  - Exon skippers, translational blockers
    - DMD, Vitravene (CMV)

- **Aptamers**
  - RNA/protein interaction
    - Macugen (wet AMD)

- **Decoy RNA**
  - Sequester viral proteins or cellular cofactors for viral replication
    - Antiviral (HIV)

- **Antisense Oligo (ASO)**
  - Rnase H mediated cleavage
    - Multiple indications and routes

- **Small interfering RNA (siRNA)**
  - RISC mediated cleavage
    - Multiple indications and routes

- **MicroRNA Therapeutics**
  - AntagoMirs and miRNA mimetics
    - Multiple indications

- **CpG ODNs**
  - Immune modulation/stimulation
    - Cancer, allergy, autoimmunity, infectious diseases
Inhaled oligos for respiratory diseases

- Access to chemically intractable targets
- Reduced lead optimisation cycle time
- Existing knowledgebase on chemistry/class toxicities (systemic)
- Potential for intermittent dosing schedule
- Increase local/reduced systemic exposure to improve therapeutic index

- For some oligo classes barriers to effective delivery are still significant
- There are some safety considerations
Inhaled Oligos – Clinical Pipeline

- Repeat dose tolerability has been good, with high achieved doses
  - e.g. TPI-ASM8 = 8mg, AIR645 = 20mg, RSV01 = 150mg

<table>
<thead>
<tr>
<th>Compound (Modality)</th>
<th>Company</th>
<th>Indication (Target)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI-2010 (ASO)</td>
<td>Epigenesis</td>
<td>Asthma (Adenosine A1R)</td>
<td>Phase II - no efficacy</td>
</tr>
<tr>
<td>AVT-01 (ASO)</td>
<td>Dolthera (Avontec) GmbH</td>
<td>Asthma (STAT-1)</td>
<td>Phase II - ongoing?</td>
</tr>
<tr>
<td>TPI-ASM8 (combination ASO)</td>
<td>Topigen/Pharmaxis Pharma</td>
<td>Asthma (CCR3:IL3, IL5, GM-CSF)</td>
<td>Phase II - ongoing</td>
</tr>
<tr>
<td>AIR645 (ASO)</td>
<td>Altair/Isis Pharma</td>
<td>Asthma (IL4-Rα)</td>
<td>Phase II - no efficacy</td>
</tr>
<tr>
<td>ALN-RSV01 (siRNA)</td>
<td>Alnylam/Cubist/Kyowa</td>
<td>RSV infection</td>
<td>Phase IIb - ongoing</td>
</tr>
<tr>
<td>Excellair (siRNA)</td>
<td>ZaBeCor Pharma</td>
<td>Asthma (Syk kinase)</td>
<td>Phase II - ongoing</td>
</tr>
<tr>
<td>ISS1018 (immunostim.)</td>
<td>Dynavax</td>
<td>Asthma</td>
<td>Phase II - no efficacy</td>
</tr>
</tbody>
</table>

Information sourced from: Seguin and Ferrari (2009). Oligonucleotides. 19:2 and various corporate websites
What do we know about the preclinical toxicology?

• Public-domain knowledgebase is limited

• Luqi Pei (FDA Pulmonary Division) presentation at DIA meeting, Mar 2010
  – Only 6 INDs open for inhaled oligos
  – Pro-inflammatory effects seen in rodent toxicology studies should be considered relevant to man until proven otherwise (Note: not formal view of the FDA)

• Experience of members of the OSWG-Inhaled Oligo Subcommittee
  – Different backbone chemistries have different toxicological profiles
  – Most contributors working with ASO class
Key lung toxicity findings following inhalation of oligos

- **Alveolar macrophage accumulation**
  - Contain intracytoplasmic basophilic granular material (oligo uptake?)

