“Effectiveness of Comparison of Different Routes of Administration of Oncolytic Virus in Abdominal Tumors”

Hideki Kasuya MD PhD FACS

Cancer Immune Therapy Research Center
Nagoya University Graduate School of Medicine
Japan
HF10 Clinical Development

To date, a total of $\geq 100$ **patients** have been treated with HF10.

<table>
<thead>
<tr>
<th></th>
<th>Phase</th>
<th>Mono or Combo</th>
<th>Tumor type</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US</strong></td>
<td>Phase I</td>
<td>monotherapy</td>
<td>Solid tumor</td>
<td>completed</td>
</tr>
<tr>
<td></td>
<td>Phase II</td>
<td>Combo with Ipilimumab</td>
<td>Melanoma</td>
<td>ongoing (enrollment completed)</td>
</tr>
<tr>
<td><strong>Japan</strong></td>
<td>Phase I</td>
<td>monotherapy</td>
<td>Solid tumor</td>
<td>ongoing</td>
</tr>
<tr>
<td></td>
<td>Phase II</td>
<td>Combo with Ipilimumab</td>
<td>Melanoma</td>
<td>Planned</td>
</tr>
<tr>
<td></td>
<td>Phase I/II</td>
<td>Combo with Gemcitabine etc.</td>
<td>Pancreatic cancer</td>
<td>Planned</td>
</tr>
<tr>
<td>Investigators’ initiated clinical study</td>
<td>Combo with Gemcitabine+Erlotinib</td>
<td>Pancreatic cancer</td>
<td>completed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>monotherapy</td>
<td></td>
<td>Breast, H/N and Pancreatic cancer</td>
<td>completed</td>
</tr>
</tbody>
</table>
# HF10 + Ipilimumab Phase II trial in unresectable stage IIIB – IV melanoma

<table>
<thead>
<tr>
<th>Title of the study</th>
<th>A Phase II Study of Combination Treatment with HF10, a Replication-competent HSV-1 Oncolytic Virus, and Ipilimumab in Patients with Stage IIIB, Stage IIIC, or Stage IV Unresected or Metastatic Malignant Melanoma</th>
</tr>
</thead>
</table>
| Objectives        | To assess efficacy and safety with repeated administration of intratumoral injections of HF10 at 1x10^7 TCID\textsubscript{50}/mL in combination with intravenous infusions of 3mg/kg ipilimumab and evaluate the following objectives:  
**Primary Objective:**  
Best overall response rate (BORR) at Week 24  
**Secondary Objectives:**  
Safety and tolerability, ORR, PFS, DRR, 1-year survival rate, Evaluation of correlative studies |
| # of patients     | It is planned that at least 43 patients will be enrolled in the study |
| Methodology       | a single arm, open label Phase II trial |
| Principal Investigator | Robert Andtbacka, University of Utah, Huntsman Cancer Institute |

**Ipilimumab 3mg/kg IV q3wks x 4**  
irRC/mWHO 12, 18, 24, 26 & 48 wks

<table>
<thead>
<tr>
<th>3 weeks</th>
<th>3 weeks</th>
<th>3 weeks</th>
<th>3 weeks</th>
<th>1 year</th>
</tr>
</thead>
</table>

**HF10 1x10^7 TCID50/mL IT q1wk x 4 wks, then q3wks up to 45 wks**
### HF10 + Ipilimumab Phase II trial - Patient Demographics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N (%)</th>
<th>Characteristics</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>67</td>
<td>Male</td>
<td>27 (59%)</td>
</tr>
<tr>
<td>Range</td>
<td>29-92</td>
<td>Female</td>
<td>19 (41%)</td>
</tr>
<tr>
<td>ECOG Status</td>
<td></td>
<td>Disease Stage</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>34 (74%)</td>
<td>IIIB</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>1</td>
<td>12 (26%)</td>
<td>IIIC</td>
<td>20 (43%)</td>
</tr>
<tr>
<td>2</td>
<td>0 (0%)</td>
<td>IV</td>
<td>17 (37%)</td>
</tr>
<tr>
<td>HSV-1 antibody</td>
<td></td>
<td>≥ 1 Prior Cancer Therapy</td>
<td></td>
</tr>
<tr>
<td>(+)</td>
<td>30 (65%)</td>
<td>Yes</td>
<td>20 (43%)</td>
</tr>
<tr>
<td>(-)</td>
<td>16 (35%)</td>
<td>No</td>
<td>26 (57%)</td>
</tr>
</tbody>
</table>

