Translatability of cytokine data: from animals to humans

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Presentation outline

- Overview of cytokines
- Factors related to cytokine biology
- Challenges associate with cytokine measurement
- Cytokines as biomarkers of toxicity
- Case study: Evaluation of cytokine release following the administration of compound X in rats, NHPs and humans

*Compound x indication: Advanced solid Tumors*
Cytokines

- Diverse group of soluble peptides that signal between cells and elicit biological responses
- Biological responses includes: cell activation, proliferation, growth, differentiation, migration, cytotoxicity and inflammation
- Cytokines were understood in the context of the immune response but they are also widely expressed by non-hematopoietic cell type
- Roles outside of immunity: development, reproduction, endocrine regulation and metabolism
- Cytokines can therefore define a pathologic response but not necessarily a tissue site of toxicity
Generally act locally at ng/pg per mL concentrations

Short half-life and transient activity

The low concentrations and predominately local activity may produce little change in cytokine levels in the systemic circulation despite considerable local perturbation, therefore, biological activity of cytokines at the cellular level in local environment may prevent their detection in the systemic circulation

Primary cytokines that drive the early response, such as pro-inflammatory and anti-inflammatory cytokines, are more commonly detectable in peripheral blood
Considerations related to Cytokines biology

- Important biological variability in concentrations of cytokines

- Variation in cytokine levels can be induced by external factors such as circadian rhythm, stress

- Redundancy: shared receptors that modulate different downstream signals. As some cytokines has multiple functions, they lack specificity for one outcome alone

- Cytokine genes have different conservation across species (e.g., rodents do not have an ortholog of human IL8)

- Cytokine release may not be equivalent in all species due to different toxicity susceptibility. Species differences is important when discussing species translatability (e.g., TGN1412)
# Pros and Cons of cytokine measurements

<table>
<thead>
<tr>
<th><strong>Advantages</strong></th>
<th><strong>Challenges</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative measurement</td>
<td>Short serum half-life</td>
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<tr>
<td>Robust modulation in proximal events of inflammation, immune response and repair</td>
<td>Low to undetectable baseline levels</td>
</tr>
<tr>
<td>Accessibility</td>
<td>Lack of tissue-specific or toxicity-specific expression</td>
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<tr>
<td>Serial monitoring</td>
<td>Complexities related to cytokine expression with multi-organ involvement</td>
</tr>
<tr>
<td>Availability of analytical methods</td>
<td>Species, strain, and inter-individual differences</td>
</tr>
<tr>
<td>Fast analytical turn-around time</td>
<td>Analytical, methodological, and study design–related variables</td>
</tr>
<tr>
<td>Blood-based markers have the potential for the translation of preclinical risk assessment to the human patient population as they are generally readily adapted to clinical trials</td>
<td>Relationship between changes in cytokine levels and the development of phenotypic or functional manifestations of toxicity not well established</td>
</tr>
</tbody>
</table>

When there is no premonitory biomarker for specific toxicity in routine panels, cytokines are often proposed as biomarkers of toxicity.
Case study

Evaluation of cytokine release following the administration of compound X in rats, NHPs and humans
First generation compound

- Compound X: designed to treat patients with advanced solid tumors
- Compound X was designed to target two distinct pathways of tumor pathology involving cell proliferation and angiogenesis.
- The safety of the first generation of compound X was investigated by running pre-clinical studies in rats and NHPs
Study design (Rat)

3 hour intravenous infusion

Whole blood sampling for IL1β, IL-6, TNF-α, INF-γ analysis
## Results

### Test related findings in the rat study (4, 7 and 10 mg/kg)

#### Findings at ≥ 7 mg/kg

<table>
<thead>
<tr>
<th>Findings</th>
<th>Details</th>
</tr>
</thead>
</table>
| Mortality      | n = 1 at 7 mg/kg and n= 4 at 10 mg/kg  
Microscopic changes in kidneys/liver  
Non treatment related death occurred for 4 animals (n= 1 at 4 mg/kg and n=3 at 10 mg/kg) |
| Clinical signs | Dose-related decreased activity and/or muscle tone, labored breathing, and red urine/red staining around the urogenital region                  |
| Food consumption | Dose-related decreased (Body weight decreases at 10 mg/kg only)                                                                            |
| Hematology     | Increases in white blood cell parameters (all cell type, except basophils)  
Reductions in red blood cell parameters                                             |
| Coagulation     | Various changes observed                                                                                                                   |
| Biochemistry    | Elevations in liver enzymes (ALT, ALP, AST) and urea                                                                                       |
| Pathology       | Reversible increases of liver and spleen weights  
Target organs identified: liver, kidneys, adrenal, spleen, lymph nodes, bone, and bone marrow (macroscopic and/or microscopic changes) |
TNF-α

