The Double Vector System
First Observation

iGEM SupBiotech Paris Project

1st Observation
Double targeting for gene therapy
### First Observation: Problem identification

**Identified Problem: The Genome**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Galenic</th>
<th>Vectorization</th>
<th>DVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Disease</td>
<td></td>
<td>Insertion of foreign genome</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td>Genome modification</td>
<td></td>
</tr>
<tr>
<td>Genetic disease</td>
<td></td>
<td>Dysfunctional genome</td>
<td></td>
</tr>
</tbody>
</table>

- **Viral Disease**: Insertion of foreign genome
- **Cancer**: Genome modification
- **Genetic disease**: Dysfunctional genome
First Observation:
Action on the genome

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>Chemical drugs</td>
<td>➞ No specificity so Toxic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-drugs (protein, siRNA, etc)</td>
<td>➞ Specific but Temporary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene insert</td>
<td>➞ Highly specific and Permanent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Best agent: gene sequence
First Observation:

**Gene characteristics**

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Very low toxicity</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Need to reach the nucleus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low stability outside the cell</td>
<td></td>
<td></td>
<td>Protection</td>
</tr>
</tbody>
</table>

Specific sequence
First Observation:
How to protect it?

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<tbody>
<tr>
<td>• <em>Ex vivo</em> Gene action</td>
<td>➤ Personal treatment</td>
<td>➤ Low protection</td>
<td>➤ Encapsulation</td>
</tr>
<tr>
<td>• Classical galenic</td>
<td></td>
<td>COST</td>
<td>DEGRADATION</td>
</tr>
<tr>
<td>• Vectorization</td>
<td>➤ GENE PROTECTION</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ideal galenic**: gene vectorization
First Observation :
What is a vector ?

• Components:  
  – Reservoir  
  – Targeting system  
  – Stealth system

• Types of vectors :  
  – Virus  
  – Phages  
  – Bacteria  
  – Lipidic nanoparticles  
  – Polymer nanoparticles
• The 6 major issues of vectorization

<table>
<thead>
<tr>
<th></th>
<th>Stability</th>
<th>Toxicity</th>
<th>Target</th>
<th>Passage through membranes</th>
<th>Immune system resistance</th>
<th>Industrialization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>Good</td>
<td>Null</td>
<td>Cell</td>
<td>Very good</td>
<td></td>
<td>Difficult</td>
</tr>
<tr>
<td>Phages</td>
<td>Good</td>
<td>Null</td>
<td>Cell</td>
<td>Very low</td>
<td></td>
<td>Easy</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Good</td>
<td>Low/High</td>
<td>Tissue</td>
<td>Very low</td>
<td></td>
<td>Easy</td>
</tr>
<tr>
<td>Lipidic NPs</td>
<td>Low</td>
<td>Null</td>
<td>Cell</td>
<td>Good</td>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>polymer NPs</td>
<td>Good</td>
<td>Medium</td>
<td>Cell</td>
<td></td>
<td></td>
<td>Medium</td>
</tr>
</tbody>
</table>

➡ Technological barrier
First Observation : Solution!

- Each issue can be resolved by a vector
- Three types of vectors have a genome

- Synthetic Biology :
  Possible to mix genomes

➡️ Solution : the Double Vector System (DVS)
First Observation:
Double Vector System

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<tr>
<td><strong>Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Tissue targeting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Immune system resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Produces phages under control</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tissue Vector:**
Specific bacterium

**Cell Vector:**
Recombinant phage

**Characteristics:**
- Cell targeting
- Passage through membranes
- Encapsidates an exogenous plasmid
First Observation:
Double Vector System

Tissue Vector:

- *Mycobacterium avium* subspecies *avium*

  - (a) Phage genome
  - (b) System of phage production control
First Observation:
Control of the Cell vector synthesis

No Doxycyclin

- TetR
- RBS
- tetP/O
- RBS
- LacI

Doxycyclin

- TetR
- RBS
- tetP/O
- RBS
- LacI

cl inhibition ➞ Production of recombinant phages
First Observation:
Double Vector System

**Cell Vector:**
- Lambda Phage
- Controlled lysogeny
- Viral Proteins on the capsid

![Diagram of cell vector system](image)

- pL: Lambda genome
- Lac P/O: cl repressor
- pR: Lambda genome
- Targeting Protein
- Protein J
- Lambda genome
- Polypeptide III
- Protein D
- Kanamycin Cassette

