Teacher Guide: Vector Selector

ACTIVITY OVERVIEW

Abstract:
Acting as employees of a vector supply company, students research a disease that is a candidate for gene therapy and determine the best vector to use. They must then present their choice and the reasoning behind it in order to help "sell" the vector to a biotech company developing a therapy for this disease.

Module:
Gene Therapy: Molecular Bandage?

Prior Knowledge Needed:
DNA structure and function; mutation; gene expression; basic cell structure and function; viral ligands bind to receptors of specific cell types; *ex vivo*; *in vivo*

Key Concepts:
Vectors; *in vivo*; *ex vivo*; cell targeting; immune response

Materials:
Student Pages, computers and/or library for research (optional), media equipment as necessary for student presentations, art supplies as needed for student presentations

Appropriate For:
Ages: 15 - 20
USA grades: 10 - 14

Prep Time:
30 minutes to review gene therapy scenarios and copy

Class Time:
2-4 class periods depending on student research and presentations

Activity Overview Web Address:
http://gslc.genetics.utah.edu/teachers/tindex/
overview.cfm?id=vselector

Other activities in the *Gene Therapy: Molecular Bandage?* module can be found at:

http://gslc.genetics.utah.edu/teachers/tindex/

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I. PEDAGOGY

A. Learning Objectives
- Students will design a solution to a problem by applying their knowledge of gene therapy and vectors.
- Students will think critically about the properties of various vectors.
- Students will communicate their thought process and rationale in choosing a specific vector for a given gene therapy scenario.

B. Background Information

Activity Information
This activity is designed for use after students have studied the different types of vectors used in gene therapy and the advantages/disadvantages of each type. Students also need a basic understanding of gene therapy delivery techniques (*ex vivo* versus *in vivo*), and the genetic disorder presented in their assigned scenario. The "Vector Types" table (page S-1), and the online portion of the *Gene Therapy: Molecular Bandage?* module on the Genetic Science Learning Center website (http://gslc.genetics.utah.edu) will provide most of the necessary background information. Basic information about the specific genetic disorder is provided in the scenarios. Also see *Additional Resources* in the online portion of the *Gene Therapy: Molecular Bandage?* module for more information on specific disorders.

There is a lot of flexibility in the type of presentation students may be assigned to give depending on available time, technology and desired complexity of presentation. Students may also work individually or in groups. Some ideas include:

- Group discussions, then oral reports about what vector they would choose
- Oral "sales pitch" including visuals
- Poster or brochure to market their vector
- Written prospectus describing the vector and its use in gene therapy
- Multi-media presentation

A rubric for presentations (page S-7) and a vector choices answer key (page 6) is provided for your convenience.

Five different gene therapy situations are included in this activity. You may wish to assign different situations to each group of students, or assign the same situation to all groups.

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Scientific Information

Scientists refer to DNA delivery vehicles as vectors. Each vector is designed to target specific cells. Traditionally, vectors have been derived from viruses, including retroviruses, adenoviruses, adeno-associated viruses, and herpes simplex viruses. Components of the virus that cause disease are removed and the gene the researcher wants to be delivered is inserted. The transfer of DNA into a cell by a modified infectious virus is called *transfection*. This distinguishes it from *infection*, by which a virus inserts its own DNA or RNA into a cell. Non-viral gene delivery approaches being examined include the use of liposomes, (lipid-based pockets that can carry plasmid DNA) or simply naked DNA with no carrier. Each vector and method of delivery varies depending on the specific disorder the therapy aims to treat. Three important factors to consider when choosing a vector for gene therapy are:

1. Will the gene fit into the vector?
2. Will the vector target the right type of tissue?
3. Does the vector work within the appropriate cell cycle stage to affect the disease?

There are two basic ways to deliver a gene into a group of cells in a patient's body. The first is to inject the vector into the body and specifically target those cells. This is called an *in vivo* approach.

The second way, called *ex vivo*, is to deliver the gene to cells while they are outside the body using the following procedure:

1. Isolate the desired cells from the body.
2. Culture the cells in a Petri dish, in the laboratory.
3. Deliver the gene to the cells (using an appropriate vector), activate it, and make sure it integrates properly into the cells.
4. Put the genetically modified cells back into the proper place in the body, ensure that they survive there, and let them get to work.

