Changing the Way We Treat Cancer with CYT-6091 (Aurimune®): A Model Cancer Nanomedicine

*International Symposium on Assessing the Economic Impact of Nanotechnology*

Washington, D.C.
27 March 2012
Problem: For many cancers, response rates of patients treated surgically first, followed by chemotherapy and/or radiation are poor
- Surgery alone does not cure most patients of cancer
- Following surgery, many patients present with metastatic disease

Need: Improve efficacy and safety, and minimize recurrent disease
- Targeting tumors
- Limit exposure of healthy tissues and organs to cytotoxics

Solution: Use nanotechnology-based therapeutics, first
- First treat patients medically to reduce tumors, use surgery only if needed
  - May lead to improved tumor regression, reduced side effects, and reduced recurrent disease
Benchmarks for a Cancer Nanomedicine

Needs to Avoid Uptake by Mononuclear Phagocyte System (MPS)
- Primarily the liver and spleen

Needs to Target Tumors (Passive and Active)
- Corollary
  - Less severity/frequency of side-effects compared to unformulated API

Needs to Be Manufactured to Defined Specifications
- Robust
- Reproducible
- Cost Effective
Design of CYT-6091 (Aurimmune®):

Water absorbed by PEG-Thiol shields nanoparticle from immune detection
Too Large for Toxic Side Effects. CYT-6091 is small enough to safely travel through healthy blood vessels, but too large to pass through blood vessel walls into healthy tissues and organs, resulting in reduced toxicity.

Small Enough to Exit Tumor Vessels. All solid tumors are fueled by new, “leaky” blood vessels that have gaps in their walls. When CYT-6091 reaches these “leaky” vessels, the nanoparticles are small enough to pass through these walls into their target, the tumor.

Due to its engineered nanometer size and targeted capabilities, CYT-6091 is able to reduce toxicity and increase efficacy.
CYT-6091: Avoids Immune Recognition and Uptake

PEG bound to gold nanoparticles prevents uptake by the liver and spleen, major organs of the MPS, (black color is aggregated gold particles)

- Uncoated nanoparticles may be safe, but do not reach tumor target
Differential Uptake of CYT-6091 in Mouse Model

Electron micrographs comparing tumor and healthy tissue

Spleen  Tumor  Liver

Bar at bottom = 200 nm
Pharmacokinetic Modeling of CYT-6091 in the Rat*

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Native TNF</th>
<th>CYT-6091</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TNF</td>
</tr>
<tr>
<td>$V_d$ (mL)</td>
<td>326</td>
<td>36</td>
</tr>
<tr>
<td>Clearance (mL/min)</td>
<td>5.08</td>
<td>0.43</td>
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<tr>
<td>Elimination Rate</td>
<td>0.027</td>
<td>0.004</td>
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<tr>
<td>Terminal Half Life (min)</td>
<td>26</td>
<td>182</td>
</tr>
</tbody>
</table>

*Study Conducted by the Nanotechnology Characterization Laboratory (NCL), NCI
Biodistribution of TNF Following CYT-6091 Rx

Tumor concentrations of TNF increase after dosing of mice with CYT-6091

- TNF levels in all major organs, including liver, decrease over same time period

![Graph showing time course of TNF concentration in various organs and tumor after CYT-6091 injection.](image)

- CYT-6091-Liver
- CYT-6091-Lung
- CYT-6091-Brain
- Native TNF - Tumor
- CYT-6091-Tumor
Previous systemic clinical testing with TNF shows

- No clinical effect at maximum tolerated dose of 0.4 mg
- At 1 mg patients experience severe hypotension, leading to complete organ failure and possibly death
- Not approved by FDA or EMA (European agency)

Isolated Limb Perfusion (ILP) procedure (EMA approved)

- Temporary surgical isolation of tumor-burdened limb -- maintain limb viability with heart-lung machine
- 1 mg TNF administered followed 30 minutes later by chemotherapy results in complete response rates = 85%

CYT-6091 systemic clinical testing

- Succeeded in rescuing TNF therapeutic potential with CytImmune platform
- CYT-6091’s targeting capability has potential to significantly improve typical chemotherapy response rates
Effect of Systemically Administered CYT-6091 on Tumor Vasculature

By delivering TNF to the tumor vasculature CYT-6091 causes vascular breakdown

- Massive vascular leak destroys high intra-tumor pressure

From: R. van Horssen et al, The Oncologist 2006;11:397-408
Selective Induction of Vascular Leak by CYT-6091

