Supplemental Materials

for

Antiviral Drug Research Proposal Activity

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Team building questionnaire

Name:
Class Standing:
Major:
GPA:
On or Off campus residency:
   If Off campus, where?
Are you technologically savvy?
What are your career interests?
Dear Students, our goal is to understand your learning in this course. To do so, we would appreciate your input. Please complete this survey. This is an anonymous survey of students in Virology lecture and although your responses will not affect your grade, completion will be worth 1 point of the antiviral drug research proposal. Thank you!

1. I am aware of the type and scale of scientific research that is occurring in labs on campus.
   AGREE  DISAGREE  DON’T KNOW
   Explain why.

2. I have an understanding of how the research process is conducted by scientific researchers working in a research lab.
   AGREE  DISAGREE  DON’T KNOW
   Explain why.

3. I am comfortable in asking questions and sharing my ideas in my science classes
   AGREE  DISAGREE  DON’T KNOW
   Explain why.

4. Team work (collaborative work) is valuable for scientific advances
   AGREE  DISAGREE  DON’T KNOW
   Explain why.

5. Research designed to address basic science questions is vital to addressing problems of global significance
   AGREE  DISAGREE  DON’T KNOW
   Explain why.

6. Research designed to address basic science questions is a vital part of the drug discovery process.
   AGREE  DISAGREE  DON’T KNOW
   Explain why.

7. Prior to releasing an antiviral drug as a treatment for disease, the drug is first tested in one animal model (for example in a mouse model or in a rabbit model), if no complications are observed, the drug is then tested in humans (clinical trials)
   AGREE  DISAGREE  DON’T KNOW
   Explain why.

8. Viruses rapidly develop resistance to antiviral drugs.
   AGREE  DISAGREE  DON’T KNOW
   Explain why.
Pre-survey

Please rate your SKILL in the following areas.

(none - 1, very low - 2, low - 3, moderate - 4, high - 5, very high - 6)

a. Able to work as an effective group member

b. Able to read and understand scientific research articles

c. Able to interpret information presented in graphs, tables and figures of a scientific research article

d. Able to build a model from data collected from research articles

e. Able to suggest a reasonable hypothesis or ask a “next question” following analysis of data (from lab or presented in a research article)

f. Able to distinguish a peer reviewed article from one that is not peer reviewed

g. Able to distinguish an article that is considered a “primary source” from an article that is considered a “secondary source”

I learn best by: (choose one)

1. Listening to a lecture

2. Working on a project with other students

3. Participating in a discussion in class

4. Solving problems presented in context of a case study

5. Working in lab

6. Other (explain)

I give my permission for responses to be used for educational purposes

Yes

No
Antiviral Drug Research Proposal (50pts)

Basic research and clinical research are both very important in leading to scientific advances. The development of antiviral drugs provides an example of how these two different kinds of research must be used together in order to achieve the final goal of treating disease.

You will work in teams of 6 to develop an antiviral drug research proposal. Each team will be assigned a virus and each member of the team will be assigned a specialty. The pre-surveys, post-surveys, quiz, specialty research report, specialty research report revisions, and presentation of the poster will be handed in individually while the poster will be graded as a team effort. Make sure that all members of the team contribute equally because your team members will be grading your participation at the end.

Pre-survey
- Take the HPI concept inventory (1pt) on Course Management System (CMS)
- Take the pre-survey (1pt) during Discussion 1

Project setup/introduction
- You will be assigned to a team of 6 people to work on the antiviral drug for a given virus
- You will be assigned a specialty within the team (1-6)

Specialty Research (Post in Specialty Research assignment page in CMS)
- Answer only the questions of your specialty in one page (3pts)
- Utilizing primary literature, answer the questions pertaining to your specialty in the context of your assigned virus.
- Your specialty research can be written as an outline, bullet points, or paragraph form, but must include citations.
- If information is not available for your particular virus, the topics should be researched more generally about the family in which the virus belongs.
- You will work individually to investigate information on your specialty in the context of your assigned virus.
- Your research will be reviewed in the Specialty Research discussion, when you will share your work with the class.

