Enhanced Pentarins For Improved Pharmacokinetics And Efficacy
Sequencing Cancer Genes Has Advanced Our Understanding Of The Disease

Stratton (2011) Science 331, 1553
Personalized Cancer Medicine; Dramatic Response To The BRAF Inhibitor Vemurafenib

Personalized Cancer Medicine; Dramatic Response To The BRAF Inhibitor Vemurafenib

A 38-year-old man with BRAF mutant melanoma and miliary, subcutaneous metastatic deposits.

BRAF mutant melanoma patients treated with vemurafenib

Personalized Cancer Medicine; Dramatic Response To The BRAF Inhibitor Vemurafenib

A 38-year-old man with BRAF-mutant melanoma and miliary, subcutaneous metastatic deposits.

There Remains A Considerable Unmet Clinical Need In Cancer

Incidence of dominantly acting driver mutations in 552 Non-Small Cell Lung Cancers*

- KRAS 24%
- EGFR 12%
- PIK3CA 4%
- ALK 5%
- CTNNB1 2%
- NRAS 1%
- BRAF 1%

* Sequist et al. ASCO Annual Meeting 2011

- Dominantly acting mutations are detected in less than half of all tumors in the most common cancer types.
- Some of the dominantly acting cancer mutations have so far proven intractable targets, e.g. KRAS.
- There is a need for medicines that target these ‘dominant driverless’ and patient populations.
Some receptors are over-expressed on the surface of cancer cells, e.g., Her2 on breast cancer cells.

These create targets for the delivery of potent cytotoxic payloads to the cancer cells.

Antibody drug conjugates are an example of delivering payloads to cancer cells.
Antibody Drug Conjugates Are Successful Drugs

- Her2 is over-expressed in ~25% of breast cancers
- The antibody drug conjugate ado-traztuzumab emtansine consists of an antibody targeting Her2 and the potent cytotoxic DM1
- Ado-traztuzumab emtansine gave 30.9 months median survival compared to 25.1 for lapatinib+ capecitabine in Her2 positive breast cancer

Ado-traztuzumab emtansine survival compared to lapatinib+ capecitabine
Antibody Drug Conjugates Can Uncouple Over Time In The Plasma

- Ado-traztuzumab emtansine has a non-cleavable linker between the traztuzumab antibody and DM1 payload.
- However in plasma the DM1 dissociates from the antibody to the extent that 70% of the traztuzumab has no DM1 attached after 14 days.
- This potentially limits the effectiveness of ado-traztuzumab emtansine.
Deeper Solid Tumor Penetration Can Be Achieved With Smaller Targeting Ligands

Penetration into HT-29 Colon Xenografts

- The size of antibodies limits solid tumor penetration compared to smaller targeting ligands.
- Smaller targeting ligands also penetrate solid tumors faster which is important for payloads that require a high tumor \( C_{\text{max}} \).

EpCAM Aptimer-PEG ~20KDa

EpCAM Antibody ~150KDa

Xiang et al Theranostics 2015; 5(10):1083-1097
The Kinetics And Depth Of Solid Tumor Penetration Is Dependent On Therapeutic Size

- **Kinetics**
  - Small molecules penetrate rapidly
  - Rapid penetration is followed by rapid clearance of small non-targeted molecules
  - Larger molecules penetrate more slowly

- **Depth**
  - Small molecules penetrate deeper
  - Larger molecules penetrate less, despite the durable kinetics

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Tumor penetration of different sized dextrans into a tumor xenograft

- **Pentarin Size** (3.3 kDa)
  - Rapid, deep penetration

- **Intermediate** (10 kDa)
  - Slow, limited penetration

- **Half Antibody Size** (70 kDa)

- **Large** (2,000 kDa)

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1 Dreher et al (2006) J NCI 98, 335
Pentarins Are Miniaturized Biologic Drug Conjugates

Selected Targeting Ligand For Optimal PK And Receptor Affinity

Linker Designed To Balance Plasma Stability With Tumor Activation

Potent Payload Chosen To Drive Cancer Cell Death

Miniature Size Maintained

Tuned Biodistribution & Penetration

Rapid Iterative Design

Final Pentarin
The somatostatin receptor family consists of 5 members; SSTR1 to SSTR5.

