Nanotechnology-based Drug Delivery Systems (nanoDDS) against Cancer Stem Cells (CSC)

Fernanda da Silva Andrade
WHY CANCER

• Cancer is a generic term for a large group of diseases that can affect any part of the body.

• The rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs.
Cancer

© 2014 Encyclopædia Britannica, Inc.
Cancer

In 2020:
- 10 million cancer related deaths
- 19.3 million new cases
- New cases are expected to rise by to 29.5 million up to 2040

Estimated number of new cases in 2020, World, both sexes, all ages

Estimated number of deaths in 2020, World, both sexes, all ages
Cancer Statistics

2040 previsions

Estimated number of new cases from 2030 to 2040. Both sexes, age 0-85+.
Cancer Statistics

Spain

Estimated number of new cases from 2020 to 2040, Both sexes, age [0-85+]
All cancers
Spain

<table>
<thead>
<tr>
<th>Year</th>
<th>2020</th>
<th>2040</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>282k</td>
<td>375k</td>
</tr>
</tbody>
</table>

Estimated number of deaths from 2020 to 2040, Both sexes, age [0-85+]
All cancers
Spain

<table>
<thead>
<tr>
<th>Year</th>
<th>2020</th>
<th>2040</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>113k</td>
<td>160k</td>
</tr>
</tbody>
</table>
Top 10 Therapy Areas in 2024, Market Share & Sales Growth

Key growth drivers:
- Upadacitinib (ABBV)
- Xeljanz (PFE)
- Filgotinib (GILD)

Key growth brakes:
- Humira (ABBV)
- Remicade (JNJ)
- Enbrel (AMGN)

Key growth drivers:
- Keytruda (MRK)
- Ibrance (PFE)
- Tagrisso (AZN)
- Imbruvica (ABBV)
- Opdivo (BMY)

Key Anti-neoplastic MAb launches:
- DS-8201 (Daiichi Sankyo) - expected 2020 launch
- Sacituzumab govitecan (IMMU) - expected 2019 launch

Key contributors to CAGR growth:
- Dupixent (SNY)
- Stelara (JNJ)
- Ozanimod (CELG)
- Bardoxolone methyl (Reata Pharmaceuticals)
## Cancer Costs

Worldwide Prescription Drug & OTC Sales by Evaluate Therapy Area (2018 & 2024: Top 10 Categories & Total Market)

<table>
<thead>
<tr>
<th>Rank</th>
<th>Therapy Area</th>
<th>WW Sales ($bn)</th>
<th>CAGR % Growth</th>
<th>WW Market Share</th>
<th>Chg. (+/-)</th>
<th>Rank Chg. (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oncology</td>
<td>123.8</td>
<td>+11.4%</td>
<td>14.3%</td>
<td>+5.0pp</td>
<td>+0</td>
</tr>
<tr>
<td>2.</td>
<td>Anti-diabetics</td>
<td>48.5</td>
<td>+2.9%</td>
<td>5.6%</td>
<td>-0.9pp</td>
<td>+1</td>
</tr>
<tr>
<td>3.</td>
<td>Anti-rheumatics</td>
<td>58.1</td>
<td>-1.0%</td>
<td>6.7%</td>
<td>-2.3pp</td>
<td>-1</td>
</tr>
<tr>
<td>4.</td>
<td>Vaccines</td>
<td>30.5</td>
<td>+6.6%</td>
<td>3.5%</td>
<td>+0.1pp</td>
<td>+1</td>
</tr>
<tr>
<td>5.</td>
<td>Anti-virals</td>
<td>38.9</td>
<td>+1.4%</td>
<td>4.5%</td>
<td>-1.0pp</td>
<td>-1</td>
</tr>
<tr>
<td>6.</td>
<td>Immunosuppressants</td>
<td>14.2</td>
<td>+16.9%</td>
<td>1.6%</td>
<td>+1.3pp</td>
<td>+6</td>
</tr>
<tr>
<td>7.</td>
<td>Dermatologicals</td>
<td>15.8</td>
<td>+12.6%</td>
<td>1.8%</td>
<td>+0.8pp</td>
<td>+4</td>
</tr>
<tr>
<td>8.</td>
<td>Bronchodilators</td>
<td>28.0</td>
<td>+1.6%</td>
<td>3.2%</td>
<td>-0.7pp</td>
<td>-2</td>
</tr>
<tr>
<td>9.</td>
<td>Sensory Organs</td>
<td>22.3</td>
<td>+5.3%</td>
<td>2.6%</td>
<td>-0.1pp</td>
<td>+0</td>
</tr>
<tr>
<td>10.</td>
<td>Anti-coagulants</td>
<td>19.3</td>
<td>+4.1%</td>
<td>2.2%</td>
<td>-0.2pp</td>
<td>+0</td>
</tr>
</tbody>
</table>
Cancer Costs