Andtbacka, RHA et al. ESMO 2016 Abstract #1872 (and poster presentation)
# HF10 + Ipilimumab Phase II trial - Safety Summary

<table>
<thead>
<tr>
<th>Treatment-Emergent Adverse Events (TEAEs)</th>
<th>Number of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety evaluable patients</td>
<td>46</td>
</tr>
<tr>
<td>With any TEAEs</td>
<td>46 (100%)</td>
</tr>
<tr>
<td>With any TEAEs related to HF10</td>
<td>42 (91%)</td>
</tr>
<tr>
<td>With severity ≥ Gr 3 for HF10 related TEAEs</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>With any TEAEs related to Ipilimumab</td>
<td>43 (93%)</td>
</tr>
<tr>
<td>With severity ≥ Gr 3 for Ipilimumab related TEAEs</td>
<td>10 (22%)</td>
</tr>
<tr>
<td>With any serious, HF10 related TEAEs</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>With any serious, Ipilimumab related TEAEs</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>With any serious, unrelated TEAEs</td>
<td>6 (13%)</td>
</tr>
<tr>
<td>Who discontinued drug due to HF10 related TEAEs</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Andtbacka, RHA et al. ESMO 2016 Abstract #1872 (and poster presentation)
HF10 + Ipilimumab Phase II trial
- Maximum Change in Tumor, Best Overall Response Rate

Presented at ESMO 2016

% Change in SPD

Note: Scale was selected for best overall clarity of the plot. Progression beyond 200% is not displayed.

<table>
<thead>
<tr>
<th>Best Overall Response (N=44)</th>
<th>24 weeks (N%)</th>
<th>Post 24 Weeks (N%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Response (irCR + PR)</td>
<td>19 (43%)</td>
<td>22 (50%)</td>
</tr>
<tr>
<td>Clinical Benefit (irCR +PR+SD)</td>
<td>30 (68%)</td>
<td>30 (68%)</td>
</tr>
<tr>
<td>Complete Response (irCR)</td>
<td>7 (16%)</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>Partial Response (irPR)</td>
<td>12 (27%)</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>Stable Disease (irSD)</td>
<td>11 (25%)</td>
<td>8 (18%)</td>
</tr>
<tr>
<td>Progressive Disease (irPD)</td>
<td>12 (27%)</td>
<td>12 (27%)</td>
</tr>
<tr>
<td>Not Evaluable (NE)</td>
<td>2 (5%)</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>

SPD = sum of the products of the two largest perpendicular diameters of all index lesions

Andtbacka, RHA et al. ESMO 2016 Abstract #1872 (and poster presentation)
HF10 + Ipilimumab Phase II trial
- Change in tumor burden

Presented at ESMO 2016

Andtbacka, RHA et al. ESMO 2016 Abstract #1872 (and poster presentation)
HF10 + Ipilimumab Phase II trial
- Timing and Durable Response

Presented at ESMO 2016

Andtbacka, RHA et al. ESMO 2016 Abstract #1872 (and poster presentation)
HF10 + Ipilimumab Phase II trial - Overall survival (preliminary)

Presented at ESMO 2016

Andtbacka, RHA et al. ESMO 2016 Abstract #1872 (and poster presentation)
HF10 + Ipilimumab Phase II trial
- Response to Uninjected Visceral lesion

Presented at AACR-NCI-EORTC 2015

Andtbacka, RHA et al. ESMO 2016 Abstract #774 (and poster presentation)
2015 AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics Conference
### HF10 + Ipilimumab Phase II trial - Comparison with other immunotherapy

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Grade ≥ 3 AEs(%)</th>
<th>Response rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monotherapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>27</td>
<td>6 – 15</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>13</td>
<td>27 – 38</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>16</td>
<td>34 – 40</td>
</tr>
<tr>
<td>T-VEC</td>
<td>11</td>
<td>26</td>
</tr>
<tr>
<td><strong>Combination therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nivolumab + ipilimumab</td>
<td>55</td>
<td>52</td>
</tr>
<tr>
<td>T-VEC + ipilimumab</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>T-VEC + pembrolizumab</td>
<td>24</td>
<td>56</td>
</tr>
<tr>
<td>HF10 + ipilimumab</td>
<td>30</td>
<td>48</td>
</tr>
</tbody>
</table>