- **Interstitial mononuclear cell infiltration in lung and associated lymph nodes**
  - Believed to be B-cell lineage

- **Occasional observations of haemorrhage, possibly secondary to tissue inflammation**

- **Fibroplasia and metaplasia in the lung or associated tissues (e.g. trachea, lymph nodes), at relatively high dose levels**

- **Findings are generally more pronounced in rodents than in monkeys**
  - In-line with systemic experience
  - De-select immunostimulatory motifs during early discovery

- **Systemic exposure too low to result in typical ‘class effects’**

- **Other (non oligo) inhaled drugs can induce similar effects, yet development has continued (e.g. Tobramycin powder for CF)**
Lung distribution of inhaled oligos in the mouse

Images courtesy of Nicolay Ferrari/Topigen, Part of the Pharmaxis Group

• Prominent uptake of fluorescently tagged oligo in alveolar macrophages, but also epithelial cells

Confocal microscopy  
- Oligo 1  
- Oligo 2  
- Nuclei
Oligo-mediated macrophage changes in rat lungs

Control

ASO

Macrophage aggregates following microsprayer i.t. instillation (0.9mg/kg PS ASO, 8x over 14 days)

Note: Lung changes observed following i.t. instillation of oligos closely models effects observed in inhalation toxicology studies
Macrophage changes in monkey lungs following inhalation of oligos

TPI-ASM8: Summary of reported histopathology changes in the lungs of cynomolgus monkeys

<table>
<thead>
<tr>
<th>Findings</th>
<th>Main Study Animals (Day 15)</th>
<th>Recovery (Day 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>Lung Mixed cell/inflammation</td>
<td>+2/6</td>
<td>+4/6</td>
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<tr>
<td>Lung Accumulation of macrophages, foamy</td>
<td></td>
<td></td>
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<tr>
<td>Lung Intra-alveolar inflammation, granulocytic</td>
<td></td>
<td></td>
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<tr>
<td>Lung Metaplasia, bronchilar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung Haemorrhage, focal</td>
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<td></td>
</tr>
<tr>
<td>Lymph node, bronchial Accumulation of macrophages, foamy</td>
<td></td>
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</tbody>
</table>

- Adapted from Guimond et al, 2008 Pulm Pharm & Ther
- Grades: + = minimal, ++ = mild
- Doses are expressed as mg/kg/day (achieved)
- Grades: + = minimal, ++ = mild
- Numbers represent incidence values, males and females combined
Are oligo-mediated macrophage changes in the lungs adverse or adaptive?

- Alveolar macrophage changes can occur without concurrent frank pathology
  - Evidence of reversibility

- Systemically administered oligos also result in macrophage accumulation which, in the absence of any other adverse pathology, are viewed as benign
  - e.g. basophilic granules in Kupffer cells of the liver or in macrophages at injection sites
  - Postulated as a natural clearance mechanism
  - Reversible observation (period depends on tissue half life of oligo)

- *In vitro* studies have been conducted in macrophage-derived cells to explore this....
The effect of *in vitro* exposure to antisense oligonucleotides on macrophage morphology and function

*Brasey et al, J Nuc Acids Investigation, 2010: vol. 2:e121*

- Murine RAW264.7 cells exposed to 18mer phosphorothioate ASO for 48 hours
  - No effect on cell viability up to 25µM
  - Modest levels of cytokine induction versus amiodarone

Cytokine analysis (ELISA)
No effect on macrophage phagocytic function

*Brasey et al, J Nuc Acids Investigation, 2010: vol. 2:e121*

- 25µM ASO exposure does not lead to significant decreased/delayed phagocytic activity in RAW264.7 cells
Unpublished findings in macrophage cell lines

Courtesy of Philippa Allen (Inhaled Sciences/Safety Assessment) and Therapeutic Oligo DPU, GSK R&D

- Effects of three chemistries assessed in rat alveolar macrophage-derived cell line and human primary macrophage cells
  - Locked nucleic acid-phosphorothioate (LNA-PS)
  - Locked nucleic acid-phosphodiester (LNA-PO)
  - 2’O-methyl-phosphorothioate (2’OMe-PS)

- No evidence of cytotoxicity following 24 hr (human cells) or 72 hr (rat cells) exposure of to oligos (up to 400 or 800µg/mL; equates to ~60 or ~120µM)
Cytokine changes in macrophage cells

*Courtesy of Philippa Allen (Inhaled Sciences/Safety Assessment) and Therapeutic Oligo DPU, GSK R&D*

- Measured rat IL-1a, IL-1b, TNFα, CXCL1, IL-13, MCP-1 and human IL-1b, IL-6, IL-8, TNFα
- No concentration-dependent increase in any cytokines in rat macrophages
- Minimal induction of IL-6, IL-8 and TNFα in human macrophages (1/3 sensitive donors)

![Graph showing fold increase in IL-6 release from human cells](image)
Could PS backbone-mediated chemotaxis initiate macrophage accumulation in vivo?