Each of the vectors and methods has its strengths and limitations for gene delivery. Success in gene therapy depends on the efficient delivery of the correct gene to the correct cells in the correct tissue. Thus, the selection of a particular vector is highly dependent upon its properties and the characteristics of the disease undergoing treatment. See Teacher Reference (page 6) for information about how each vector type would or would not work for each situation included in this activity.

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C. Teaching Strategies
1. Timeline

- One day before activity:
    - Decide what type of presentation to assign (see suggestions in Background, page 1)
    - Decide which situation or situations to use
    - Copy situations (Student Pages S-2 through S-7), one per student or group
    - Copy the Assessment Rubric (page S-7) and Vector Types Table (page S-1), make one copy per student (optional)

- Day of activity:
    - Divide class into groups
    - Hand out copies of situations (Pages S-2 through S-6)
    - Provide time for students to complete the task and prepare a presentation

2. Classroom Implementation

- Introduce the overall assignment background to the class (located at the top of each situation).
- Divide the class into groups.
- Assign each group a specific gene therapy situation.
- Explain the presentation requirements.

    ◦ If using it, hand out the presentation assessment rubric (Page S-7).

- Hand out copies of the Vector Types Table (page S-1).
- Provide time for groups to research and prepare their presentations.
- Provide time for groups to present.

3. Extensions

- Have students research gene therapies currently being studied and their relative success.

4. Adaptations

- You may want to have students fill out the blank Vector Types table (S-8) on their own. This information is located online in the Vector Toolbox found in the "Tools of the Trade" section of the _Gene Therapy: Molecular Bandage?_ module on the Genetic Science Learning Center website (http://gslc.genetics.utah.edu). You may project the Vector Toolbox to the whole class or bring your class to the computer lab to visit this section individually.

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- Use this activity as a prompt for students to research basic information about gene therapy on their own.
- Have students carry out this activity individually, rather than in groups.

5. Assessment Suggestions

- Use the presentations as an assessment. (See page S-7 for rubric)
- Because this activity requires students to use what they know about gene therapy, it may be used as an overall assessment for a gene therapy unit.

6. Common Misconceptions

- Students sometimes think that gene therapy *replaces* a faulty gene with a functional copy. It is important that they understand this is not the case. Gene therapy endeavors to *add* a functional copy of the gene in the hope that its function will counteract the effect of the faulty gene.

II. ADDITIONAL RESOURCES

A. Activity Resources linked from the online Activity Overview at:
http://gslc.genetics.utah.edu/teachers/tindex/overview.cfm?id=vselector

- <u>Website</u>: *Gene Therapy: Molecular Bandage?* - information about the idea behind gene therapy, techniques, current status of trials and other related issues.

- <u>Website</u>: *Gene* Therapy: Molecular Bandage? - *Additional Resources* - information about the specific genetic disorders addressed in this activity's scenarios.

- <u>Website</u>: *Genetic Disorder Corner: What is NF 1*? - information about Neurofibromatosis 1.

III. MATERIALS
A. Detailed Materials List

- Student Pages S-1 through S-6, one per student or groups
- Assessment rubric, page S-7 (optional)
- Computers (optional) if providing time for online research
- Art supplies (optional) as necessary for presentations
- Media equipment (optional) as necessary for presentations

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IV. STANDARDS

A. U.S. National Science Education Standards

<u>Grades 9-12</u>:
- Content Standard C: Life Science - The Molecular Basis of Heredity; in all organisms, the instructions for specifying the characteristics of the organism are carried in DNA.

B. AAAS Benchmarks for Science Literacy

<u>Grades 9-12</u>:

- The Living Environment: Heredity - genes are segments of DNA molecules; inserting, deleting, or substituting DNA segments can alter genes; an altered gene may be passed on to every cell that develops from it; the resulting features may help, harm, or have little or no effect on the offspring's success in its environment.
- The Human Organism: Physical Health - faulty genes can cause body parts or systems to work poorly.
- The Designed World: Health Technology - knowledge of genetics is opening whole new fields of health care.