All SSTRs are G protein couple receptors with 7 transmembrane segments.

SSTR2 and 5 are expressed in normal pancreas, pituitary, stomach and CNS.

SSTR2 is over expressed in neuroendocrine cancers.

Somatostatin is a peptide hormone of 14 or 28 amino acids that regulates the endocrine system and affects neurotransmission.

Analogs of somatostatin have been developed with receptor specific binding, including octreotide that is selective for SSTR2 and 5.
Somatostatin Pentarins Target Cancer Cells with High Affinity And Are Internalized

- Designed Pentarins demonstrated high affinity for target receptor
  - Binding is specific as control non-specific ligand Pentarin shows very low affinity for the receptor

- Pentarins induces internalization of the target receptor at low concentration
  - Non-specific Pentarin conjugate shows very large shift in internalization

Pentarins bind cancer cells with high affinity

![Binding Ki (nM)](chart)

![Conjugate Induced Internalization](chart)

**Ligand control**
**Pentarín**
**Non-specific Pentarin control**
Somatostatin Pentarin Has Minimal Effects In Cells Not Expressing The Somatostatin Receptor

- The cell proliferation IC$_{50}$ of a somatostatin Pentarin is 12 fold lower in non-expressing cell line than expressing cell-line
- The growth effect in non-expressing cell-line is not altered when excess unconjugated ligand is included with the Pentarin in a competition assay

Cancer Cell Proliferation Inhibition

<table>
<thead>
<tr>
<th></th>
<th>NCI-H524</th>
<th>NCI-H460</th>
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<tbody>
<tr>
<td>Receptor</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Competition</td>
<td>-</td>
<td>+</td>
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Somatostatin Pentarin Show a Significant And Sustained Biological Effect \textit{In Vivo}

- Histone 3 phosphorylation (pH3) was demonstrated as a biological, pharmacodynamic, biomarker for a somatostatin Pentarin \textit{in vitro}

- \textit{In vivo} a somatostatin Pentarin shows a measurable and sustained pH3 biological effect in tumor cells

pH3 response in tumors at 24h

<table>
<thead>
<tr>
<th>Vehicle control</th>
<th>Pentarin</th>
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<tr>
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pH3 response in tumors from 2hr to 72h

<table>
<thead>
<tr>
<th>Time</th>
<th>Phospho-H3 signal (fluorescence)</th>
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<tbody>
<tr>
<td>2hr</td>
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</tr>
<tr>
<td>6hr</td>
<td>0</td>
</tr>
<tr>
<td>24hr</td>
<td>20000</td>
</tr>
<tr>
<td>48hr</td>
<td>25000</td>
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<tr>
<td>72hr</td>
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</table>
The biological effects from treatment are seen deep in the tumor in the NCI-H69 xenograft model and distant from blood vessels.

**Histological Sections from Center of the Tumor**

- **Cell Cycle Arrest**
- **Cell Death**

**Vehicle**

**Somatostatin Receptor Targeting Pentarim**

- PhosphoHistone H3 level
- Cleaved caspase-3 level

**Complete Tumor NCI-H69 Xenograft**
Complete Tumor Regression From A Somatostatin Receptor Targeting Pentarin

- Pentarin cause tumor regression when dosed at 2 mg/kg

NCI-H69 Human Small Cell Lung Cancer Cell Line Expresses SSTR2
One cycle of SSTR2 targeting Pentarin causes complete regression

Cisplatin/Etoposide combination with one or two cycles fails to cause regression

Octreotide/DM1 combination shows no efficacy
Strategies To Optimize The Pharmacokinetics Of Pentarins For Efficacy

