Molecular Diapapeutics: Phospholipid Ether Analogs for Broad Spectrum Cancer and Cancer Stem Cell Detection and Treatment

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Coinventor of CLR1404 technology and technical founder/CSO/Director of Novelos, Inc (Madison, WI) which owns all rights to CLR1404 and related technologies.

\[ \text{NM404=CLR1404} \]
Improving Outcomes in Cancer Therapy

- **Diagnosis**
  - Sensitive, early detection and localization of primary tumors and metastases for disease staging and treatment planning

- **Therapy**
  - Tumor kill and metastasis blockade while addressing phenotypic heterogeneity and cancer stem cells, all with low toxicity/side effects
### Ether Cleavage Enzyme Activity in Normal Liver and Neoplastic Tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Host Animals</th>
<th>Activity $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Liver</td>
<td>Buffalo $^b$</td>
<td>7.3</td>
</tr>
<tr>
<td>Morris Hepatoma 7794A</td>
<td>Buffalo $^b$</td>
<td>5.8</td>
</tr>
<tr>
<td>Morris Hepatoma 7777</td>
<td>Buffalo $^b$</td>
<td>1.4</td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>HA/ICR $^c$</td>
<td>0.42</td>
</tr>
<tr>
<td>Melanoma B-16</td>
<td>C57BL/6 $^c$</td>
<td>0.31</td>
</tr>
<tr>
<td>Ehrlich Ascites Carcinoma</td>
<td>HA/ICR $^c$</td>
<td>0.14</td>
</tr>
<tr>
<td>KHZ Mammary Tumor</td>
<td>C3H $^c$</td>
<td>0.11</td>
</tr>
<tr>
<td>Walker-256</td>
<td>Carsworth Farms Nelson $^b$</td>
<td>0.10</td>
</tr>
</tbody>
</table>

$^a$ Expressed as $\mu$mol of ether cleaved/20 min/mg protein
$^b$ Rat strains
$^c$ Mouse strains

Ref. Soodsma, Piantadosi, and Snyder
>30 analogs synthesized, radiolabeled, and evaluated

Diapeutic Phospholipid Ether Analogs

131I-CLR1404
Molecular Radiotherapy

124I-CLR1404
PET Imaging

CLR1401
Cytotoxic Chemotherapy

CLR1501
Optical 500 nm

CLR1502
Optical 800 nm (Near IR)

Phase 1b MTD
Preclinical stage
Preclinical stage
Preclinical stage

Complete safety pharmacology & toxicology package
**PLE Tumor Imaging Agents**

**CLR1404** *=* I-131

**CLR1401** *=* I-127

**NM404** *=* I-124

**CLR1404** *=* I-131

**PET/Therapy**

**CLR1502**

**NIR** (ex 780/em 800 nm)

**CLR1501**

**(ex 495/em 515 nm)**

**Fluorescent**

**Indocyanine**

**Bodipy**

**Alkylphosphocholine Class**
Small molecule simplicity and advantages
- Stabile aromatic iodine resists *in vivo* deiodination
- Can be radiolabeled with any iodine isotope
  - Iodine-124 (PET isotope with 4.2 day half-life)
  - Iodine-131 (SPECT and therapy isotope with 8 day half-life)
  - Iodine-125 (low E gamma/therapy isotope with 60 day half-life)
- CLR1404 is taken up and **selectively retained** by 52/54 xenograft, orthotopic, and transgenic solid tumor models examined to date.

  adenoma vs hyperplasia vs malignancy

  No  No  Yes

- Tumor uptake is independent of anatomic location with little or no tumor clearance
- Avoids inflammatory lesions
- GLP safety/pharm/tox study (>20 total studies) results in rodents and non-human primates indicate an exceedingly high safety index even at >800 times the anticipated human mass dose.
Tumor Imaging with $^{124}$I-CLR1404 PET
CLR1404 Tumor Time Course

$^{124}$I-CLR1404 in PC3 SCID Mouse

µPET scans: Head down/tail up with flank tumor
Fiducial markers (arrows)

Tumor uptake evident in about 9h
Inflammation: CLR1404 vs FDG PET

PC3 Prostate CA

$^{18}$F-FDG

$^{124}$I-CLR1404

3D cine projection (I=carrageenan induced inflammatory lesion, H=heart, T=human PC3-prostate tumors, SCID mouse)
CLR1404 PET/MRI U87 MG-nuRat

48h
2 mm tumor

72h
4.7T MRI
3D Hybrid microPET/CT image of an anesthetized orthotopic BxPC3 pancreatic tumor-bearing Nude mouse 48h post iv administration of $^{124}$I-CLR1404, The presence of the primary pancreatic tumor (P) as well as a spontaneous liver metastasis (M) is evident on the 3D scan. The presence of both tumors was verified at necropsy.