C. Utah Secondary Science Core Curriculum

Intended Learning Outcomes for Ninth to Twelfth Grade Science
   Students will:
   4. Demonstrate Awareness of the Social and Historical Aspects of Science
    a. Provide relevant data to support their inferences and conclusions

<u>Biology (9-12)</u>
   Standard 4: Students will understand that genetic information coded in DNA is passed from parents to offspring by sexual and asexual reproduction. The basic structure of DNA is the same in all living things. Changes in DNA may alter genetic expression.
   Objective 3: Explain how the structure and replication of DNA are essential to heredity and protein synthesis.
      - Research, report, and debate genetic technologies that may improve the quality of life (e.g., genetic engineering, cloning, gene splicing).

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V. CREDITS

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Teacher Reference: Vector Choices Answer Key

	RETROVIRUS	ADENOVIRUS	ADENO-ASSOCIATED VIRUS	HERPES SIMPLEX VIRUS	LIPOSOME	NAKED DNA
Situation One NFS Co.	**NOT POSSIBLE** *The NF1 gene is encoded by 8,454 base pairs. The gene is too large to fit in this type of vector.*	**NOT POSSIBLE** *The NF1 gene is encoded by 8,454 base pairs. The gene is too large to fit in this type of vector.*	**NOT POSSIBLE** *The NF1 gene is encoded by 8,454 base pairs. The gene is too large to fit in this type of vector.*	**NOT POSSIBLE** *Although the NF1 gene contributes to parts of the nervous system, the HSV will not integrate into the host cell's genome. An immune response is possible.*	**NOT POSSIBLE** *Vector has no cell target specificity*	**NOT POSSIBLE** *The NF1 gene is encoded by 8,454 base pairs. The gene is too large to fit in this type of vector.*
Situation Two CellMart	**POSSIBLE** *The vector can be introduced into blood stem cells, which divide frequently. However, the gene may integrate into the host cell's genome in random locations and disrupt another gene. An immune response is possible.*	**POSSIBLE** *The vector can be introduced into blood stem cells, which divide frequently. However, the gene might cause an immune response.*	**NOT POSSIBLE** *The blood-clotting gene is encoded by 7,350 base pairs. The gene is too large to fit in this type of vector.*	**NOT POSSIBLE** *Blood clotting factors are not part of the nervous system. They are part of the circulatory system.*	**NOT POSSIBLE** *Vector has no cell target specificity*	**NOT POSSIBLE** *Vector has no cell target specificity.*
Situation Three Oma, Inc.	**NOT POSSIBLE** *Vector infects only dividing cells. Lung cells divide slowly, if at all.*	**POSSIBLE** *The genes of this vector do not integrate into the genome. Repeated treatments would be necessary. An immune response is possible.*	**POSSIBLE** *Vector is effective at entering lung cells, and typically does not cause an immune response.*	**NOT POSSIBLE** *Lung cells are not part of the nervous system. They are part of the respiratory system*	**NOT POSSIBLE** *Vector has no cell target specificity.*	**NOT POSSIBLE** *Vector has no cell target specificity.*
Situation Four SBG, Inc.	**POSSIBLE** *The virus infects dividing cells, but integrates into the host cell's genome in random locations, which might disrupt another gene. An immune response is possible.*	**POSSIBLE** *The virus does not integrate into the genome, and so, repeated treatments would be necessary. Also, may cause an immune response.*	**POSSIBLE** *Vector infects cells efficiently, usually integrates into a specific, non-disruptive location, and typically does not cause an immune response.*	**NOT POSSIBLE** *T-cells are not part of the nervous system. They are part of the immune system.*	**NOT POSSIBLE** *Vector has no cell target specificity. Enters cells less effectively than viruses.*	**NOT POSSIBLE** *Vector has no cell target specificity. Enters cells less effectively than viruses.*
Situation Five CF, Inc.	**NOT POSSIBLE** *Vector infects only dividing cells. Lung cells divide slowly, if at all*	**POSSIBLE** *The vector is effective at entering non-dividing cells like lung cells, but can cause an immune response.*	**POSSIBLE** *The vector is effective at entering non-dividing cells like lung cells, and typically does not cause an immune response.*	**NOT POSSIBLE** *Lung cells are not part of the nervous system. They are part of the respiratory system.*	**NOT POSSIBLE** *Vector has no cell target specificity. Enters cells less effectively than viruses.*	**NOT POSSIBLE** *Vector has no cell target specificity. Enters cells less effectively than viruses.*

