Serum Cytokine Induction After a Single Mycobacterium Cell Wall Fraction (MCWF) treatment in Tumor Bearing Dogs

Martins, M; Losowski, L; Masic A; Prunic B, Rodrigues, LCS

WVCC – 2016
Foz do Iguassu
Breakthrough of the Year

Cancer Immunotherapy

T cells on the attack
Nonspecific Immunotherapies

Cytokines - Stimulate the immune system

Checkpoint inhibitors – Release the brakes on the immune system, i.e. PD-1/PD-L1

Immunomodulating drugs – Boost the immune system, i.e.
Goal of this Study

The aim of this study was to evaluate the ability of MCWF to induce serum cytokine synthesis after single intravenous treatment in dogs with different solid tumors.
Mycobacterium cell wall fraction (MCWF)

Biological Modulator

Induce cytokine production

Induce apoptosis

Cytokines: IL-6, IL-8, IL-10, IL-12, IL-18, TNF-α

Indirect Anti-cancer activity

Direct

Cancer cells

Human and canine TCC in vitro: HT-1376; HT-1197 and K99

Apoptosis or cell lysis

Filion et al., 1999; Morales et al., 2015
Study Design

10 naïve dogs with solid tumors

- Tumor Diagnosis
- Complete Staging (3 view Chest X-Ray, ABD-US)
- CBC, Biochemistry, Urinalysis
- Body Condition Score and ECOG performance Status

MCWF treatment

- 50µg/Kg in 100mL of 0.9%NaCl over 45min as slow I.V infusion

Cytokine Analysis
Study Design

Pre-treatment
- PE
- CBC + Biochemistry
- Urinalysis
- Cytokines

2 hours
- PE
- Cytokines

4 hours
- PE
- Cytokines

6 hours
- PE
- Cytokines

12 hours
- PE
- Cytokines

24 hours
- PE
- Cytokines

48 hours
- PE
- CBC
- Cytokines

168 hours
- PE
- CBC + Biochemistry
- Urinalysis
- Cytokines
Study Design

- IL-2
- IL-6
- IL-10
- GM-CSF
- IFN-γ
- TNF-α
- IL-12
- MyBiosource®

**Milliplex® MAP CCYTOMAG-90K-06**

**450nm Luminex® 200 and Xponent (Software)**
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Weight (Kg)</th>
<th>BCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient#1</td>
<td>Mixed</td>
<td>female spayed</td>
<td>17</td>
<td>8.70</td>
<td>2</td>
</tr>
<tr>
<td>Patient#2</td>
<td>Mixed</td>
<td>female spayed</td>
<td>13</td>
<td>6.75</td>
<td>4</td>
</tr>
<tr>
<td>Patient#3</td>
<td>Mixed</td>
<td>female spayed</td>
<td>15</td>
<td>4.70</td>
<td>4</td>
</tr>
<tr>
<td>Patient#4</td>
<td>Mixed</td>
<td>female spayed</td>
<td>10</td>
<td>14.60</td>
<td>4</td>
</tr>
<tr>
<td>Patient#5</td>
<td>Pit Bull</td>
<td>Male intact</td>
<td>7</td>
<td>34.00</td>
<td>2</td>
</tr>
<tr>
<td>Patient#6</td>
<td>Labrador</td>
<td>Male castrated</td>
<td>10</td>
<td>30.10</td>
<td>4</td>
</tr>
<tr>
<td>Patient#7</td>
<td>Labrador</td>
<td>female spayed</td>
<td>15</td>
<td>32.90</td>
<td>4</td>
</tr>
<tr>
<td>Patient#8</td>
<td>Mixed</td>
<td>female spayed</td>
<td>12</td>
<td>6.85</td>
<td>4</td>
</tr>
<tr>
<td>Patient#9</td>
<td>Mixed</td>
<td>Male Intact</td>
<td>13</td>
<td>18.00</td>
<td>4</td>
</tr>
<tr>
<td>Patient#10</td>
<td>French Bulldog</td>
<td>female spayed</td>
<td>2.5</td>
<td>9.70</td>
<td>5</td>
</tr>
<tr>
<td>Patient ID</td>
<td>Diagnosis</td>
<td>Location</td>
<td>Clinical Staging</td>
<td>Tumor Size</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------</td>
<td>----------------</td>
<td>------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>Patient#1</td>
<td>Oral Melanoma</td>
<td>left mandible</td>
<td>V</td>
<td>20 x 23 mm</td>
<td></td>
</tr>
<tr>
<td>Patient#2</td>
<td>Mammary Tumor</td>
<td>right M5</td>
<td>II</td>
<td>32 x 32 mm</td>
<td></td>
</tr>
<tr>
<td>Patient#3</td>
<td>Mammary Tumor</td>
<td>right M5</td>
<td>II</td>
<td>32 x 30 mm</td>
<td></td>
</tr>
<tr>
<td>Patient#4</td>
<td>Mast Cell Tumor</td>
<td>left leg</td>
<td>Ia</td>
<td>20 x 18mm</td>
<td></td>
</tr>
<tr>
<td>Patient#5</td>
<td>Osteosarcoma</td>
<td>left femur</td>
<td>II</td>
<td>85 x 80mm</td>
<td></td>
</tr>
<tr>
<td>Patient#6</td>
<td>Oral Melanoma</td>
<td>right mandible</td>
<td>II</td>
<td>50 x 48 mm</td>
<td></td>
</tr>
<tr>
<td>Patient#7</td>
<td>Hepatocellular Carcinoma</td>
<td>left lobe</td>
<td>I</td>
<td>47.2 x 29.7 mm</td>
<td></td>
</tr>
<tr>
<td>Patient#8</td>
<td>Mammary Tumor</td>
<td>right M4</td>
<td>II</td>
<td>33 x 29 mm</td>
<td></td>
</tr>
<tr>
<td>Patient#9</td>
<td>Mast Cell Tumor</td>
<td>left leg</td>
<td>IV</td>
<td>56 x 42 mm</td>
<td></td>
</tr>
<tr>
<td>Patient#10</td>
<td>Mast Cell Tumor</td>
<td>inguinal area</td>
<td>IV</td>
<td>10 x 7 mm</td>
<td></td>
</tr>
</tbody>
</table>
Results

