Target-to-Treat Pancreatic Cancer using Nanotechnology

Who we are?

- Health
- Human Disease
- Research
- Discovery
- Development
- Medicines
- Translation of Multidisciplinary Research
- Generating Innovative Health Technologies
- Diagnosis
- Treatment
- Monitoring
- Prevention
...developing new drug delivery systems for specific and clinically relevant situations (inflammation, infections, genetic, autoimmune diseases and cancer). ...conventional and new materials, such as lipids, polymers, metals, hybrid systems, natural products, agri-food byproducts...Both invasive and non-invasive routes of administration...Special focus is addressed to the establishment of in vitro (including 3D), ex vivo and in vivo experimental models...."
Drug Discovery and Development

Nanotechnology is an idea that most people simply didn't believe.

Ralph Merkle

“There’s Plenty of Room at the Bottom”

Richard Feynman, Nobel 1965
But We should believe…
Pancreatic Cancer

Target-to-Treat...

Facts & Numbers

Pancreatic Cancer Prognosis remains very poor with 5-year survival less than 5% in most reports!
Gemcitabine First-line Treatment

Combinational chemotherapies FOLFIRINOX

Only effective in 23.8% of these cases → (5-year survival rate: 2%)

Surgical resection → Only 20% of patients with early disease diagnosis*

99% 


Leading Therapies

Effect on healthy cells?

Natural products: Source of Lead Molecules

Plectranthus species → Significant biological activities

Parvifloron D (PvD) → Naturally occurring diterpene → Strong cytotoxic properties

Structure of PvD, MW 434 g/mol.

24 h incubation in PBS pH 7.4 (Ph. Eur. 7.0):
25°C = 3.7 ± 0.8 μg/mL
37°C = 4.9 ± 0.3 μg/mL

Natural Related 30%
Natural Derived 27%
Natural Product 6%
Synthetic 37%
How can Nanotechnology be a Strategy?

**Passive targeting**
- Small particle size → Easy escape through leaky endothelial tissue
- Higher tumor accumulation

**Active targeting**
- Ligand attachment to the NPs' surface
- Higher affinity to EGFR than endogenous ligands
- Tyrosine Kinase Inhibitor → Prevent ligand-induced receptor activation

**Objectives**
1. Isolation of PvD from *P. ecklonii* Benth
2. Optimization of a preparation method of PvD-loaded NPs
3. Development of two functionalized PvD-loaded NPs
Extraction of PvD from *P. ecklonii* Benth

**Analysis by TLC**

Extract yield of 14.5%

**Analysis by HPLC-DAD**

PvD as *P. ecklonii* Benth major compound

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**How BSA NPs were produced?**

By a new method

Commonly used conditions:
Albumin solution → addition of the desolvating agent ethanol (500 rpm at room temperature).

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How PvD-loaded BSA NPs were produced?

By a new method

PvD addition

FTIR assays
DSC assays

Characterization of PvD-loaded BSA NPs

<table>
<thead>
<tr>
<th></th>
<th>Mean Diameter (nm) ± SD</th>
<th>Polydispersivity Index (Pdi)</th>
<th>Mean Zeta Potential (mV) ± SD</th>
<th>Loading Capacity (%)</th>
<th>Encapsulation Efficiency (EE) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PvD-loaded BSA NPs</td>
<td>280 (± 86)</td>
<td>&lt;0.370</td>
<td>-42 (± 4)</td>
<td>2.75</td>
<td>78.5</td>
</tr>
<tr>
<td>Empty BSA NPs</td>
<td>393 (± 131)</td>
<td>&lt;0.120</td>
<td>-37 (± 6)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

AFM analysis of PvD-loaded BSA NPs

SEM analysis: PvD-loaded BSA NPs

Haemolytic activity: Below 1%  Safe for I.V. administration  No hemolytic risk!

More than half of PvD was released after 48 h.

• Isolation of PvD from *P. ecklonii* Benth

• Optimization of a preparation method of PvD-loaded NPs

• Development of two functionalized PvD-loaded BSA NPs

How PvD-loaded BSA NPs were functionalized?