Vector Types

	RETROVIRUS	ADENOVIRUS	ADENO-ASSOCIATED VIRUS	HERPES SIMPLEX VIRUS	LIPOSOME	NAKED DNA
How the vector carries genetic material	In the form of RNA rather than DNA. The most famous is Human Immunodeficiency Virus (HIV), which causes AIDS.	Double-stranded DNA. The common cold virus.	Single-stranded DNA	Single-stranded DNA	Plasmid DNA packaged into miniature lipid–based pockets that can fuse to the cell's own membranes, The DNA is released and transported into the nucleus	Plasmid DNA molecule all by itself that can be taken up by some cells, and transported into the nucleus
Maximum length of DNA that can be inserted into vector	8, 000 base pairs	7,500 base pairs	5,000 base pairs	20,000 base pairs		
ADVANTAGES	+ Infects only dividing cells	+ Infects both dividing and non-dividing cells very effectively + Possible to target specific cell types by engineering proteins on the virus surface to recognize special proteins on the target cell's surface	+ Does not cause illness in humans + Infects a wide range of dividing and non-dividing cell types very effectively + Need the assistance of a "helper" virus to replicate themselves inside cells + Possible to target specific cell types by engineering proteins on the virus surface to recognize special proteins on the target cell's surface + Integrates into the host cell's genome; 95% of the time, it will integrate into a specific region on Chromosome 19, greatly reducing the chance that integration will disrupt the function of other genes in the cell + Typically will not cause an immune response	+ Infects cells of the nervous system + Will not integrate into the host cell's genome, but is a circular piece of DNA that replicates when the cell divides; will stay in the cell's nucleus for a long time + Will not disrupt the function of other genes in the host cell	+ Will not generate an immune response + Better suited for *ex vivo* gene therapy approaches	+ Will not generate an immune response + Generally not toxic
DISADVANTAGES	- Integrates into the host cell's genome in random locations; might integrate into a place where it disrupts another gene - Can cause an immune response	- Will not integrate into the host cell's genome; the cell will discard the virus and gene activation will be lost - Can cause an immune response	- 5% chance of integrating and disrupting the function of other genes in the cell	- Can cause an immune response in the patient	- Not specific for any cell type - Enter, cells far less effectively than viruses - Will not integrate into the host cell's genome	- Not specific for any cell type - Enter, cells far less effectively than viruses - Will not integrate into the host cell's genome - Unstable in most body tissues

vector selector

You are a researcher at Advanced Bio Delivery Systems (ABDS), a company that supplies vectors for use in gene therapy. The marketing department has come to you for assistance. They would like to sell the vectors made by ABDS to biotech companies pursuing specific gene therapies.   It is your job to research the disorders these biotech companies are targeting and decide on a vector that has potential to work. You will be presenting your findings to the marketing department for approval. There may be more than one vector possibility, so your job is to choose what you feel is the best one and defend your position. Your company stands to make a considerable profit if you are able to "sell" their vector for use in research.
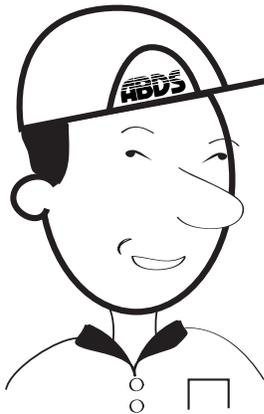
Some things to consider:
   - What are important characteristics of the disease being investigated (genetic defect, body system affected…)?
   - Will the gene fit into the vector?
   - What types of cells would the therapy need to target (dividing/non-dividing)?
   - What will happen to the genetic material once it is inserted into the cells?
   - Does gene expression need to last a long time?
   - What kinds of safety issues need to be considered before recommending a vector type?