**Medicinal Chemistry**
- Engineering the chemistry of the Pentarin to achieve the optimal PK for efficacy

**Nanoparticle Incorporation**
- Proteins and peptides traditionally difficult to incorporate in nanoparticles at levels that are therapeutically and economically viable
- Existing methods to incorporate peptides/proteins include double emulsion techniques and HIP modulation
- Pentarins exhibit higher solubility in the aqueous phase and can form aggregates which lead to complications in nanoparticle incorporation
- Blend has developed unique nanoparticle strategies that can overcome these challenges and tune the performance of the nanoparticles to deliver a range of PK profiles
Generalized Process For Polymeric Nanoparticle Production

Stage 1: Emulsion formation

Stage 2: Purification and fill finish of nanoparticle formulation

Understanding of complex interplay between various formulation variables
Optimizing The Incorporation Of Pentarins Into Nanoparticles

**Organic Phase**
Pentarins and polymers

**Challenge**
Thermodynamic phase stability

**Solution**
Multi-component solvent system

**Aqueous Phase**
Emulsifiers/surfactants

**Challenge**
Toxicity / complexity

**Solution**
Reduce/eliminate

Control of nanoparticle properties such as size, rate of release and loading of Pentarins

**Emulsification**
Challenges
- Multiple steps
- Scale

**Solutions**
- Optimize
- Increase efficiency

Nanoparticles
Achieving The Target Physical Properties Of Pentarin Nanoparticles

Size distribution of Pentarin, nanoparticle 3

TEM image of Pentarin, nanoparticle 3
Nanoparticle Development From Bench Top To Pilot Scale

- Early formulation and process capabilities have to be robust to scale to clinical supply
- Integrating chemistry, analytical, biology and engineers and drives the iterative development of nanoparticles
Rapid *In Vitro* Nanoparticle Assessment Translates To *In Vivo* PK

- Optimal efficacy from Pentarins incorporated in nanoparticle is dependent on the plasma pharmacokinetics.
- Tunable *in vitro* release is directly translatable to *in vivo* rat pharmacokinetics – AUC and half-life.

**In vitro:** Pentarin stability in plasma

**In vivo:** Pentarin PK
Achieving Pentarin Nanoparticle Properties For Therapeutic Evaluation

Tunable plasma half-life and AUC achieved with nanoparticle incorporation using optimized polymers, co-solvents and reversible modification of Pentarin lipophilicity.

**In vivo Rat Plasma Pharmacokinetics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pentarin</th>
<th>NP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (μM)</td>
<td>11.1</td>
<td>36.9</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>1.1</td>
<td>4.8</td>
</tr>
<tr>
<td>$AUC_{0-inf}$ (μM*h)</td>
<td>18.3</td>
<td>256</td>
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</tbody>
</table>

NP3 Properties

- NP size: 63 nm
- Polydispersity index: 0.15
- Drug load: 4.9%
- Incorporation efficiency: >99%
Enhanced Efficacy With A Pentarin Incorporated Into A Nanoparticle

Pentarln Accumulates In Xenograft Tumor Tissue

Modest Efficacy With Free Pentarin

![Graph showing tumor payload concentration and average tumor volume over time](image-url)
Enhanced Efficacy With A Pentarin Incorporated Into A Nanoparticle

Elevated payload accumulation in tumor xenograft

Nanoparticle incorporation results in tumor regressions, a marked improvement in effect over the Pentarin alone.

![Graph showing tumor payload concentration and average tumor volume over time for different treatments.](image-url)
One in three people will be diagnosed with cancer

Targeting the unique aspects and vulnerabilities of cancer cells has been successful however there is still considerable unmet need

Pentarins are miniaturized biologic drug conjugates that penetrate deep into solid tumor tissue and target cancer cells

A Pentarin targeting of the somatostatin receptor 2 on small cell lung cancer leads to complete xenograft regressions

Nanoparticle incorporation of Pentarins has been used to tune the pharmacokinetics of the Pentarin and increase efficacy in xenograft models
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