![Graph showing TNF-α concentrations over time for different groups.](image)
IL-6

Concentration (pg/mL)

Timepoints:
- 3 hrs
- Day 3
- Day 15
- Day 17
- Day 36

Groups:
- Group 1
- Group 2
- Group 3
- Group 4
INF-γ

The graph shows the concentration of INF-γ over time for different groups. The x-axis represents time points: 3 hrs, Day 3, Day 15, Day 17, and Day 36. The y-axis represents concentration in pg/mL. Different symbols represent different groups:
- Black circles: Group 1
- Red triangles: Group 2
- Green squares: Group 3
- Yellow diamonds: Group 4

The data points indicate variations in concentration across different time points and groups.
Study design (NHP)

3 hour intravenous infusion

**Compound X**

- **Day 1**: Pre-dose
- **Day 7**: 1, 2
- **Day 22**: 22, 23
- **Day 28**:

**Dosing phase (4 weeks)**

- Group 1 – 0 mg/kg
- Group 2 – 1.0 mg/kg
- Group 3 – 5.0 mg/kg*
- Group 4 – 10.0 mg/kg**

**Recovery (6 weeks)**

*Whole blood sampling for IL1β, IL-6, TNF-α, INF-α analysis*

*Dosing changed to 3 mg/kg following the first dose

**Dosing changed to 6 mg/kg following the 1st dose**
## Results

**Test related findings in the NHP study (1, 5 (3), and 10(6) mg/kg)**

*Findings at ≥ 1 mg/kg*

<table>
<thead>
<tr>
<th>Findings</th>
<th>Details</th>
</tr>
</thead>
</table>
| Mortality         | n = 1 ♀ at 5(3) mg/kg and n= 4 ♂, 5 ♀ at 10(6) mg/kg  
Microscopic changes in liver/adrenals/kidney (cause of death was not determined for some animals). Severe clinical signs, hematology changes, elevations in liver-associated enzyme, elevation in cytokines and complement factors |
| Clinical signs    | Hunched posture, decreased muscle tone, thin body condition, skin lesions (muzzle and gums) with discharge, swollen gums, salivation, partly closed eyes, and swollen periorbital region. |
| Food consumption  | No clear changes in food consumption or body weight                                                                                       |
| Hematology        | Reversible changes in white blood cells (neutrophils, monocytes, lymphocytes)  
Decreases in RBC, hemoglobin and hematocrit  
Bone marrow hypercellularity and increases in erythroid series                                                                 |
| Coagulation       | Minimal changes                                                                                                                          |
| Biochemistry      | Increases in liver enzymes (CRP, ALT, ALP, AST and GGT)                                                                                   |
| Complement        | Increases in C3a  
Increases in Bb (highest increases 15 min. post-Rx on Day 1 and 22)  
CH50 appeared generally decreased                                                   |
| Pathology         | Decreases in thymus weight and lymphoid atrophy  
Target organs: adrenals, liver, spleen, thymus, bone marrow, lung, kidneys, and infusion site (macroscopic and/or microscopic changes) . Reversibility or partial reversibility (not all tissues) |
TNF-α

![Graph showing concentration over time for different groups]
IL-6

Day 1 Day 2 Day 20 3hrs Day 23
Pre-dose 3hrs Day 2 Day 20

Concentration (pg/mL)
16384.00 8192.00 4096.00 2048.00 1024.00 512.00 256.00 128.00 64.00 32.00 16.00

Group 1
Group 2
Group 3
Group 4

Pre-dose 3hrs Day 1
Day 2 Day 20
3hrs Day 22
Day 23

Charles River
IL1-β

![Graph showing concentration of IL1-β over time for different groups.]
Summary from 1st generation

- Similar clinical signs observed in rats and NHP

- TNFα results were non-conclusive in rats due to high levels observed in control animals. In NHPs the results were also difficult to interpret due to high levels observed at pre-dose, although a higher incidence of animals in dosed groups presented higher TNF-α levels

- IL-6 in both species showed a dose dependent increase following dosing. The responses obtained in the NHP were generally higher than those obtained in the rat

- IL-1β was not detected in the rat and only mildly in the NHP

- INFγ showed a nice dose dependent increase in the rat. No data was available for the NHP

- Based on the data obtained in both tox studies, it was decided to modify compound X
2nd generation compound

- 2nd generation of compound X was still targeting two distinct pathways of tumor pathology involving cell proliferation and angiogenesis
- Compound X was modified in order to reduce the toxicity
- Dosing was reduced and infusion time was reduced from 3 hrs to 15 minutes
- Investigate the potential toxicity of compound X by running pre-clinical studies in rats and NHPs
Study design (Rat)