With Verma/Hafeez
Pan Cancer Imaging with $^{124}$I-CLR1404

Primary+Mets in 52/54 xenograft and spontaneous models
Radiotherapy or Diapertic Potential of $^{131}$I-CLR1404

Prolonged Tumor Retention

RL251 Adrenal CA
Starting tumor size was 200 mm³ for both treated and control groups.

Control Groups (n=6, RED) received equal mass dose of CLR1401 when tumor size reached 200 mm³.

Treated groups (n=6, BLUE) received single 100 µCi dose of ¹³¹I-CLR1404 except where noted (U87MG).
124/131I-CLR1404 Diaphoretic Response

PET/CT time course of an LS180 colon CA xenograft bearing mouse injected simultaneously with a mixture of 124,131I-NM404 (200 µCi each). Time course is from 3h to 10 days. Animal lost weight near the end of the study. Images are not normalized for exposure levels.

1 cm tumor regression over 10 days following single CLR1404 injection
CLR1501 Selectively Targets Cancer Cells

CLR1501 incubated 24h with cells then washed 2X with PBS prior to z-stack confocal microscopy. All exposure settings are the same. Blue is nuclear stain.
Lipid Rafts are Over-Expressed in Cancer Cells

CLR1501 is taken up by cells via “lipid rafts”, specialized regions of cell plasma membranes (= red; fluorescent-labeled cholera toxin subunit B). Methyl-β-cyclodextrin selectively disrupts rafts.
Fluorescence micrograph of a brain section (20 µm) 24h post CLR1501 (1 mg, iv) injection. 22T cell line blue is a nuclear stain (To-Pro-3) and 1501 is green.

22T Glioma Margins: Confocal
Green stain: CLR1501 and Blue stain: Hoechst 33452 (nucleus)
Red arrow=Tumor
Blue arrow=Normal brain parenchyma
Injected with 1 mg of CLR1502. Monitored the intensity in vivo over time. The color reflects the intensity. At 48 hours, animals euthanized and organs excised and scanned ex vivo. The organs clockwise starting from top left corner: heart, tumor, spleen, lung; middle: GI tract (not flushed); skin, kidneys and liver. The signal intensity in tumor is 200 times higher than signal from liver.
Intraoperative Tumor Margin Illumination

CLR1502
96h post injection HCT116 Xenograft

In vivo (IVIS Spectrum)

In vivo (Fluobeam)

In vivo Post Partial Dissection (Fluobeam)

Fluobeam™ Fluoptics
Mouse Brain Tumor Illumination-Fluobeam

Fluobeam Image of excised mouse brain and tumor 96h post CLR1502 injection

Photograph of excised brain and GSC-derived tumor
Cancer stem cells have now been firmly associated with most if not all major cancer types.

Numerous recent reports confirm that cancer stem cells do exist and are chemotherapy resistant.

Glioma stem cells are also known to be up to 30% more radiation resistant relative to normal cancer cells.

These cells affiliated with tumor regrowth and metastasis following chemo and radiation therapy.

Tumor hypoxia stimulates CSC propagation leading to increased resistance and metastatic potential.

“Any new cancer treatment paradigm must address tumor heterogeneity including cancer stem cells” - Jeremy Rich and others

CSCs extremely tumorigenic: (1 cell → tumor in melanoma)
## Cancer Stem Cell Isolation and Properties

### Isolation of Cancer Stem Cells

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Marker(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>CD34</td>
<td>Lapidot et al. 1994 Bonnet and Dick 1997</td>
</tr>
<tr>
<td>Brain</td>
<td>CD133 Neurospheres</td>
<td>Singh et al., 2004 Hemmati et al., 2003 Clark et al., 2007</td>
</tr>
<tr>
<td>Breast</td>
<td>CD24, CD44</td>
<td>Al Hajj et al., 2003</td>
</tr>
<tr>
<td>Colorectal</td>
<td>CD133</td>
<td>O'Brien et al., 2007 Ricci-Vitiani et al., 2007</td>
</tr>
<tr>
<td>Prostate</td>
<td>CD44, CD133</td>
<td>Collins et al., 2005</td>
</tr>
</tbody>
</table>