- No difference in PE and clinical parameters
- Treatment did no change biochemistry profile
  - ALT; ALP; ALB, GLOB, Tbil, TB
  - CRE, BUN, PHOS, K+, Na+
  - AMY, PHOS, GLU, Ca
Results

IL-10

GM-CSF

p=0.0306

IL-12

INF-g
Results

IL-2

IL-6

TNF-alpha

Cytokines (pg/mL)

Timepoints: 0h, 2h, 4h, 6h, 12h, 24h, 48h, 168h
IL-10

Inhibitory actions on macrophages and DC
Inhibits the production of IL-12 and IL-2
Decrease the expression of class II MHC

Produced by:
- T and B Cells
- Monocytes
- DC
- NK

Stimulation of macrophage phagocytosis and NK cytotoxicity

Carson et al., 1995
Moore et al., 2001; de Waal et al., 1993
# Inhibitory effects of IL-10 on tumor growth in animal models

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Method of delivery</th>
<th>Role</th>
<th>Best result reported</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10-transfected hamster ovarian tumour implants</td>
<td>Local</td>
<td>Local antitumour effect</td>
<td>Inhibition of tumour growth</td>
<td>Richter et al., Cancer Res, 1993</td>
</tr>
<tr>
<td>IL-10-transfected spontaneous mammary carcinoma</td>
<td>Local</td>
<td>Induction of antitumour immune response</td>
<td>Protection</td>
<td>Giovarelli et al., J Immunol, 1995</td>
</tr>
<tr>
<td>IL-10-transfected tumour implants</td>
<td>Local</td>
<td>Induction of antitumour immune response</td>
<td>Protection</td>
<td>Barth et al., Ann Surg Oncol, 1996</td>
</tr>
<tr>
<td>Various types of established (7-day) murine tumours</td>
<td>Systemic</td>
<td>Induction of antitumour immune response</td>
<td>Treatment</td>
<td>Berman et al., J Immunol, 1996</td>
</tr>
<tr>
<td>Subcutaneous implants of B16 melanoma transduced with IL-10</td>
<td>Local</td>
<td>Local antitumour effect</td>
<td>Protection</td>
<td>Gerard et al., Hum Gene Ther, 1996</td>
</tr>
<tr>
<td>IL-10-transfected murine mammary cancer subcutaneous implants</td>
<td>Local</td>
<td>NK-dependent inhibition of tumour</td>
<td>Inhibition of tumour growth</td>
<td>Kundu et al., K Natl Cancer Inst, 1996</td>
</tr>
<tr>
<td>B16 and spontaneous melanoma lung metastases</td>
<td>Systemic (i.p.)</td>
<td>NK-dependent inhibition of tumour</td>
<td>Inhibition of tumour growth</td>
<td>Zheng et al., J Exp Med, 1996</td>
</tr>
<tr>
<td>Breast cancer cells transduced with murine IL-10</td>
<td>Local</td>
<td>Recruitment of leukocytes</td>
<td>Inhibition of tumour growth</td>
<td>Di Carlo et al., Eur Cytokine Netw, 1998</td>
</tr>
<tr>
<td>IL-10-transfected CT26 colon carcinoma-cell implants</td>
<td>Local</td>
<td>T-cell-dependent inhibition of tumour</td>
<td>Inhibition of tumour growth</td>
<td></td>
</tr>
<tr>
<td>IL-10-transfected A375 human melanoma subcutaneous and pulmonary metastases</td>
<td>Local</td>
<td>Local antitumour effect</td>
<td>Inhibition of tumour growth</td>
<td></td>
</tr>
<tr>
<td>ß²-Galactosidase-expressing 3-day pulmonary metastases</td>
<td>Systemic (i.p.)</td>
<td>Adjuvant to poxvirus-based vaccine</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Human prostate cancer vertebral metastases xenografts</td>
<td>Systemic (i.m.)</td>
<td>Inhibition of vasculogenesis</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Ovalbumin-expressing subcutaneous implants</td>
<td>Systemic (i.p.)</td>
<td>Adjuvant to peptide-pulsed APC vaccine</td>
<td>Protection and treatment</td>
<td></td>
</tr>
</tbody>
</table>