T-VEC: Treatment naïve patients  
HF10: ≥ 2nd line patients

Andtbacka RHI, et al. ASCO 2016: Abstract 9543  
Daud A, et al. ASCO 2015 Abstract 9005  
Puzanov I, et al. ASCO 2015: Abstract 9063 (and poster)  
Long, et al. SMR 2015
<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Organization (PI)</th>
<th>Study period</th>
<th># of Pts</th>
<th>Dose</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>Nagoya University (Nakao A)</td>
<td>2003/5～9</td>
<td>6 pts</td>
<td>$\sim 5 \times 10^5$ pfu/day x 3 days</td>
<td>HF10 injection was well tolerated. Oncolytic activity demonstrated by histopathology.</td>
</tr>
<tr>
<td>Head and Neck Cancer</td>
<td>Nagoya University (Fujimoto Y)</td>
<td>2004/7～2005/7</td>
<td>3 pts</td>
<td>$\sim 1 \times 10^5$ pfu/day x 3 days</td>
<td>HF10 injection was well tolerated. Oncolytic activity demonstrated by histopathology.</td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td>Nagoya University (Nakao A)</td>
<td>2005/1～2011/3</td>
<td>8 pts</td>
<td>$\sim 1 \times 10^6$ pfu/day x 3 days (+ 1 day x 3 wks)</td>
<td>See pages 10-13.</td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td>Nagoya University (Kasuya H, Hirooka Y)</td>
<td>2013/5～2015/12</td>
<td>9 pts</td>
<td>$\sim 1 \times 10^7$ pfu/day x 4 times/2months</td>
<td>completed</td>
</tr>
</tbody>
</table>

Kasuya H et. al., Hepatogastroenterology (2014) 61:599-605
HF10 structure

Natural mutated HSV virus, no artificial gene
Function: UL43, 49.5, 55, 56, LAT are inactivated
Oncolytic viruses and their application to cancer

First phase
OV infection/replication

Second phase
OV-directed antitumor immune responses

Innate responses (IFN-α/β)

Tumor cell

Virus replication

Immune stimulation

DC

B cell

T cell

OV

Innate cells

Tumor

GM-CSF

Transgene product

© 2014 American Association for Cancer Research

Cancer Immunology Research: Cancer Immunology at the Crossroads

Attenuated virulence of HF10

Virulence of HSV-1 & 2 after Intraperitoneal Administration to Adult Mice (LD$_{50}$ (pfu))

<table>
<thead>
<tr>
<th>Virus</th>
<th>Characteristics</th>
<th>LD$_{50}$ (pfu)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 KOS</td>
<td>Wild-type</td>
<td>2,200</td>
<td>1</td>
</tr>
<tr>
<td>HSV-1 hrR3</td>
<td>UL39-deletion</td>
<td>&gt; 5,000,000</td>
<td>&gt; 2,272.7</td>
</tr>
<tr>
<td>HSV-1 SP23</td>
<td>Wild-type</td>
<td>5,600</td>
<td>2.5</td>
</tr>
<tr>
<td>HSV-1 N38</td>
<td>US9,10,11,12-deletion</td>
<td>100,000</td>
<td>45.5</td>
</tr>
<tr>
<td><strong>HSV-1 HF 10</strong></td>
<td>3.9kb-deletion including UL56</td>
<td>&gt; 5,000,000</td>
<td>&gt; 2,272.7</td>
</tr>
<tr>
<td>HSV-2 186</td>
<td>Wild-type</td>
<td>63</td>
<td>0.03</td>
</tr>
<tr>
<td>HSV-2 L1BR1</td>
<td>US3-deletion</td>
<td>&gt; 1,000,000</td>
<td>&gt; 454.5</td>
</tr>
<tr>
<td>HSV-2 Y7</td>
<td>Clinical isolate</td>
<td>21</td>
<td>0.01</td>
</tr>
<tr>
<td>HSV-2 YN</td>
<td>Clinical isolate</td>
<td>55</td>
<td>0.03</td>
</tr>
</tbody>
</table>

→ LD$_{50}$ reflect mainly neurovirulence

HF10 showed strongly attenuated virulence
Antitumor effect in vivo

Only back side injection shows systemic anti tumor effect

1. Liver Metastasis Control Group
2. Liver Metastasis Subcutaneous Therapy Group
3. Peritoneal Metastases Control Group
4. Peritoneal Metastases Subcutaneous Therapy Group

Survival rate

Tumor volume on back side (㎟)

Survival days

Survival rate

Liver tumor

Peritoneal tumor

P = 0.001

P = 0.0066
Conclusion

1. We observed strong antitumor effect against back side tumor and abdominal tumor even if only back side tumor injection.