### Cancer Stem Cell Properties

<table>
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<tr>
<th>Property</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetically/Phenotypically similar to parental tumor</td>
<td>Lee et al., 2006</td>
</tr>
<tr>
<td>Enhanced chemoresistance</td>
<td>Eramo et al., 2006 Liu et al., 2006</td>
</tr>
<tr>
<td>Enhanced radioresistance</td>
<td>Bao et al., 2006a Diehn et al., 2009</td>
</tr>
<tr>
<td>Release angiogenic factors</td>
<td>Bao et al., 2006b Bruno et al., 2006 Calabrese et al., 2007</td>
</tr>
</tbody>
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**Glioma**

1. Stem cell medium + EGF + bFGF
2. Growth medium + Serum
3. Single cell suspension
4. GBM CSC
5. GBM cells (non-CSC)

**Orthotopic injection into SCID mouse**

**Kuo, Clark**
Glioma Stem Cell Results-Kuo Group

Comparative uptake of 1501 in normal vs malignant cells

In vivo uptake and serial GSC regrowth (3wk) with 1501
Prolonged retention in GSCs

Cells pretreated with 1401 or 1501-24h followed by ortho-inoculation with subsequent MRI monitoring. Survival: Control 59±6.1 days; CLR1401 94±4.4 days.

Glioma Stem Cell Lipid Raft Status (Alexa Fluor-594)
124I-PET Imaging Phase 1-2 Clinical Trials (UW IND)
- Lung Cancer (Traynor/Perlman) *(Image and dose optimization)*
- Glioma/ Brain tumors or Mets *(Image and dose optimization)*
- Multiple Tumor Protocol (Liu) *(Image and dose optimization)*
  - Pancreas
  - Breast
  - Prostate *(Wilding, Liu, Med Onc)*
  - Head and neck *(Speer/Harari. Rad Onc)*
  - Others-9 total

131I-Therapy Phase 1b MTD Trial (Novelos IND)
(UW, Georgetown, City of Hope)
- 20-40-60 etc *(12.5 mCi/m²)*
- 2nd dose cohort completed *(3rd cohort ongoing)*

Phase 1a dosimetry completed in 2010: 8 patients, 10 mCi ¹³¹I-CLR1404, 4 tumor types
  - No adverse events
  - Low coefficient of variance among subjects
  - Strong visual evidence of tumor uptake
3 Previously unknown brain mets were discovered with CLR1404 (5 mCi) in this lung cancer patient.

- Extremely low background activity afforded tumor to brain ratio of >28 (3-5 typically considered adequate)
- Tumor uptake at 24h

Treatment plan was altered because of this finding
Human CLR1404 Brain Tumor Imaging

Lung Mets

Recurrent Glioma
Grade 3
Newly diagnosed GBM. CE-MRI (left), 48h $^{124}$I-CLR1404 PET (center), and fused PET/MRI image (right). Blood pool activity from venous sinus (BP).

“NM404 PET shows heterogeneous avidity throughout the tumor, likely showing more uptake in viable parts of tumor and lack of uptake in areas of necrosis. If NM404 PET can better identify viable tumor and tumor infiltration compared to MRI, this could have a positive impact on treatment strategies and patient survival.” Lance Hall
CLR1404 PET vs F-DOPA PET

18F-DOPA Image of Recurrent Glioma
JNM Cover Image-March, 2012
53:393-98.

T1-MRI PET

124I-CLR1404 Image of Recurrent Glioma
(Tumor uptake at 6h)
Surgical efficacy due to lack of tumor clearance?
High Grade Astrocytoma Previously Resected and Radiated

MRI demonstrated new small enhancing lesion (<1 cm) in posterior right temporal lobe/hippocampus.

Radiation changes also evident throughout right hemisphere on T2 FLAIR MRI. No 1404 uptake.
Phase 1b MTD Patient SPECT

SPECT/CT image of Phase 1b patient with colorectal cancer metastases to the liver (multiple) and lung 21 days post injection of 25 mCi of $^{131}$I-CLR1404 confirming prolonged tumor retention.
Diapeutic Treatment Paradigm

Find-Treat-Follow

124I-NM404 PET Image

Image Quantification/Dose Calculation

131I-CLR1404 Therapy Dose Injection

Monitor Response Imaging

Tumor
A major goal of oncology today is to predict which patients will respond to a molecularly targeted drug. This is done by using biomarkers or imaging surrogates which are selective for the pathway or target of interest. Limitations still exist.

The PLE based diapeutic treatment paradigm has the following advantages over existing approaches:

- **Identical biomarker and therapy molecule** (CLR1404) which are administered in nearly the same mass dose.

- **PET/CT allows full body quantitative 4-D mapping of in vivo biodistribution**

- **PET/CT based dosimetry may predict personalized therapy dose or no treatment if imaging shows suboptimal tumor or normal organ uptake.**
Unique preclinical tumor targeting and retention properties of CLR1404 appear to translate to primary and metastatic human cancers (lung and others?)