Situation I

The ABDS marketing department has identified NFS Co. as a biotech company that is developing gene therapies. NFS Co. is focusing on neurofibromatosis type 1 which is caused by a mutation in the *NF1* gene (8,454 base pairs) on chromosome 17.   This disorder causes tumors to grow on nerves, skin changes and bone deformities. NFS Co. is looking for a vector that could target non-dividing nerve cells and insert a transgene stably into the chromosome.

Prepare a sales pitch to suggest a vector.

vector selector

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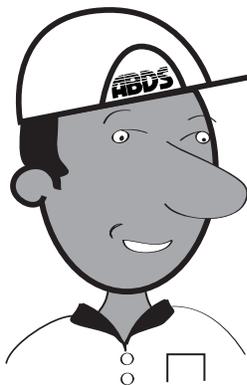
Some things to consider:
- What are important characteristics of the disease being investigated (genetic defect, body system affected…)?
- Will the gene fit into the vector?
- What types of cells would the therapy need to target (dividing/non-dividing)?
- What will happen to the genetic material once it is inserted into the cells?
- Does gene expression need to last a long time?
- What kinds of safety issues need to be considered before recommending a vector type?

Situation 2

The ABDS marketing department has identified CellMart as a biotech company that is developing gene therapies. CellMart is searching for a vector to treat hemophilia, a bleeding disorder caused by a deficiency in one of the blood clotting factors. This results in excessive bleeding. CellMart intends to isolate blood stem cells, which divide frequently, and introduce the vector *ex vivo*. The gene they plan to use is 7,350 base pairs long.

Prepare a sales pitch to suggest a vector.

vector selector

You are a researcher at Advanced Bio Delivery Systems (ABDS), a company that supplies vectors for use in gene therapy. The marketing department has come to you for assistance. They would like to sell the vectors made by ABDS to biotech companies pursuing specific gene therapies. It is your job to research the disorders these biotech companies are targeting and decide on a vector that has potential to work. You will be presenting your findings to the marketing department for approval. There may be more than one vector possibility, so your job is to choose what you feel is the best one and defend your position. Your company stands to make a considerable profit if you are able to "sell" their vector for use in research.

Some things to consider:
- What are important characteristics of the disease being investigated (genetic defect, body system affected…)?
- Will the gene fit into the vector?
- What types of cells would the therapy need to target (dividing/non-dividing)?
- What will happen to the genetic material once it is inserted into the cells?
- Does gene expression need to last a long time?
- What kinds of safety issues need to be considered before recommending a vector type?

Situation 3

The ABDS marketing department has identified Oma, Inc. as a biotech company that is developing gene therapies. Oma Inc. would like to target non-small cell lung cancer (75% of all lung cancers) with gene therapy. They need a vector to introduce *p53*, a tumor suppressor gene (2,150 base pairs long), directly into the tumor. The vector must target the lung cells directly and the potential immune response must be considered.

Prepare a sales pitch to suggest a vector.

vector selector

You are a researcher at Advanced Bio Delivery Systems (ABDS), a company that supplies vectors for use in gene therapy. The marketing department has come to you for assistance. They would like to sell the vectors made by ABDS to biotech companies pursuing specific gene therapies. It is your job to research the disorders these biotech companies are targeting and decide on a vector that has potential to work. You will be presenting your findings to the marketing department for approval. There may be more than one vector possibility, so your job is to choose what you feel is the best one and defend your position. Your company stands to make a considerable profit if you are able to "sell" their vector for use in research.
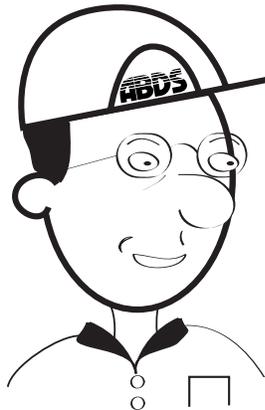
Some things to consider:
- What are important characteristics of the disease being investigated (genetic defect, body system affected…)?
- Will the gene fit into the vector?
- What types of cells would the therapy need to target (dividing/non-dividing)?
- What will happen to the genetic material once it is inserted into the cells?
- Does gene expression need to last a long time?
- What kinds of safety issues need to be considered before recommending a vector type?