2. The tumor suppressing effect was observed against liver and peritoneal metastasis, while HF10 was not detected in abdominal tumor.

3. CTL-mediated cytotoxicity to MC26 cells was observed on MTT assay. CTL secreted cytokine on Elispot assay.

4. Our results suggest that HF10 induces the tumor specific host immunity, and metastasis tumor is suppressed by anti tumor immunity.
Clinical Trial using HF10 in Japan

- Intratumoral injection of herpes simplex virus HF10 in recurrent head and neck squamous cell carcinoma.
  

- Pilot study of oncolytic viral therapy using mutant herpes simplex virus (HF10) against recurrent metastatic breast cancer
  

- A phase I dose-escalation clinical trial of intraoperative direct intratumoral injection of HF10 oncolytic virus in non-resectable patients with advanced pancreatic cancer
  

- Phase I Dose-escalation Clinical Trial of HF10 Oncolytic Herpes Virus in 17 Japanese Patients with Advanced Cancer
  
“Different Routes of Administration of Oncolytic Virus in Abdominal Tumors”
Classification of histological response

Grade 2: Marked response; marked change of more 66% cancer cells.
Grade 3: Complete response; necrosis or disappearance of 100% cancer cells.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Gender</th>
<th>Contents (pfu)</th>
<th>Times</th>
<th>Histologic response</th>
<th>Histopathology</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>Female</td>
<td>$1 \times 10^4$</td>
<td>× 1</td>
<td>Grade 1b</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>Female</td>
<td>$1 \times 10^5$</td>
<td>× 1</td>
<td>Grade 1a</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>Female</td>
<td>$1 \times 10^5$</td>
<td>× 3</td>
<td>Grade 2</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>Female</td>
<td>$5 \times 10^5$</td>
<td>× 1</td>
<td>Grade 1b</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>Female</td>
<td>$5 \times 10^5$</td>
<td>× 3</td>
<td>Grade 2-3</td>
<td>Mucinous carcinoma</td>
<td>(-)</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>Female</td>
<td>$5 \times 10^5$</td>
<td>× 3</td>
<td>Not applicable</td>
<td>Scirrhous carcinoma</td>
<td>(-)</td>
</tr>
</tbody>
</table>
Design of Clinical Trial for Breast Cancer

HF10 Clinical Trial for 2 weeks (14 days)

Virus Injection

HF10 x 1 ~ x 3 days

Resection after 2 weeks of Injection

X
Saline

Control 14d.

HF10 (10⁵ pfu × 3 days), 14d.

Control 14d specimen.

Saline

HF10 \((5 \times 10^5 \text{ pfu} \times 3 \text{ days}), 14d.\)
Ultrasonography finding of breast Cancer.

(A) Subcutaneous metastasis of the breast cancer. 
(B) The same lesion 10 days after HF10 injection. Its length decreased approximately 30% compared with length of (A).

At 10 days after virus injection
(This is Before rejection)
### Recurrent Head and Neck cancer

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Sex</th>
<th>Squamous cell carcinoma</th>
<th>Adverse drug reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>58</td>
<td>Male</td>
<td>1 x 10(4) / x 3</td>
<td>(-)*</td>
</tr>
<tr>
<td>8</td>
<td>79</td>
<td>Female</td>
<td>1 x 10(5) / x 3</td>
<td>(-)*</td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>Male</td>
<td>1 x 10(5) / x 3</td>
<td>(-)*</td>
</tr>
</tbody>
</table>

* Low grade fever

CD4-positive or CD8-positive cells were much greater than in untreated nodules. Immunohistochemical staining showed evidence of viral infection.