- Effect of PO-CpG and PS-CpG oligos on murine peritoneal macrophages
  - Chemotaxis assay demonstrated only PS-CpG increased migration across 8µm membrane
  - Both PO and PS backbones resulted in macrophage activation (IL12p70 release and CD86)
  - Chemotaxis was dependent on endosomal maturation (chloroquine inhibition)

A Novel Function of Phosphorothioate Oligodeoxynucleotides as Chemoattractants for Primary Macrophages

Kwan Hyuck Baek, Sang Jun Ha, and Young Chul Sung

Phosphorothioate cytosine-guanine oligodeoxynucleotides (CpG PS-ODNs) has been reported to induce Th1 immune responses against coadministered Ags more efficiently than phosphodiester CpG ODNs (CpG PO-ODNs). Here, we demonstrated that PS-ODNs, but not PO-ODNs, have a chemotactic effect on primary macrophages, which is independent of the CpG motif. In addition, the conjugation of a hexameric dG run (dG₆ run) at the 3' terminus reduced the concentration required for the optimal chemotactic activity of PS-ODNs by ~10-fold. Endosomal maturation blockers, such as monensin and chloroquine, inhibited the chemotactic effect of PS-ODNs. The inhibition of the activities of p38 mitogen-activated protein (MAP) kinase, and extracellular signal-related kinases (ERKs) as well as phosphoinositide 3-kinase with their specific inhibitors also resulted in suppressing the chemotaxis of primary macrophages induced by PS-ODNs. These results indicate that the PS-ODN-mediated chemotaxis requires the activation of ERKs, p38 MAP kinase, and phosphoinositide 3-kinase as well as endosomal maturation. In addition, the phosphorylations of the p38 MAP kinase, ERKs, and protein kinase B, Akt, were induced by PS-ODN, which were further enhanced by the presence of both a dG₆ run and CpG motifs. Our findings suggest that the chemotactic activity of PS-ODNs may be one of the mechanisms by which PS-ODNs exhibit stronger immunomodulatory activities than PO-ODNs in vivo. *The Journal of Immunology*, 2001, 167: 2847–2854.
Summary

• Inhaled delivery of therapeutic oligos offers great potential in respiratory diseases

• Limited preclinical safety knowledgebase, although toxicities are generally reversible (not fibrosis) and good clinical tolerability to date

• Foamy macrophage changes are associated with basophilic granules, believed to be accumulation of oligo-related material
  – Highly soluble – description not indicative of drug ‘particle’ accumulation

• Studies in macrophage cell lines suggest single stranded ‘non-immunostimulatory’ oligos do not induce an adverse phenotype
  – No evidence of cytotoxicity/minimal cytokine induction with multiple chemistries
  – No change in phagocytic function following RAW264.7 cells
  – PS backbone may promote macrophage chemotaxis

“Based on the existing knowledgebase the Inhaled Oligo Sub-committee believe that, in the absence of any concurrent adverse pathology, macrophage changes observed in preclinical oligo inhalation toxicology studies represent a benign clearance mechanism of the therapeutic class”
Outstanding issues

- *In vivo* assessment of markers of alveolar macrophage activation and/or function
- *In vitro* studies in primary alveolar macrophages
- Are all findings identified preclinically relevant for clinical safety?
- How best to monitor for toxicities identified preclinically in the clinic?
- Chronic effects of inhaled oligos are yet to be defined
Acknowledgements

• All participants of the OSWG Inhaled Oligo Subcommittee

• Nicolay Ferrari (formally of Topigen, part of the Pharmaxis Group) for providing dual stained microscopy images of mouse lungs

GSK R&D

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• Inhaled Sciences, Respiratory TAU, Stevenage
  – David Hassall
Thank you for your attention!

Questions?