Situation 4

The ABDS marketing department has identified SCID-BE-GONE (SBG), Inc. as a biotech company that is developing gene therapies. SBG is interested in developing a delivery system that will insert a good *ADA* gene (1,498 base pairs long) in T-cells of patients who have SCID (severe combined immune deficiency). SBG plans to harvest T-cells from a patient and use an *ex-vivo* technique to insert a functional *ADA* gene. As the transfected cells begin to produce the ADA protein, they will be retuned to the patient. The company is having trouble finding a vector to deliver their gene.

Prepare a sales pitch to suggest a vector.

SBG
SCID-BE-GONE

vector selector

You are a researcher at Advanced Bio Delivery Systems (ABDS), a company that supplies vectors for use in gene therapy. The marketing department has come to you for assistance. They would like to sell the vectors made by ABDS to biotech companies pursuing specific gene therapies.   It is your job to research the disorders these biotech companies are targeting and decide on a vector that has potential to work. You will be presenting your findings to the marketing department for approval. There may be more than one vector possibility, so your job is to choose what you feel is the best one and defend your position. Your company stands to make a considerable profit if you are able to "sell" their vector for use in research.
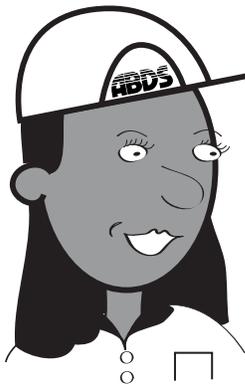
Some things to consider:
- What are important characteristics of the disease being investigated (genetic defect, body system affected…)?
- Will the gene fit into the vector?
- What types of cells would the therapy need to target (dividing/non-dividing)?
- What will happen to the genetic material once it is inserted into the cells?
- Does gene expression need to last a long time?
- What kinds of safety issues need to be considered before recommending a vector type?

Situation 5

The ABDS marketing department has identified CF, Inc. (CFI) as a biotech company that is developing gene therapies. CFI is interested in developing a delivery system that will insert a good *CFTR* gene in the lung cells of patients who have cystic fibrosis. The *CFTR* gene (4,443 base pairs long) codes for the protein that controls ion transfer across cell membranes. CFI is developing an *in vivo* system to deliver the functional gene copy. Challenges that face CFI are getting the vector to the lung cells and dealing with the immune response mounted as the vector enters the lungs.

Prepare a sales pitch to suggest a vector.

Vector Selector: Assessment Rubric

CATEGORY	4	3	2	1
Amount of information on disease	Important characteristics of the disease are addressed and described in detail.	Most of the important characteristics of the disease are addressed and described.	Important characteristics of the disease are addressed and described marginally.	Only one characteristic of the disease is addressed.
Amount of information on vector	Important characteristics of the vector are addressed and described in detail.	Most of the important characteristics of the vector are addressed and described.	Important characteristics of the vector are addressed and described marginally.	Only one characteristic of the vector is addressed.
Targeting issues	Vector chosen will bind to the target cells. Description of targeting strategy is detailed.	Vector chosen will bind to the target cells. Description of targeting strategy is somewhat vague.	Vector chosen will bind to the target cells. Description of targeting strategy is incomplete.	Vector chosen will not bind to the target cells.
Integration of genetic material	Includes detailed description of what will happen to the DNA.	Description of what will happen to the DNA is correct, but described in less detail	Description of what will happen to the DNA is lacking some information.	Description of what will happen to the DNA is unclear or incorrect.
Vector Choice	Provides ideas that support choice of vector.	Provides ideas that support choice of vector.	Provides one idea that supports choice of vector.	No ideas presented that support choice of vector.

Vector Types

	RETROVIRUS	ADENOVIRUS	ADENO-ASSOCIATED VIRUS	HERPES SIMPLEX VIRUS	LIPOSOME	NAKED DNA
How the vector carries genetic material						
Maximum length of DNA that can be inserted into vector						
ADVANTAGES						
DISADVANTAGES						