Acta Oto-Laryngologica 2006; 126: 1115-1117
Design of Clinical Trial for Heads & Neck Cancer

- HF10 x 3 days
- Virus Injection
- HF10 Clinical Trial for 2 weeks (13 or 15 days)
- Resection after 2 weeks of Injection

Performed by Dept. of Oto Rhino Laryngology
Non-resectable pancreatic cancer patient profiles

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>CHOI</th>
<th>Gender</th>
<th>Contents (pfu) x Time</th>
<th>Histopathology</th>
<th>Toxicity</th>
<th>Non-resectable cause</th>
<th>Survival</th>
<th>Chemo 1 M later</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>PD</td>
<td>Male</td>
<td>$1 \times 10^5 \times 3$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>P, S</td>
<td>200 days</td>
<td>Gem</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>SD</td>
<td>Male</td>
<td>$1 \times 10^5 \times 3$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>P</td>
<td>166 days</td>
<td>Gem</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>SD</td>
<td>Male</td>
<td>$5 \times 10^5 \times 3$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>L</td>
<td>318 days</td>
<td>Gem</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>PD</td>
<td>Male</td>
<td>$1 \times 10^6 \times 3$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>L</td>
<td>98 days</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>PR</td>
<td>Male</td>
<td>$1 \times 10^6 \times 3$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>L</td>
<td>209 days</td>
<td>TS-1</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>SD</td>
<td>Male</td>
<td>$1 \times 10^6 \times 3$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>P, C</td>
<td>315 days</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>PD</td>
<td>Male</td>
<td>$1 \times 10^6 \times 6$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>L</td>
<td>206 days</td>
<td>Gem</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>PD</td>
<td>Male</td>
<td>$1 \times 10^6 \times 6$</td>
<td>Invasive ductal carcinoma</td>
<td>(-)</td>
<td>P</td>
<td>113 days</td>
<td>-</td>
</tr>
</tbody>
</table>

P: Peritoneal dissemination, S: Superior mesenteric artery invasion, C: Common hepatic artery invasion, L: Liver metastasis
Design of Clinical Trial for Pancreatic Cancer

Add EUS from 7th patients

Virus injection

HF10 clinical trial for 30 days

Until 6th patients
Virus Injection into Non Resectable Pancreatic Cancer
Pancreatic cancer tumor marker movement

(Patient 1)  CA19-9

HF10 treatment x 3

800  1000  1200  1400  1600  1800  2000
7  14  21

↑↑↑  : Consecutive 3days HF10 injection

(Patient 3)  CA19-9

HF10 treatment x 3

3450  3510  3570  3630  3690  3750  3810
14  21  28

(Patient 5)  CA19-9

HF10 treatment x 3

8000  7000  6000  5000  4000  3000
pre  14  28

: 30days observation period without any other therapeutics
Patient 5 (PR)
**Patient 3**

HSV envelope protein Ab of Autopsy specimen (318 days)

**Patient 8**

HSV envelope protein Ab of EUS needle biopsy specimen (14 days)

HSV 1 Inclusion Bodies (14 days)
Monotherapy of HF10 clinical trial

• Sufficient safety data and some efficacy even if mono therapy
• Symptom of angiogenesis (inflammation) and tumor rebound

2009

Translational Research

• Gemcitabine inhibited MDSC (Myeloid Derived Suppressor T cell)
• Erlotinib inhibited angiogenesis

2013

Combination therapy of HF10 clinical trial

• Sufficient safety data and more efficacy than mono therapy
• Non-resectable → resectable pancreatic cancer (2 Surgical CR)

2015

Back Ground → Next Steps

50% (PR+SD) @ target tumor

100% (PR+SD) @ target tumor
In vivo experiment

Tumor Volume

Survival rate

Translational Research
Tumor volume

\[ \text{Tumor volume} = \{ (\text{length}) \times (\text{wide})^2 \} \times \frac{1}{2} \]