Normal Vasculature
No Vascular Leak

Tumor Neovasculature
Vascular Leak
Killing Tumors: CYT-6091 Pre-Clinical Mouse Data

Stealthy.  PEG-Thiol bound to colloidal gold nanoparticles avoids immune detection by the MPS

Targeted.  CYT-6091 delivers TNF to solid tumors:

- *Passively* by extravasating from the tumor vasculature
- *Actively* by binding to TNF receptors on tumor endothelial cells

Accumulation.  CYT-6091 accumulates TNF in TNF sensitive and insensitive tumors

- For TNF sensitive tumors:
  - One treatment induces potent anti-tumor responses at lower doses
- For TNF insensitive tumors:
  - One treatment induces transient anti-tumor response
  - Multiple doses causes cytostasis
  - Combination with doxorubicin is additive
Effect of CYT-6091 and TNF on Tumor Growth

Single treatment of C57BL/6 mice with TNF-sensitive MC-38 tumors

- High dose TNF effective, but causes 40% mortality
- High dose CYT-6091 equally effective with no mortalities
- Low dose CYT-6091 just as effective as high dose, shows potential of tumor targeting
CYT-6091 Clinical Trial in Cancer Patients

Water absorbed by PEG-Thiol shields nanoparticle from immune detection.
Clinical Grade CYT-6091

Current production capacity scaled 10-fold from Phase I to Phase II

- Solved manufacturing challenge for a nanomedicine
- Process is robust, reproducible and cost effective
- 3-year shelf life as a freeze-dried product
Goal of CYT-6091 (Aurimune) Clinical Trial

Safely administer 1 mg of TNF formulated as CYT-6091 to cancer patients without inducing hypotension
CYT-6091 Phase I Trial: Clinical Observations

Safe, systemic delivery. Delivered 1.2 mg of TNF with no dose limiting toxicity

- No Hypotension, the dose-limiting toxicity associated with TNF use in man
- No Serious Adverse Events that were unexpected and related to treatment

Tumor targeted. Drug accumulation at tumor sites

- Gold particles seen in tumors but few if any in healthy tissues

Not Antigenic. No antibody response

- Titer checks after CYT-6091 treatments show no anti-TNF antibodies
### CYT-6091 Phase I Patient Population

<table>
<thead>
<tr>
<th>Patient ID and Dose</th>
<th>Histology</th>
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<tbody>
<tr>
<td>01 (50 µg/m2)</td>
<td>Cutaneous Melanoma</td>
</tr>
<tr>
<td>02 (50 µg/m2)</td>
<td>Colon Adenocarcinoma</td>
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<tr>
<td>03 (50 µg/m2)</td>
<td>Ocular Melanoma</td>
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<tr>
<td>04 (100 µg/m2)</td>
<td>Colon Adenocarcinoma</td>
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<td>05 (100 µg/m2)</td>
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<td>06 (100 µg/m2)</td>
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<td>07 (150 µg/m2)</td>
<td>Lung Adenocarcinoma</td>
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<td>08 (150 µg/m2)</td>
<td>Pancreatic Adenocarcinoma</td>
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<td>09 (150 µg/m2)</td>
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<tr>
<td>10 (200 µg/m2)</td>
<td>Invasive Ductal Carcinoma</td>
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<tr>
<td>11 (200 µg/m2)</td>
<td>Leiomyosarcoma</td>
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<td>12 (200 µg/m2)</td>
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<td>15 (250 µg/m2)</td>
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<td>21 (400 µg/m2)</td>
<td>Desmoplastic Small Round Cell</td>
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<td>22 (400 µg/m2)</td>
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<td>28 (600 µg/m2)</td>
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<td>29 (600 µg/m2)</td>
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<tr>
<td>30 (600 µg/m2)</td>
<td>Adrenocortical carcinoma</td>
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</table>
Effect of Pre-Treatment on CYT-6091 Induced Fever

Acetaminophen/indomethacin/benadryl pretreatment used for all subsequent patient dosings
Effect of CYT-6091 on Blood Pressure

![Graph showing the effect of CYT-6091 on blood pressure over time for different doses.](image-url)

- **Systolic Blood Pressure**
- **Diastolic Blood Pressure**

The graph illustrates the change in systolic and diastolic blood pressure following the injection of CYT-6091 at various doses. The x-axis represents time after injection (hours), and the y-axis represents blood pressure (mm Hg). Different doses are indicated by distinct colors and markers.
Pharmacokinetics of CYT-6091 in Humans

**Graph Description:**
- The graph illustrates the pharmacokinetics of CYT-6091 in humans, showing the concentration of TNF (ng/ml) over time (min) after injection.
- The x-axis represents the time after injection in minutes, ranging from 0 to 1500.
- The y-axis represents the TNF concentration in ng/ml, ranging from 0.1 to 100.0.
- Different concentrations of CYT-6091 (50 µg/m2 to 600 µg/m2) are represented by distinct lines and markers.