  Specialty 1:
  1. How would the development of this antiviral drug benefit the global community?
  2. Describe/diagram the life cycle of your virus (or family of viruses)
  3. Identify 3 targets for an antiviral drug

  Specialty 2:
  1. Explain the purpose of high throughput screening
  2. Give examples from primary literature of a cell-based screen and a mechanism-based screen using viruses within the same family as your assigned virus
  3. Identify 3 different compound sources

  Specialty 3:
1. Explain what *in vitro* (cell-based) screening is and why it is important?
2. Give 3 examples from primary literature of *in vitro* screens using viruses within the same family as your assigned virus
3. What does it mean for a drug to have low toxicity? How would you screen for your compounds’ toxicity?

- **Specialty 4:**
  1. Explain 3 different *in vivo* (animal) models.
  2. Give 3 examples from primary literature of *in vivo* screens using viruses within the same family as your assigned virus
  3. What needs to be shown in the animal model before you can proceed to clinical trials? (Think about toxicity and efficacy)

- **Specialty 5:**
  1. Define clinical trial phases I, II, and III
  2. Give one example from primary literature of each of the three phases of clinical trials
  3. List 3 possible ethical issues involved with clinical trials

- **Specialty 6:**
  1. Give 2 examples of antiviral drugs on the market where the drug has gained resistance (preferably to treat at least one virus in the family of your assigned virus)
  2. Give 3 examples of *in vitro* or *in vivo* experiments in primary literature where viruses developed resistance to a drug
  3. List 3 organizations that fund antiviral drug research for viruses in you assigned virus’ family

**Specialty Research Discussion** (participation - 2pts)
- Meet with members of your Specialty team
- Share the information you acquired while completing your Specialty Research Report with your Specialty team
- As a specialty team, help each person individually with their ideas while discussing the information that all members acquired
- Report an overview of your specialty team’s discussion to the class
- **After discussion 2, post Specialty research report in the wiki in the CMS for your specialty to share your work within your specialty team**

**Specialty Research Revisions** (1pt) (post in Specialty Research Revisions assignment page in CMS)
- Specialty Research Report with changes/revisions after specialty team input from Specialty Research discussion
- Prepare to present your finding to your Virus team
**Virus Discussion** (participation – 2pts)
- Meet with members of your Virus team
- Present the information you compiled in completing your Specialty Research Revisions Report
- As a team, make the following decisions, in order:
  1. What will be the target of your antiviral drug?
  2. Which high-throughput screen and compound source will you use?
  3. Which in vitro screen will you use?
  4. Which in vivo model will you use?
  5. From which phase of clinical trials will you assess data?
  6. From which organization will you request grant funding?
- Report an overview of your Virus team’s decisions to the class
- **After the Virus discussion, post your Specialty Research Revisions in the wiki in the CMS to share your work within your Virus team**
  - The wiki for your Virus team is a place for your team to store information related to your project that only members of your team can see

**Proposal Preparation and Poster Design** (27pts) (post in Proposal assignment page the CMS)
- Work with your Virus team to design a poster to present your antiviral drug research proposal to the organization you will request funding from.
- The poster should be designed in Microsoft PowerPoint™ and posted on CMS before the poster session.
- The presentation should be printed so that it can be attached to a provided tack board.
- An example poster can be found on CMS to give you a place to start with the format and content. Your poster should have more creativity and information than the example.
- Write 3 questions you think other students should be able to answer after visiting your poster.

**Poster session** (10 pts)
- Each team will be given a number and will set up their poster at their numbered spot.
- Each Virus team will be divided into three teams of two and the class time was apportioned into three twelve-minute sessions.
- While one team of two is presenting their poster, the other four members will visit the posters of other teams.
- At the end of each twelve minutes session, the two presenters will visit other posters and a new team of two will present.
- Instructors will visit all posters to assess presentation of the material by all members of each team.