In 2010:
○ Costs of cancer care: $157 billion

In 2020:
○ Costs of cancer care projected: $174 billion (calculated $200 billion)

In 2030:
○ Costs of cancer care projected: $246 billion

Data from: USA
Medicines under Development

Data from: 2020

* Some medicines are in more than one category.
Medicines and Vaccines in Development for Cancer by Type

- Bladder: 30
- Brain: 67
- Breast: 108
- Colorectal: 47
- Gastric: 33
- Head and Neck: 46
- Hematologic Malignancies: 109
- Kidney: 26
- Leukemia: 145
- Liver: 40
- Lung: 141
- Lymphoma: 129
- Multiple Myeloma: 72
- Myelodysplastic Syndromes: 46
- Ovarian: 49
- Pancreatic: 50
- Prostate: 85
- Sarcoma: 25
- Skin: 59
- Solid Tumors, Unspecified: 84
- Others: 34
- Unspecified: 84

Note: Some medicines may be in more than one category.

Data from: 2020
Nanomedicine

- Passive – Enhanced Permeability and Retention (EPR) effect

- Active Targeting

Nanomedicine

Non Liposomal Drug  Liposomal Drug
Biopharmaceutical classification system (BCS)

- **Class I**: High Solubility, High Permeability
  - Diazepam, Nifedipine, Diltiazem, Verapamil, Quinidine, Midazolam

- **Class II**: Low Solubility, High Permeability
  - Aciclovir, Captopril, Amoxicillin, Penicillin

- **Class III**: High Solubility, Low Permeability
  - Atorvastatin, Cyclosporin, Tamoxifen, Ketoconazole

- **Class IV**: Low Solubility, Low Permeability
  - Paclitaxel, Amphotericin B

Increase solubility and dissolution rate: increase surface area/size reduction; solid solutions/dispersions; solvents/surfactants

Increase trans-epithelial permeability: permeation enhancers; nanotechnology
Doxil® - The first FDA-approved nano-drug (1995)
Stealth liposomes of doxorubicin
Ovarian cancer
AIDS-related Kaposi’s Sarcoma
Multiple Myeloma

Cardiotoxicity dose:
Doxorubicin: 570 mg/m²
Doxil: 785 mg/m²

Abraxane® - FDA-approved nano-drug (2005)
Albumin nanoparticles of paclitaxel
Advanced breast cancer
Advanced non–small cell lung cancer
Advanced pancreatic cancer

Maximum tolerated dose:
Taxol: 175 mg/m²
Abraxane: 260 mg/m²
**Cancer Stem Cells (CSC)**

**CSC properties**
- Support the metastatic spread and tumor resistance reducing overall survival.
- **Self-renewal ability**
- **Multi-lineage differentiation potential**
- **Tumor-forming ability**
- **Non-adhesion survival**
- ↑ **invasion potential**
- Infrequent: 1-10% of total tumor cells
- Abnormal activation of proliferating signaling pathways *(Wnt/β-Catenin, Notch and Hedgehog)*
- **CD44⁺/CD24⁻/low ALDH1, CD133...**
- Resistant to conventional anticancer therapies
- Urgent need to identify efficient **targeted** anticancer therapies

*Urgent need to identify efficient targeted anticancer therapies*
Targeting Cancer Stem Cells (CSC)

CSC-specific drugs of systems

- Targeting cell surface markers of CSC (CD44, ALDH1..)
- Targeting CSC signalling pathways

Advanced Breast Cancer
Development of CSC fluorescently traceable model

Breast Cancer – most prevalent cancer worldwide (WHO).

ALDH1A1-tdTomato reporter system
- Permanent expression of reporter in CSC allowing
  - Isolation of CSC from regular cultures
  - Monitoring of CSC within cell culture

ALDH1A1 is overexpressed in bCSCs

Zileuton™ – Anti-asthmatic drug – inhibitor of ALOX5 – overexpressed in CSC

Gener, P. et al, Nanomedicine. 2020 Feb;24:102106
Polymeric Micelles: Zileuton™ – characterization

- Morphology (TEM and CryoTEM)
- Size and Stability over time (day 0 and day 30)
- Stability in serum

PM-Zil 23.86 ± 0.89 0.226 ± 0.016

PM-Zil 23.93 ± 0.20 0.176 ± 0.004

Gener, P. et al, Nanomedicine. 2020 Feb;24:102106
Polymeric Micelles: Zileuton™ – in vitro results

Increased activity of Zileuton™ when encapsulated in PM

Gener, P. et al, Nanomedicine. 2020 Feb;24:102106
Polymeric Micelles: Zileuton™ — in vivo results

A) Reduction of CSC content in ensuing tumor
B) Abolishment of CTC and reduction of the number of metastasis detected by BLI.