**Key Points:**
- The graph visually demonstrates the decay of TNF concentration over time for various injection doses.
- The significance of the pharmacokinetic profile in understanding the drug's efficacy and duration of action.

**Legend:**
- 50 µg/m2
- 100 µg/m2
- 150 µg/m2
- 200 µg/m2
- 250 µg/m2
- 300 µg/m2
- 400 µg/m2
- 500 µg/m2
- 600 µg/m2

**Additional Information:**
- The graph provides a comprehensive view of how different doses affect the TNF concentration over time, which is crucial for clinical applications and research.
- Understanding these pharmacokinetic properties helps in optimizing the dosing regimen and predicting side effects.
# Clinical Studies: Systemic TNF Vs. CYT-6091

## Analysis of CYT-6091 Pharmacokinetic Data:
Comparison with the Historical Data on the Pharmacokinetics of rhTNF in Man


** Source: HR Alexander in *Biologic Therapy of Cancer*: Chapter 13 Page 331 Copyright 1995

<table>
<thead>
<tr>
<th>Dose Range (µg/m2)</th>
<th>T 1/2 (min)</th>
<th>AUC (ng-min/ml)</th>
<th>Gamm et. al.*</th>
<th>T 1/2 (min)</th>
<th>AUC (ng-min/ml)</th>
<th>Alexander et. al.**</th>
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-- = Not Tested
Electron Micrographs* of a Patient’s Biopsies

Patient diagnosed with inoperable breast cancer
- Patient had no prior treatment; samples taken 24h after treatment
- Drug accumulated in tumor, not in healthy breast tissue

Healthy Breast  
Tumor

*Magnification = 20,000x
Strategy for Phase II Clinical Trial Design

Isolated Limb Perfusion (ILP) of TNF + chemotherapy is 85% effective
- Phase II protocol mimics ILP combination protocol

CYT-6091 Phase I Success + ILP Efficacy → Dramatic improvement in Overall Response Rates
## Potential CYT-6091 Phase II Clinical Trial Sites

<table>
<thead>
<tr>
<th>Cancer Indication</th>
<th>Chemotherapy</th>
<th>Principal Investigator</th>
</tr>
</thead>
</table>
| Non-small cell lung cancer/Ovarian | Taxotere®    | Steven K. Libutti, M.D., FACS  
Director, Montefiore-Einstein Center for Cancer Care  
Montefiore Medical Center/Albert Einstein College of Medicine  
Bronx, NY 10467                                                                 |
| Pancreatic                | Gemcitabine  | Professor John P Neoptolemos, FMedSci  
Head of School of Cancer Studies  
Head Division of Surgery and Oncology  
The Duncan Building, The University of Liverpool  
Liverpool L69 3GA, UK                                                                 |
| Melanoma                  | DTIC         | Prof. Alexander M.M. Eggermont, MD, PhD  
Head Surgical Oncology  
Erasmus MC - Daniel den Hoed Cancer Center  
The Netherlands                                                                 |
| Soft Tissue Sarcoma/Breast | Doxil®       | Prof. Alberto A. Gabizon, M.D., Ph.D.  
Head, Oncology Institute, Shaare Zedek MC  
Hebrew University - School of Medicine,  
Jerusalem, ISRAEL                                                                 |
The Promise of Cancer Nanomedicines

Deliver potent anti-cancer agents directly to the site of disease
- Reduced or no toxicity
- Improved efficacy

Treat cancer as a medical disease first
- Dose intravenously prior to surgery
- Limited biodistribution due to leaky tumor blood vessels
- Reduce tumor burden by tumor-targeted nanomedicines
- Reduce or eliminate sophisticated surgical procedures
- Improve patient outcome

Treat cancer as a chronic medical disease
- Treat periodically to destroy nascent tumor neovasculature
- Suppress metastatic disease
CYT-6091: An Ideal Cancer Nanomedicine

Designed to meet critical requirements for tumor targeted therapy

- Not picked-up by liver and spleen
- Targets tumor endothelial cells
- Manufacturing process robust, reproducible and cost-effective

![Diagram showing CYT-6091 targets tumors and avoids MPS uptake.](image-url)

- Targets Tumors
- Avoids MPS Uptake
- Manufactured to Defined Specifications