**Post-survey and quiz**
- Take 3 of the 10 quizzes designed by other teams (4.5pts)
- Take graphical data comprehension quiz (0.5pt)
- Take self-reported learning survey (1pt)
- Assess contribution from team members
Antiviral Drug Research Proposal Rubric
50pts total

Section 1 (Pre-surveys)

HPI concept inventory (2pt)
2pt – Completed in CMS on time
1pt – Completed in CMS late
0pt – Not completed

Pre survey (1pt)
1pt – Completed anonymously and signed attendance sheet
0pt – Not completed

Section 2 (Specialty research)

Specialty research report (3pts)
3pts – All three topics within the specialty description were addressed and primary literature was used for example data (see Project Setup within Procedure section of manuscript for descriptions)
2pts – Two topics were addressed
1pt – One topic was addressed
0pt – No posted assignment

Specialty Research Discussion (2pts)
2pts – Contributed to both online and in-class discussion
1pt – Contributed either online or in-class discussion
0pt – Contributed neither online or in-class discussion

Specialty research report revisions (1pt)
1pt – Posted assignment differs from specialty research report in that it contains additional correct information revealing that they have reconciled and evaluated research models considering ethics, efficacy, and feasibility and come to a consensus on the team topic
0pt – Posted assignment is the same as the Specialty research report

Section 3 (Virus research)

Virus Research Discussion (2pts) – explain what participate meant (see table)
2pts – Contributed to both online and in-class discussion
1pt – contributed either online or in-class discussion
0pt – contributed neither online or in-class discussion
Proposal Development (26.5pts)

Section 4a (Drug development process)

Target (3pts)
3pts – Student’s research model strongly supports (1) life cycle, (2) choice of target, and (3) reasoning behind drug development
2pts – One of the three concepts is explained incorrectly
1pt – Two of the three concepts is explained incorrectly
0pts – Student’s research model does not support life cycle, choice of target, and reasoning behind drug development

High-throughput screen (3pts)
3pts – Student’s research strongly supports choice of screen and compound source
2.5pts – Explanation of the choice of screen or compound source is partially correct
2pts - Explanation of the choice of screen or compound source is incorrect
1.5pts – Either choice of screen or compound source is not explained
1pt - Only choice of screen or compound source is explained, but is partially correct
0.5pts - Only choice of screen or compound source is explained, but is incorrect
0pts - Student’s research does not explain choice of screen and compound source

Cell-based screen (3pts)
3pts – Student’s research strongly supports effectiveness screen and toxicity screen
2.5pts – Explanation of effectiveness screen or toxicity screen is partially correct
2pts - Explanation of effectiveness screen or toxicity screen is incorrect
1.5pts – Either effectiveness screen or toxicity screen is not explained
1pt - Only effectiveness screen or toxicity screen is explained, but is partially correct
0.5pts - Only effectiveness screen or toxicity screen is explained, but is incorrect
0pts - Student’s research does not explain choice of effectiveness screen and toxicity screen

Animal models (3pts)
3pts – Student’s research strongly supports animal models and steps to clinical trials
2.5pts – Explanation of animal models or steps to clinical trials is partially correct
2pts - Explanation of animal models or steps to clinical trials is incorrect
1.5pts – Either animal models or steps to clinical trials is not explained
1pt - Only animal models or steps to clinical trials is explained, but is partially correct
0.5pts - Only animal models or steps to clinical trials is explained, but is incorrect
0pts - Student’s research does not explain animal models and steps to clinical trials
Clinical trials (3pts)
3pts – Student’s research strongly supports method to employ clinical trials and ethical issues to consider.
2.5pts – Explanation of method to employ clinical trials and ethical issues to consider is partially correct.
2pts - Explanation of method to employ clinical trials and ethical issues to consider is incorrect.
1.5pts – Either method to employ clinical trials and ethical issues to consider is not explained.
1pt - Only method to employ clinical trials and ethical issues to consider is explained, but is partially correct.
0.5pts - Only method to employ clinical trials and ethical issues to consider is explained, but is incorrect.
0pts - Student’s research does not explain method to employ clinical trials and ethical issues to consider.