15 minutes intravenous infusion

Compound X

Day 1

Day 14

Day 29

Day 43

Dosing phase (8 weeks)

Recovery (4 weeks)

Group 1 – 0 mg/kg
Group 2 – 0.3 mg/kg
Group 3 – 1.0 mg/kg
Group 4 – 3.0 mg/kg
Group 5 – 6.0 mg/kg*

* Dosing changed to 5 mg/kg following the first dose

Whole blood sampling for IL1β, IL-6, TNF-α, INF-γ analysis
### Results

**Test related findings in the rat study (dose levels = 0, 0.3, 1, 3 and 5-6 mg/kg)**

**Findings at 5 and 6 mg/kg**

<table>
<thead>
<tr>
<th>Findings</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>n=1 in the 0 mg/kg non treatment related death</td>
</tr>
<tr>
<td></td>
<td>n=8 in the 6 mg/kg; high dose was reduced to 5 mg/kg</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>Dose-related decreased activity and/or muscle tone, labored breathing, pale skin, wet fur, dehydration, red urine/red staining around the urogenital region</td>
</tr>
<tr>
<td>Food consumption</td>
<td>Lower body weight gain + food consumption following 1\textsuperscript{st} dose</td>
</tr>
<tr>
<td>Hematology</td>
<td>Increase in WBC parameters</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Various changes observed</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Elevations in liver enzymes and urea</td>
</tr>
<tr>
<td>Pathology</td>
<td>Increases of liver and spleen weights</td>
</tr>
<tr>
<td></td>
<td>Target organs identified: liver, kidneys, spleen, thymus, testes (macroscopic and/or microscopic changes)</td>
</tr>
</tbody>
</table>
Results

Cytokine profile

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1β</td>
<td>All animals &lt; LLOQ, with the exception of: One 5.0 mg/kg animal on Day 44; elevated levels</td>
</tr>
<tr>
<td>IL-6</td>
<td>All animals &lt; LLOQ, with the exception of: One 5.0 mg/kg animal on Day 44; elevated levels</td>
</tr>
<tr>
<td>TNF-α</td>
<td>All animals &lt; LLOQ, with the exception of: Four 3.0 mg/kg animals on Day 1; slightly above the LLOQ</td>
</tr>
<tr>
<td>INF-γ</td>
<td>All animals &lt; LLOQ</td>
</tr>
</tbody>
</table>

Improvement from the 1st generation compound
Study design (NHP)

15-minute intravenous infusion

Compound X

Day 1
Day 15
Day 29
Day 43

Dosing phase (8 weeks)

Recovery (4 weeks)

Group 1 – 0 mg/kg
Group 2 – 0.3 mg/kg
Group 3 – 1.0 mg/kg
Group 4 – 3.0 mg/kg
Group 5 – 6.0 mg/kg

Whole blood sampling for IL1β, IL-6, TNF-α, INF-α analysis
## Results

Test related findings in the NHP study (dose levels = 0, 0.3, 1, 3 and 6 mg/kg)

### Findings at 3 and 6 mg/kg

<table>
<thead>
<tr>
<th>Findings</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>No death</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>Dose-related decreased activity and/or muscle tone, labored breathing, pale skin, wet fur, dehydration, red urine/red staining around the urogenital region</td>
</tr>
<tr>
<td>Food consumption</td>
<td>No finding</td>
</tr>
<tr>
<td>Hematology</td>
<td>Increase in WBC parameters in the 6mg/kg group; recovered</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Various changes observed</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Elevations in liver enzymes and urea in the 3 and 6 mg/kg group; recovered</td>
</tr>
</tbody>
</table>
| Pathology           | Increases of liver and spleen weights  
Target organs identified: liver, kidneys, spleen, bone marrow (macroscopic and/or microscopic changes) |
### Activation of complement proteins

<table>
<thead>
<tr>
<th>Complement protein</th>
<th>Dose</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bb fragment</td>
<td>1.0mg/kg</td>
<td>↑ in 3 ♀ 6 hrs post dose on Days 1 and 43 and on Day 2 and 44</td>
</tr>
<tr>
<td></td>
<td>3.0 mg/kg</td>
<td>↑ in ♀ and ♂ 6 hrs post-dose on Days 1 and 43 and on Day 2 and 44</td>
</tr>
<tr>
<td></td>
<td>6.0 mg/kg</td>
<td>↑ in ♀ and ♂ 6 hrs post-dose on Days 1 and 43 and on Day 2 and 44</td>
</tr>
<tr>
<td>C3a</td>
<td>0.3</td>
<td>↑ 15 min post-dose in 2 ♂ on days 1 and 43</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>↑ 15 min post-dose in 2 ♂ on days 1 and 43</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>↑ 15 min post-dose in 2 ♂ on days 1 and 43; ↑ in 4 ♀ at all timepoints</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>↑ 15 min and 6 hrs post-dose in all ♂ and ♀ on days 1 and 43</td>
</tr>
<tr>
<td>C4d</td>
<td>-</td>
<td>No finding</td>
</tr>
</tbody>
</table>
## Results