**Diagram:**

- **A)**
  - **MDA-MB-231**
  - **MCF7**
  - Comparison of tdTomato (%) between Vehicle and Zileuton-PM.

- **B)**
  - **Lung BLI Intensity (ph/s/µg) (Mean±SEM)**
  - **MDA-MB-231**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CTC incidence</th>
<th>CSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6/9</td>
<td>35.3</td>
</tr>
<tr>
<td>PM-Zileuton</td>
<td>0/7</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

Gener, P. et al, Nanomedicine. 2020 Feb;24:102106
Advanced Colorectal Cancer
CD44v6 in Colorectal Cancer

- Colorectal Cancer (CRC) – 2nd leading cause of cancer mortality worldwide (WHO).
- Metastatic CRC – non-responsive to treatments due to intrinsic and acquired drug resistance.

In gastrointestinal cancers:
Tumor niche reprograms CD44v6− CRC progenitors into metastatic CD44v6+ CSC.

Wang, et al, Oncotarget, 2017, 8(8), 12866-12876

CD44v6 – Biomarker of CSC

Ma, et al, Cell Death & Disease 2019, 10:30
In vitro validation of CD44v6 as targeting for CSC

qPCR

Flow Citometry

Grey: HCT8 cells  
Red: HT29 cells  
Blue: HCT116 cells

Andrade, F. et al, J Contr. 2021;331(2021):198-121
In vitro validation of CD44v6 as targeting for CSC

**Migration**

CD44v6+ CD44v6-

**Proliferation**

**CD44v6 high expression cells present stemness properties**

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Objective

Kennedy et al, Acta Biomaterialia, 2018, 81, 208-218

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Niclosamide (NCS) present activity against CD44v6+ cells and is more potent than the standard 5-FU.

<table>
<thead>
<tr>
<th>Drug/Cell subpopulation</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU/CD44v6 +</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>5-FU/CD44v6 -</td>
<td>0.99 ± 0.26</td>
</tr>
<tr>
<td>NCS/CD44v6 +</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>NCS/CD44v6 -</td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td>8-Q/CD44v6 +</td>
<td>1.37 ± 0.05</td>
</tr>
<tr>
<td>8-Q/CD44v6 -</td>
<td>0.57 ± 0.14</td>
</tr>
</tbody>
</table>


Andrade, F. et al, J Contr. 2021;331(2021):198-121
Drug Selection

Niclosamide (NCS) present activity against CD44v6+ cells and is more potent than the standard 5-FU.

<table>
<thead>
<tr>
<th>Drug/Cell subpopulation</th>
<th>IC_{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU/CD44v6 +</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>5-FU/CD44v6 -</td>
<td>0.99 ± 0.26</td>
</tr>
<tr>
<td>NCS/CD44v6 +</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>NCS/CD44v6 -</td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td>8-Q/CD44v6 +</td>
<td>1.37 ± 0.05</td>
</tr>
<tr>
<td>8-Q/CD44v6 -</td>
<td>0.57 ± 0.14</td>
</tr>
</tbody>
</table>


Andrade, F. et al, J Contr. 2021;331(2021):198-121
Polymeric Micelles Design
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean Diameter (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
<th>AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>23.2 ± 1.1</td>
<td>0.332 ± 0.067</td>
<td>-0.7 ± 0.3</td>
<td>N.A.</td>
</tr>
<tr>
<td>PM-NCS</td>
<td>24.4 ± 0.7</td>
<td>0.206 ± 0.011</td>
<td>-3.4 ± 2.8</td>
<td>99.8 ± 4x10^5</td>
</tr>
<tr>
<td>PM-NCS:Fab</td>
<td>29.7 ± 1.2</td>
<td>0.338 ± 0.055</td>
<td>-6.5 ± 0.7</td>
<td>99.7 ± 4x10^5</td>
</tr>
</tbody>
</table>

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Drug release dependent on pH
Preferential release at tumor microenvironment

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Internalization: Fab-CD44v6 PM surface modification increase internalization in CD44v6+ (CSC) population

Andrade, F. et al, J Contr. 2021;331(2021):198-121
**Efficacy**: NCS encapsulation into PM increase its efficacy and Fab presence increase the efficacy in CD44v6+ (CSC) population

---

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Polymeric Micelles-Niclosamide:CD44v6 Fab – in vitro results

**Efficacy**: PM-NCS:Fab impairs colonpheres formation

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Hemocompatibility: PM-NCS: Fab are hemocompatible and well tolerated.