Resistance (3pts)
3pts – Student’s research strongly supports drug resistance issues to consider and a target funding agency.
2.5pts – Explanation of drug resistance issues to consider or a target funding agency is partially correct.
2pts - Explanation of drug resistance issues to consider or a target funding agency is incorrect.
1.5pts – Either drug resistance issues to consider or a target funding agency is not explained.
1pt - Only drug resistance issues to consider or a target funding agency is explained, but is partially correct.
0.5pts - Only drug resistance issues to consider or a target funding agency is explained, but is incorrect.
0pts - Student’s research does not explain choice of drug resistance issues to consider and a target funding agency.

Section 4b (Proper use of primary literature)
Primary literature comprehension (2pts)
2 pts – All information presented from primary literature is interpreted as the original authors had intended.
Deduct 0.5 pts for every two misinterpreted pieces of data.
0pts – No information presented from primary literature is interpreted as the original authors had intended.
Citations (1.5pts)
1.5pts – All materials cited properly
1pt – Citations were not in the proper format (Journal of Virology)
0.5pts – No in-text citations
0pts – No citations

Appropriate use of terminology (2pts)
2pts – Proper use of terminology
Deduct 0.5pts for every two misuses of terminology
0pt – Many mistakes with use of terminology

Section 4c (Communication and presentation skills)
Organization (1pt)
1pt – Poster presented a clear message and flowed logically
0.5pts – Some specialties were presented out of order
0pt – Poster message was not clear

Written questions (2pts)
2pts – Questions reflected upper levels of Bloom’s taxonomy \(^1\)
1pt – Questions reflected lower levels of Bloom’s taxonomy
0.5pt – Fewer than three questions were submitted
0pt – Questions were not submitted

Poster session (6pts)
Content and Clarity (4pts)
2pts – Presenter explained poster content correctly and clearly
1pt - Presenter explained **some** poster content correctly and clearly
0pt - Presenter explained **no** poster content correctly and clearly

Questions (2pts)
2pts – Participant showed effort when asking questions to presenters and answering questions as a presenter
1pt – Participant showed **little** effort when asking questions to presenters and answering questions as a presenter
0pt – Participant showed **no** effort when asking questions to presenters and answering questions as a presenter

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Section 5

**Post assessments (6.5pts)**

**Poster session quiz (4.5pts)**
Deduct 0.5pts per answer to each of the nine questions

**Post survey (1pt)**
1pt – Completed anonymously and signed attendance sheet
0pt – Not completed

**Team assessment and HPI survey (1pt)**
1pt – Completed both
0pt – Both not completed
Post-survey
Dear Students, our goal is to understand your learning in Virology lecture. To do so, we would appreciate your input. Please complete this survey. This is an anonymous survey of students in Virology lecture and although your responses will not affect your grade, completion will be worth 1 point of the antiviral drug research proposal. Thank you!

* Required

1. I am aware of the type and scale of scientific research that is occurring in labs at the University of Maryland
   • [ ] Agree
   • [ ] Disagree
   • [ ] Don't know

1a. Please explain your above response

2. I have an understanding of how the research process is conducted by scientific researchers working in a research lab.
   • [ ] Agree
   • [ ] Disagree
   • [ ] Don't know

2a. Please explain your above response

3. I am comfortable in asking questions and sharing my ideas in my science classes
   • [ ] Agree
   • [ ] Disagree
• Don't know

3a. Please explain your above response

4. Team work (collaborative work) is valuable for scientific advances
• Agree
• Disagree
• Don't know

4a. Please explain your above response

5. Research designed to address basic science questions is vital to addressing problems of global significance
• Agree
• Disagree
• Don't know

5a. Please explain your above response
6. Research designed to address basic science questions is a vital part of the drug discovery process.