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13
Type III polypeptide

- From adenovirus penton base
- Fused with the D protein
- Contain RGD motives recognize integrins
- Clustering of integrins
- Internalization of the cell vector into the eukaryotic cell
First Observation :

Double Vector System

**Therapeutic Plasmid:**

- COS sequence for encapsidation
- DTS sequence for nucleus targeting
- Sequence of therapeutic aim

Azithromycin Cassette

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DVS Project
First Observation:
**DVS advantages**

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</thead>
<tbody>
<tr>
<td>Double targeting</td>
<td>Highly specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penetration into tissues then into cells</td>
<td>Vectorized gene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encapsulated gene</td>
<td>Protected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living organism</td>
<td>Stability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prokaryotic organisms</td>
<td>Low Cost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune system resistance</td>
<td>Low clearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of a dangerous bacterium</td>
<td>Hazardous</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Injection of doxycyclin ➔ induces bacterial lysis
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2nd observation

Cancer is only wrong information!
Second Observation: Cancer

- Cell containing an issue
- Problem of genetic information
- Wrong information blocking a system
- Often apoptosis system

Solution: To bring the right information!
Right information is the non-mutated version of a gene.

What happens if you bring this information?

Cell can activate the gene pathway.

Anticancer Solution: To provide the missing genetic information
Second Observation :
How to control the genetic information?

• Promoters control genetic information response.

• Genetic information is expressed only if required.

⇒ Providing wild type promoter allows cells to choose the right regulation
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DVS application on Lung Cancer
Implementation:

DVS versus Lung Cancer

Tissue vector:
• Pulmonary tropism

Cell vector:
• Unspecific targeting

Therapeutic plasmid:
• “Wild type” version of tumor suppressor gene + “wild type” promoter
Penetration into the lung

Macrophage infected by Tissue Vector

Macrophage

Tissue Vector

Lung

Blood vessel
Dispersion in the Lung
Implementation: Tissue Targeting

- 3 murine models: immunodeficient, normal, cancerous.

<table>
<thead>
<tr>
<th>Day -7</th>
<th>Day 0</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Inoculation of fibroblastic cancer cells</td>
<td>Inoculation of $10^6$ CFU.ml$^{-1}$ of ( M. avium ),</td>
<td>Mice sacrifice, organs extraction and cell suspension from</td>
</tr>
<tr>
<td>- subcutaneously</td>
<td>- IV route in the tail</td>
<td>the lungs, tumors</td>
</tr>
<tr>
<td>- normal mice</td>
<td>- 3 mice models</td>
<td></td>
</tr>
</tbody>
</table>

- Cell suspension is analyzed by flow cytometry.
  Size and granularity: eukaryotic murine cells $\neq M. avium$
Implementation:
Tissue Targeting

<table>
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<tr>
<th>Presentation</th>
<th>Tissue</th>
<th>Cell</th>
<th>Apoptosis</th>
</tr>
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</table>

Nod Scid mouse:  
Black 6 mouse:  
Cancerous Black 6 mouse:
### Implementation: Tissue Targeting

<table>
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<tr>
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- **Our experiments**
  - Too short to prove the presence of *M. avium* in lung
- **Literature**
  - Proven many times

→ **DVS can be used on lung cancer**
Cell Vector Dispersion and Cancer Cells Targeting
Membrane Receptor Targeting
Cell Vector Internalization
Paradigm of therapeutic plasmid release is not elucidated

Insertion of the gene into the target cell
Implementation:
Cell Targeting

- Wild Type Lambda phage

- Recombined Lambda phage with penton bases on D proteins

Source: Stefania Piersanti et al., 2004
Implementation:

Cell Targeting

- Fusion of adenovirus 5 penton base with D protein
  - From a plasmid coding for the virus
  - From Lambda phage genome
Implementation: Cell Targeting

- Fusion protein not built in time.
- Capacity to infect eukaryotic cells and to quit endosomes.
- RGD fragment alone: higher efficiency of interaction with integrins of eukaryotic cells.
- In our application: use of the complete sequence

Possible to use a recombined Lambda phage to insert therapeutic genes
NLS recruitment by DTS sequence
Nuclear insertion
Therapeutic gene expression

Chromosome

p53 wt

Apoptosis pathway induction

Nucleus

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Implementation:

Apoptosis Induction

- **Cell population:** Prostatic cancer mutated p53 DU-145

- Kinetic monitoring of apoptosis induction: annexin V assay every 6 hours for 48 hours after electroporation
  - Control population
  - pcDNA3 CMV+p53wt
Implementation: Apoptosis Induction

• Apoptosis detection

Source: Chunlin Yang et al. Adenovirus-mediated Wild-Type p53 Expression Induces Apoptosis and Suppresses Tumorigenesis of Prostatic Tumor Cells 1995

⇒ Specific fixation of the annexin V coupled with a fluorophore and analysis by flow cytometry.
Application of DVS in non small cell lung cancer is confirmed
Conclusion

Lung: natural tropism of *Mycobacterium avium*

DVS: reactivation of apoptotic pathway in tumor cells by bringing the wild-type version of tumor suppressor genes.

DVS is a straight alternative to current treatments.
To confirm the concept on lung cancer

To adapt it on other diseases
Our compilation system is not easy but really useful!

This new concept brings new outlooks for synthetic biology
Thank you for your attention!

Have you got any questions?
Acknowledgements

- **Our instructors:**
  - Pierre Ougen, Project director at Sup’Biotech Paris
  - Gavin Browne, in charge of international relationship

- **Scientists:**
  - Lluis M Mir
  - Karim Benhioud
  - Bassim Al-sakere
  - Brian D Roberston
  - Nicolas Veziris
  - Srinivas Kaveri
  - Vladimir Lazar
  - Benyoussef Naimi
  - Franck Griceli
  - Claudie Bourgaux
  - Claudia Nobrega
  - Jean-Yves Trosset
  - And the others...

- **Our sponsors:**

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DVS Project
Annexs
Control of the Cell vector synthesis

No Doxycyclin

Doxycyclin

cl inhibition ➔ Production of recombinant phages
About:
Mycobacterial membrane
About:
Side effects of bacterial lysis
• Insertion sequences: insertion of both the phage genome and the control plasmid into the bacterial genome

• Just one plasmid, the therapeutic one, replicated and transmitted to the daughter bacterial cells
About:
Encapsidation mechanism of COS sequences

Genes | Galenic | Vectorization | DVS
• Bioluminescence protocol, which allows the real-time monitoring.
• Good reporter system to analyze the mycobacterial implantation and the clearance \textit{in vivo}.
Exemple of obtained images by the CCD camera:

Tropisms: Implantation in different tissue

Quantification:

Extracted from: Bioluminescent Monitoring of In Vivo Colonization and Clearance Dynamics by Light-Emitting Bacteria, Brian D.R.
• Introduction of the fusion protein in a BioBrick plasmid.

  + Antibiotic resistance
to confirm the transfection into bacteria

  + GFP reporter gene with CMV promoter
to confirm the transfection into eukaryotic cells
About:
Therapeutic plasmid

- Presentation
- Tissue
- Cell
- Apoptosis

Healthy cell vs Cancer cell:

- Wild type gene
  - Cell promoter
  - Functional protein

- Mutated gene
  - Cell promoter
  - Dysfunctional protein

- Genomic DNA
- Synthetic DNA

No Apoptosis:
- No plasmid expression

Apoptosis:
- Functional protein
- Death signals
## About:

**Treatment efficiency Modeling**

- \( Nc(t) \), the number of cancer cells depending on time,
- \( V(t) \), tumor volume,
- \( V1 \) and \( V2 \), two tumors volumes respectively times \( t1 \) and \( t2 \),
- \( Vcc \), the volume of a cancer cell,
- \( Nbi \), the number of injected tissue vectors,
- \( Pp \), the lung percentage of tissue vectors relative to the injected dose,
- \( DTB \), the doubling time of tissue vector,
- \( tinj \), injection time of the tissue vectors,
- \( Npl \), the number of cell vectors released by bacteria.
- \( \lambda \), phage efficiency

\[
Nc(t) = \left[ \frac{V(t_2) + \left( \frac{\ln(V_2 - V_1)}{t_2 - t_1} \right)(t - t_2)}{Vcc} \right] - \left[ \left( \frac{Nbi \times Pp}{100} \times 2 \left( \frac{t-tinj+DTB}{DTB} \right) \right) \times Npl \right] \times \lambda
\]
iGEM for us!