Mocelli et al., The dual role of IL-10, 2003
Mocelli et al., The dual role of IL-10, 2003

Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience.
Huang S1, Ullrich SE, Bar-Eli M.

Interleukin-10 inhibits tumor metastasis through an NK cell-dependent mechanism.
Results

**Appetite**

- Greatly Decreased
- Decreased
- No Change
- Increased
- Greatly Increased

**Sleeping Time**

- Greatly Decreased
- Decreased
- No Change
- Increased
- Greatly Increased

**Activity Level**

- Greatly Decreased
- Decreased
- No Change
- Increased

**Playfulness**

- Greatly Decreased
- Decreased
- No Change
- Increased
- Greatly Increased
Results

Urinary and Fecal Accidents

- Number of dogs
- Greatly Decreased: Before - 1, After - 10
- Decreased: Before - 0, After - 5
- No Change: Before - 0, After - 0
- Increased: Before - 0, After - 0
- Greatly Increased: Before - 0, After - 0

General Responsiveness

- Number of dogs
- Greatly Decreased: Before - 1, After - 4
- Decreased: Before - 0, After - 2
- No Change: Before - 0, After - 0
- Increased: Before - 0, After - 5
- Greatly Increased: Before - 0, After - 0

General Health Compared to the Last Evaluation

- Number of dogs
- Greatly Decreased: Before - 1, After - 10
- Decreased: Before - 0, After - 5
- No Change: Before - 0, After - 0
- Increased: Before - 0, After - 0
- Greatly Increased: Before - 0, After - 0

General Health Compared to Before the Diagnosis

- Number of dogs
- Greatly Decreased: Before - 1, After - 4
- Decreased: Before - 0, After - 2
- No Change: Before - 0, After - 0
- Increased: Before - 0, After - 5
- Greatly Increased: Before - 0, After - 0
Conclusions

• Single dose of MCWF (50 μg/Kg) IV increases IL-10 serum levels 2 hours post-treatment with statistically significant difference (p=0.0306)

• Dogs treated with MCWF demonstrated significantly improved quality of life
  – appetite, activity level, playfulness and general health improvement

• The clinical effectiveness against various malignancies, potential activation of NK and tumor control in dogs with spontaneous tumors are still undetermined
Study Limitations

• Small number of dogs
• No healthy dogs as a control

Future Directions

• Evaluate the biological activity in patients with solid tumors
• Compare the cytokine production after local treatment
ACKNOWLEDGMENT

Mirtes Martis
Larissa Losowski
Denise Samia
Kaique Braz
Ana Carolina Faria
Daiana Sousa
Felipe Fernandes
Graziela Santos
Elena Toazza
Elis Monti
Carolina Fabri
Larissa Lopes
Giza Cabral
Kladine Monique
Sthefany Chagas
Joyce, Ana Paula, Karen,
Barbara, Michele, Charles
Aline Andrade - Gisele Paim

lucasrodrigues@estima.vet.br