### Characterization of functionalized PvD-loaded BSA NPs

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<td>PvD-loaded BSA NPs</td>
<td>280 (± 88)</td>
<td>&lt;0.370</td>
<td>-42 (± 4)</td>
</tr>
<tr>
<td>ERL-functionalized empty BSA NPs</td>
<td>336 (± 91)</td>
<td>&lt;0.090</td>
<td>-36 (± 5)</td>
</tr>
<tr>
<td>ERL-functionalized PvD-loaded BSA NPs</td>
<td>349 (± 59)</td>
<td>&lt;0.450</td>
<td>-39 (± 10)</td>
</tr>
<tr>
<td>CET-functionalized empty BSA NPs</td>
<td>42 (± 5)</td>
<td>&lt;1</td>
<td>-32 (± 6)</td>
</tr>
<tr>
<td>CET-functionalized PvD-loaded BSA NPs</td>
<td>43 (± 4)</td>
<td>&lt;1</td>
<td>-43 (± 4)</td>
</tr>
<tr>
<td>ERL-CET-functionalized empty BSA NPs</td>
<td>502 (± 36)</td>
<td>&lt;1</td>
<td>-32 (± 6)</td>
</tr>
<tr>
<td>ERL-CET-functionalized PvD-loaded BSA NPs</td>
<td>1466 (±155)</td>
<td>&lt;0.560</td>
<td>-48 (± 6)</td>
</tr>
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</table>

ERL conjugated BSA NPs as best formulation!


### Characterization of functionalized PvD-loaded BSA NPs

![AFM images]

Non-functionalized NPs

ERL-functionalized NPs

CET-functionalized NPs

ERL-CET-functionalized NPs

**In vitro efficacy assessment towards tumor cells**

<table>
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<tr>
<th>Samples</th>
<th>BxPC3 cells</th>
<th>PANC-1 cells</th>
</tr>
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<tbody>
<tr>
<td>Free PvD</td>
<td>10.6 ± 3.6</td>
<td>21.7</td>
</tr>
<tr>
<td>Empty BSA NPs</td>
<td>&gt; 30</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>PvD-loaded BSA NPs</td>
<td>&gt; 30</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>ERL-functionalized empty BSA NPs</td>
<td>&lt; 20</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>ERL-functionalized PvD-loaded BSA NPs</td>
<td>21.5 ± 2.2</td>
<td>16.8</td>
</tr>
<tr>
<td>CET-functionalized empty BSA NPs</td>
<td>&gt; 30</td>
<td>&gt; 40</td>
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<tr>
<td>ERL-CET-functionalized PvD-loaded BSA NPs</td>
<td>6.9 ± 1.1</td>
<td>&gt; 40</td>
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Cell viability assays results using MTT, (n=6).

ERL-functionalized BSA NPs: Selected formulation for the following assays...


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**Characterization of functionalized PvD-loaded BSA NPs**

BSA-CET molecular complex

SLAS/ MMER - BSA-E RL complex

Yet BSA-E RL complex was considered energetically stabilized: \( \Delta E_{\text{binding}} \approx -25.355 \) kcal/mol.

BSA-E RL complex \( \Rightarrow \) Functional groups responsible for bioactivity are not involved in covalent bonding

CET is a complex molecule with light and heavy chains \( \Rightarrow \) molecular docking studies will clarify those interactions.

Decrease of cell viability likely results from cell cycle arrest.

Final remarks

1. PvD was efficiently extracted and isolated from *P. ecklonii* Benth.
2. PvD nanoencapsulation in BSA NPs was crucial to allow suitable parenteral dosage form.
3. PvD-loaded BSA NPs were successfully produced and showed high EE%.
4. ERL-functionalized PvD-loaded BSA NPs to be considered for further studies as promising approach for the treatment of Pancreatic Cancer.
5. The Role of Science...

THANK YOU!

http://imed.ulisboa.pt/

Faculdade de Farmácia, Universidade de Lisboa
Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
E-MAIL: catarinareis@ff.ulisboa.pt
T (+351) 217 946 400 (EXT 14244)
F (+351) 217 946 470