### Cytokine profile

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1β</td>
<td>All animals &lt; LLOQ</td>
</tr>
<tr>
<td>IL-6</td>
<td>Dose dependant increase</td>
</tr>
<tr>
<td>TNF-α</td>
<td>All animals &lt; LLOQ</td>
</tr>
<tr>
<td>INF-α</td>
<td>All animals &lt; LLOQ</td>
</tr>
</tbody>
</table>

**Improvement from the 1st generation compound**
IL-6 results
Summary from 2\textsuperscript{nd} generation

- Less cytokine release with the second generation than with the first generation

- IL-6 showed a dose dependent increase following dosing. The responses obtained were generally lower than those observed following treatment with the 1\textsuperscript{st} generation compound.

- No cytokine modulations was noticed in the rat: was the rat less sensitive?

- The NHP generally seems to be more sensitive than the rat to cytokine release.
Study design (Clinical study)

15 minutes intravenous infusion

**Compound X**

**Cycle 1**
- Day 1: Pre-Dose
- Day 14: Pre-Dose
- Samples at 2, 6, 24 hrs post-dose

**Cycle 2**
- Day 1: Pre-Dose
- Day 14: Pre-Dose
- Samples at 2, 6, 24 hrs post-dose

**Cycle 3**
- Day 1: Pre-Dose
- Day 14: Pre-Dose
- Samples at 2, 6, 24 hrs post-dose

**Cycle 4**
- Day 1: Pre-Dose
- Day 14: Pre-Dose
- Samples at 2, 6, 24 hrs post-dose

4 cycles of 4 weeks

Whole blood sampling IL-1β, IL-1RA, IL-6, IL-12, IP-10, IFN-α, IFN-γ, TNF-α, and G-CSF analysis

Cohort 1 – 0.1 mg/kg
Cohort 2 – 0.2 mg/kg
Cohort 3 – 0.4 mg/kg
Cohort 4 – 0.7 mg/kg
Cohort 5 – 1.0 mg/kg
Cohort 6 – 1.25 mg/kg
Cohort 7 – 1.50 mg/kg
# Results

## Cytokine profile

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>IL1β</td>
<td>All patients &lt; LLOQ</td>
</tr>
<tr>
<td>IL-12</td>
<td>Most patients &lt; LLOQ</td>
</tr>
<tr>
<td>INF-α</td>
<td>Most patients &lt; LLOQ</td>
</tr>
<tr>
<td>INF-γ</td>
<td>Most patients &lt; LLOQ</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Most patients &lt; LLOQ</td>
</tr>
<tr>
<td>IP-10</td>
<td>Modulations observed in many patients but no clear dose dependant relationship could be established</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Variability in the data was observed. Elevated concentrations were observed in pre-dose and no dose dependency was noticed</td>
</tr>
</tbody>
</table>
IL-6
IL-1RA

A graph showing concentration (µg/mL) over time points (Pre-dose, 2hrs, 6hrs, 24hrs) for different cohorts.
Summary from clinical study

- INF-α results were comparable to those obtained in the NHP study with the second generation compound.
- INF-γ results were comparable to those obtained in the rat study with the second generation compound.
- IL-1β results were comparable to those obtained in the rat and NHP study with the second generation compound.
- IL1RA serum concentration correlates with IL-6 concentrations.
- Dose dependent response observed with IL-6 and IL1-RA at 6 hours post-dose. These results correlate with those obtained in the NHP study with the second generation compound.
Summary

- Based on this case study and on this class of compound, we see that there is a certain degree of correlation observed between the cytokine data generated in pre-clinical and clinical studies (e.g., IL-6 and in cytokines that were undetected).

- Based on the limited cytokine data available from the pre-clinical studies, the NHP demonstrated some predictability.

- Identification of a specific cytokine or panel of cytokines to be included in a study in order to determine the systemic toxicity levels of a compound is challenging.

- Cytokine data on its own is difficult to interpret. It needs to be linked to toxicology data.
Acknowledgements

- Simon Lavallée, Associate Scientific Director, Charles River
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