**HF10 + Erlotinib**

BxPC-3
Pancreatic cancer

Additive/Synergic effect

<table>
<thead>
<tr>
<th>Day</th>
<th>HF10 group</th>
<th>Erlotinib group</th>
<th>Expected (E-FTV)</th>
<th>Observed (O-FTV)</th>
<th>E-FTV / O-FTV</th>
<th>Synergic</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.726107</td>
<td>0.58957</td>
<td>0.428091</td>
<td>0.455616</td>
<td>0.939586</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>0.594617</td>
<td>1.08548</td>
<td>0.645445</td>
<td>0.35141</td>
<td>1.83673</td>
<td>+</td>
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<tr>
<td>21</td>
<td>0.264146</td>
<td>0.878868</td>
<td>0.232149</td>
<td>0.15504</td>
<td>1.497346</td>
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</tr>
<tr>
<td>28</td>
<td>0.270763</td>
<td>0.647756</td>
<td>0.175389</td>
<td>0.128337</td>
<td>1.366625</td>
<td>+</td>
</tr>
<tr>
<td>35</td>
<td>0.376833</td>
<td>0.650825</td>
<td>0.245252</td>
<td>0.128268</td>
<td>1.912029</td>
<td>+</td>
</tr>
<tr>
<td>42</td>
<td>0.30703</td>
<td>0.487905</td>
<td>0.149802</td>
<td>0.127546</td>
<td>1.174489</td>
<td>+</td>
</tr>
</tbody>
</table>

FTV; fractional tumor volume: mean tumor volume experimental / mean tumor volume control
E-FTV: mean FTV of HF10 × mean FTV of Erlotinib

Reduce the numbers of MDSC in spleen with GEM

Shinichi Esaki et al. in International Journal of Cancer

It causes prominent appearance of tumor antigen

Tumor bearing mice

Shinichi Esaki et al. in International Journal of Cancer
New Protocol of HF10 with Erlotinib and Gemcitabine Against Non-resectable Pancreatic cancer

Approval in Feb. 2013 in Nagoya University Hospital

**Erlotinib**

100mg/day

**Gemcitabine**

1,000mg/m²/4weeks

**HF10**: $1 \times 10^{6-7}$

Every 2 weeks

1 course

1

2 course

2

3 course

3

4 course

4

Follow up 4 weeks
## Combination therapy

### Evaluation (RECIST ver1.1)

<table>
<thead>
<tr>
<th></th>
<th>Dose (pfu)</th>
<th>Toxicity</th>
<th>Survival date</th>
<th>Target Response</th>
<th>Overall Response</th>
<th>Surgical CR</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF-1-02</td>
<td>1 x 10^6</td>
<td>(-)</td>
<td>150days</td>
<td>SD</td>
<td>PD</td>
<td></td>
<td>119days</td>
</tr>
<tr>
<td>HF-1-04</td>
<td>1 x 10^6</td>
<td>(-)</td>
<td>465days</td>
<td>SD</td>
<td>PD</td>
<td></td>
<td>91days</td>
</tr>
<tr>
<td>HF-1-05</td>
<td>1 x 10^6</td>
<td>(-)</td>
<td>611days</td>
<td>PR</td>
<td>PR</td>
<td>CR</td>
<td>335days</td>
</tr>
<tr>
<td>Cohort 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF-2-01</td>
<td>3 x 10^6</td>
<td>(-)</td>
<td>919days@2016/10/7</td>
<td>SD</td>
<td>SD</td>
<td></td>
<td>663days</td>
</tr>
<tr>
<td>HF-2-02</td>
<td>3 x 10^6</td>
<td>(-)</td>
<td>891days@2016/10/7</td>
<td>PR</td>
<td>PR</td>
<td>CR</td>
<td>456days</td>
</tr>
<tr>
<td>HF-2-03</td>
<td>3 x 10^6</td>
<td>(-)</td>
<td>336days</td>
<td>SD</td>
<td>SD</td>
<td></td>
<td>48days</td>
</tr>
<tr>
<td>Cohort 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF-3-02</td>
<td>1 x 10^7</td>
<td>(-)</td>
<td>694days</td>
<td>PR</td>
<td>PR</td>
<td></td>
<td>217days</td>
</tr>
<tr>
<td>HF-3-03</td>
<td>1 x 10^7</td>
<td>(-)</td>
<td>273days</td>
<td>SD</td>
<td>SD</td>
<td></td>
<td>69days</td>
</tr>
<tr>
<td>HF-3-04</td>
<td>1 x 10^7</td>
<td>(-)</td>
<td>255days</td>
<td>PR</td>
<td>PR</td>
<td></td>
<td>189days</td>
</tr>
</tbody>
</table>