Optical and radioactive CLR1404 analogs target and undergo prolonged retention in glioma stem cells.

The longer half-life of I-124 coupled with the prolonged tumor cell retention of CLR1404 may enable tumor resection efficacy quantification by utilizing pre- and post surgical image comparisons. (see residual tumor)

The unique diapheutic treatment paradigm we are attempting to define continues to progress and show promise.

The optical PLE platform shows early promise for intraoperative tumor margin illumination and staging.
Thank you!

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Glen Liu
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UW
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Radiology
Medical Physics
Human Oncology
WARF
Clinical Trial Group

National Cancer Institute
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2 R21 (Breast and Lung)
RO1-CA158800
(Glioma and Brain mets)

Clinical Trial Sites
City of Hope
Duke
Johns Hopkins
Georgetown
UW

Patient Volunteers
Supplemental Slides
PET Scanning 48h post CLR1404 injection
Fused 2D microCT projection (A) and $^{124}$I-CLR1404 microPET image (B) and fused microPET/microCT image (C) of excised PIRC rat colon filled with 2% barium. Fiducial marker (M), Tumor (arrow)
Virtual Biopsy?

with Dove/Amos-Landgraph
Triple Negative Breast Cancer Images

124I-CLR1404 microPET/CT image of a mouse with triple negative breast cancer (MB-231).

**Triple negative Breast Cancer**
- Estrogen receptor negative
- Progesterone receptor negative
- Her2 receptor negative
- 15-20% of breast cancer patients have this form

*Doesn’t respond to hormonal or epidermal growth factor targeted therapies*

First Demonstration of Triple Neg Breast Cancer Imaging
### 131I-NM404 Max Tolerable Dose in Rats

<table>
<thead>
<tr>
<th>Rat Dose (mCi)</th>
<th>Human (70kg) Equiv dose (mCi)</th>
<th>Rad tox findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>150*</td>
<td>None</td>
</tr>
<tr>
<td>2.5</td>
<td>750</td>
<td>None</td>
</tr>
<tr>
<td>4.0</td>
<td>1200</td>
<td>Slight platelet drop and recovery</td>
</tr>
<tr>
<td>5.0</td>
<td>1500</td>
<td>Grade 3 platelet drop and recovery</td>
</tr>
<tr>
<td>7.0</td>
<td>2100</td>
<td>Grade 4 platelet drop and death</td>
</tr>
</tbody>
</table>

Normal rats, N=6 for each cohort

* Anticipated max human dose
MicroPET/CT Based 4D-Treatment Planning for IV Radiotherapeutics: $^{124}$I-CLR1404

Siemens 2008 Inveon Image of the Year-World Molecular Imaging Congress
124I-CLR1404 MicroPET of Human Glioma Stem Cell Derived Orthotopic Brain Tumor

24h after injection
[Tumor/Brain]=5.4

48h after injection
[Tumor/Brain]=7.0

With Kuo and Clark
Eight patients enrolled; four cancer types; 10 mCi 131I-CLR1404

Zero drug attributable serious adverse events

Consistent distribution from patient to patient – increases safety profile

Distribution and elimination exactly as predicted from animal studies; minimal renal elimination increases safety profile

Strong visual evidence of tumor uptake

Results provided starting dose (12.5 mCi/m²) for MTD therapy trial
Potential Uses for CLR1404 Analogs

- Diagnosis, characterization, and staging of tumor masses regardless of location
- Guiding or in conjunction with Tomotherapy
  - Endo/Exo Radiotherapy Synergy
  - PET Guided Tomotherapy
- Monitoring tumor response to therapies
- Radiotherapy *(Diapeutic)* [*\(^{131}\text{I}, \, ^{125}\text{I}, \, \text{Both}\)]
- Dual Modality Virtual Colonoscopy *(Virtual Biopsy)*
- Optical Versions for detecting surface-oriented cancers
  - Colorectal / esophageal / cervical / melanoma / nodes
  - Intraoperative tumor margin illumination
CLR1404 Summary

- Preclinical imaging has shown selective tumor uptake and prolonged retention in 52/54 tumor types in mice.

- Preliminary radiotherapy results with $^{131}$I-CLR1404 in mice (>12 models) are very promising and show significant life extension.

- Rat MTD studies suggest acceptable dosimetry tolerance profile.

- Phase 1a dosimetry/safety study (8 cancer patients) with 10 mCi $^{131}$I-NM404 - safe and low variance pkinetics). Phase 1b MTD study and imaging trials now FDA approved and ongoing at UW (main site), COH and Georgetown.

- Initial cancer stem cell results appear very promising.