**Hemolysis**

NV: < 5% of hemolysis

**Plasma Coagulation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>PT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Patient Control</td>
<td>12.4 ± 0.0</td>
<td>15.9 ± 0.0</td>
</tr>
<tr>
<td>Sick Patient Control</td>
<td>19.45 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>Negative Control</td>
<td>11.65 ± 0.1</td>
<td>17.3 ± 0.3</td>
</tr>
<tr>
<td>Control PBS</td>
<td>12.15 ± 0.1</td>
<td>17.45 ± 0.1</td>
</tr>
<tr>
<td>Control Methanol</td>
<td>13.1 ± 0.1</td>
<td>15.3 ± 0.1</td>
</tr>
<tr>
<td>NCS free</td>
<td>13.8 ± 0.1</td>
<td>19.05 ± 0.2</td>
</tr>
<tr>
<td>PM-NCS</td>
<td>12.35 ± 0.2</td>
<td>15.15 ± 0.1</td>
</tr>
<tr>
<td>PM-NCS:Fab</td>
<td>12.4 ± 0.1</td>
<td>15.3 ± 0.3</td>
</tr>
</tbody>
</table>

NV: PT ≤ 13.4s and TT ≤ 21s

Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Polymeric Micelles-Niclosamide:CD44v6 Fab — *in vivo* results

**In vivo tumor accumulation**

- Non-treated Bkg control
- CD44 targeted MP-DiR 0.8 mg DiR/kg
- “without” tumor
- 24 h
- 48 h

**Ex vivo tumor accumulation**

- PM-NCS:Fab reach and accumulates in tumor for at least 48h

**Ex vivo organs accumulation**

- Biodistribution: PM-NCS:Fab reach and accumulates in tumor for at least 48h

NOD-SCID mice bearing subcutaneous HCT116 tumors

Andrade, F. et al, J Contr. 2021;331(2021):198-121

Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis
Survival

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Number of doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NCS free</td>
<td>0.5</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>60%</td>
</tr>
<tr>
<td>PM-NCS</td>
<td>3</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>33%</td>
</tr>
<tr>
<td>PM-NCS:Fab</td>
<td>3</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0%</td>
</tr>
</tbody>
</table>

Body Weight

- PM-NCS:Fab 3mg/kg
- PM-NCS:Fab 4mg/kg
- PM-NCS 3mg/kg
- PM-NCS 4mg/kg

NOD-SCID mice bearing subcutaneous HCT116 tumors

Experiment conducted with the collaboration of FVPR/U20 ICTS Nanbiosis

Andrade, F. et al, J Contr. 2021;331(2021):198-121
**in vivo Efficacy**: PM-NCS:Fab decrease the tumor circulating cells and are a promising therapeutic adjuvant of CRC treatment to prevent development of mCRC.

### Tumor circulating cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/Kg)</th>
<th>% of dose injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCS free</td>
<td>0.5</td>
<td>45.33 ± 0.54</td>
</tr>
<tr>
<td>PM-NCS</td>
<td>4</td>
<td>5.69 ± 0.06</td>
</tr>
<tr>
<td>PM-NCS:Fab</td>
<td>4</td>
<td>1.13 ± 2.52</td>
</tr>
</tbody>
</table>

NOD-SCID mice bearing subcutaneous HT29 tumors

Andrade, F. et al, J Contr. 2021;331(2021):198-121
Conclusions

Cancer Stem Cells are responsible for resistance to treatment and tumor relapse.

Targeting CSC through nanomedicine improve treatment outcomes through reduction of circulating tumor cells and metastasis.
Aknowlegments

Drug Delivery & Targeting (DDT)
Area of Functional Validation & Preclinical Research (FVPR)

- Diana Rafael
- Francesc Martinez
- Joquín Seras-Franzoso
- Zamira V Díaz-Ríascos
- Júlia German
- Diego Baranda
- Marc Moltó
- Begoña Fernández
- Belén García
- Sandra Mancilla
- Laura García
- Simó Schwartz Jr.
- Ibane Abasolo

Collaborations

Bruno Sarmento
Marika Nestor
Thank You!

Any questions?

fernanda.silva@vhir.org
fernanda.dasilva@ub.edu