### Surgical Response (CR): 2/9: 22%

### Target lesion effective response (PR+SD): 9/9: 100%

### Overall effective response (PR+SD): 7/9: 78%

### Median PFS 189 days 6.3M

### Median OS: >465 days (15.5 months).

## Evaluation (CTCAE ver4.0)

<table>
<thead>
<tr>
<th></th>
<th>Original T size (cm)</th>
<th>TNM</th>
<th>Adverse effect (other)</th>
<th>Toxicity of HF10</th>
<th>DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1</td>
<td>HF-1-02 7.9 x 6.5 x 8.0</td>
<td>IVa</td>
<td>Grade III Plt, WBC ↓, Perforation Peritonitis</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>HF-1-04 3.0 x 2.6 x 2.3</td>
<td>IVa</td>
<td>Grade II fever, TB ↑, Interstitial Pneumonitis</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>HF-1-05 2.6 x 3.0 x 1.9</td>
<td>IVa</td>
<td>Chemotherapy leakage</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Cohort 2</td>
<td>HF-2-01 1.0 x 1.0 x 1.0</td>
<td>IVa</td>
<td>Grade III WBC Neutro ↓</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>HF-2-02 1.1 x 1.4 x 1.9</td>
<td>IVa</td>
<td>Grade III WBC Neutro ↓</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>HF-2-03 3.7 x 3.4 x 3.2</td>
<td>IVa</td>
<td>Grade II liver dysfunction, Grade III WBC Neutro ↓</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Cohort 3</td>
<td>HF-3-02 2.5 x 1.9 x 2.2</td>
<td>IVa</td>
<td>NA</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>HF-3-03 2.0 x 4.0 x 2.0</td>
<td>IVa</td>
<td>NA</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>HF-3-04 2.8 x 2.6 x 2.2</td>
<td>IVa</td>
<td>Grade III WBC Neutro ↓</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

Patient Info.: women, 66 years old
Tumor: Pancreas head
Tumor size: 26.0 × 30.0 mm

Therapy

= Chemotherapy =
GEM dose: 1640 mg × 9 times
Erl dose (internal use): 98 times

= Virus therapy =
HF10: 1.0 × 10^6 pfu × 4 times
Dose volume: 1.0 × 10^6 pfu/day

= Radiation therapy =
( After clinical trial)
1.8 Gy × 28 times (Total 50.4 Gy)
HF10 administration into the tumor

HF-1-05
Improvement of SMA, SMV invasion
HF-1-05

Lymph％

WBC (3.8〜8.5)

NEUTRO
HF10 leakage check in plasma by DNA detection (qPCR)

Result for 3 days after injection: All negative
The effect of HF10 is bigger than the effect of only chemotherapy.
99% cancer had disappeared

Tumor tissue (pathological diagnosis)
Low density area in CT

Radiologist ad pointed out as 2cm tumor
Normal 1

Normal 2

fibrosis

CD4

CD8

< HF-1-05 >

[Graph showing CD4 and CD8 levels in Normal 1, Normal 2, and fibrosis conditions, with 200um scale]

Patient Info. : man, 65 years old
Tumor : Pancreas body
Tumor size : 11 × 14 × 19mm

● Therapy
  = Chemotherapy =
  GEM dose                        1640mg × 9 times
  Erl dose (internal use)                       99times
  S-1
  = Virus therapy =
  HF10 3.0 × 10^6 pfu × 4 times
  Dose volume 3.0 × 10^6 pfu/day

= Radiation therapy =  December.2013 ~ January.2014
( Before clinical trial)
50Gy × 28 times (Total 1400Gy)
HF-2-02

**Lymph%**

-28D -14D 0D 14D 28D 42D 56D 70D
Administration (day)

**WBC (3.8〜8.5)**

-28D -14D 0D 14D 28D 42D 56D 70D
Administration (day)

**NEUTRO**

-28D -14D 0D 14D 28D 42D 56D 70D
Administration (day)
Radiotherapy 50Gy x28

The effect of HF10 is bigger than the effect of only chemotherapy.

<Reference :HF-2-02>
Cancer cells: diffuse type over 90% cancer had disappeared
Tissue 1

Tissue 2

Tissue 3

Arrow: Cancer cells
Combination therapy against pancreatic cancer

Conventional Therapy

Gemcitabine + Erlotinib + HF10

Gemcitabine + Abraxane + HF10

Oncolytic virus

Adverse effect

Efficiency
Conclusion

- Virus injection directly into skin surface tumor or lymph node
- Virus injection at open abdominal surgery
- Virus injection using catheter
- Virus injection using ultra sound Endoscopy
- May be CT guide injection
Thank you very much for your attention

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