- [ ] Agree
- [ ] Disagree
- [ ] Don't know

6a. Please explain your above response

7. Prior to releasing an antiviral drug as a treatment for disease, the drug is first tested in one animal model (for example in a mouse model or in a rabbit model), if no complications are observed, the drug is then tested in humans (clinical trials)

- [ ] Agree
- [ ] Disagree
- [ ] Don't know

7a. Please explain your above response

8. Viruses rapidly develop resistance to antiviral drugs.

- [ ] Agree
- [ ] Disagree
- [ ] Don't know
8a. Please explain your above response

Please rate your SKILL in the following areas

a. Able to work as an effective group member

1 2 3 4 5 6
none ☐ ☐ ☐ ☐ ☐ ☐ very high

b. Able to read and understand scientific research articles

1 2 3 4 5 6
none ☐ ☐ ☐ ☐ ☐ ☐ very high

c. Able to interpret information presented in graphs, tables and figures of a scientific research article

1 2 3 4 5 6
none ☐ ☐ ☐ ☐ ☐ ☐ very high

d. Able to build a model from data collected from research articles

1 2 3 4 5 6
none ☐ ☐ ☐ ☐ ☐ ☐ very high

e. Able to suggest a reasonable hypothesis or ask a “next question” following analysis of data (from lab or presented in a research article)

1 2 3 4 5 6
none ☐ ☐ ☐ ☐ ☐ ☐ very high

f. Able to distinguish a peer reviewed article from one that is not peer reviewed
g. Able to distinguish an article that is considered a “primary source” from an article that is considered a “secondary source”

I learn best by: (choose one)

- Listening to a lecture
- Working on a project with other students
- Participating in a discussion in class
- Solving problems presented in context of a case study
- Working in lab
- Other: 

In which step of developing the antiviral drug proposal did you learn the most? i.e. searching for/reading journal articles, discussing with your group, asking the TA/prof questions, synthesizing the information you collected...

How would you change the antiviral drug research proposal project to improve it?

I give my permission for responses to be used for educational purposes *

- Yes
Post survey Honor Pledge

* Required

By typing my name below I swear on my honor that I have completed the online post survey for the BSCI437 antiviral project. *
Antiviral poster quiz: Please answer 3 of the following questions that DO NOT pertain to your group's poster. You may ONLY use information from the POSTER SESSION.

Your name *

Explain one fact you learned about antiviral drug development for Xenotropic Murine Leukemia Virus-Related Virus.

Explain one fact you learned about antiviral drug development for O'nyong-nyong virus.

Explain one fact you learned about antiviral drug development for Nounane virus.
Explain one fact you learned about antiviral drug development for Desert Shield virus.

Explain one fact you learned about antiviral drug development for Human Papillomavirus 13.

Explain one fact you learned about antiviral drug development for Yaba monkey tumor virus.

Explain one fact you learned about antiviral drug development for Thogotovirus.
Explain one fact you learned about antiviral drug development for Parvovirus B19.

Explain one fact you learned about antiviral drug development for Coltivirus.
Team assessment
Please type each group member's name then rate their level of participation in the following question.

Your name

What group number are you in?
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10

1a. Name one person in your group.

1b. How would you rate the above person's group participation?

1 2 3 4 5 6 7 8 9 10

This member did 10% of the work expected of them.

This person did 100% of the work expected of them.

1c. Explain your above rating (Required if lower than 8)

2a. Name one person in your group.
2b. How would you rate the above person's group participation?

1  2  3  4  5  6  7  8  9  10

This member did 10% of the work expected of them.

This person did 100% of the work expected of them.

2c. Explain your above rating (Required if lower than 8)

3a. Name one person in your group.

3b. How would you rate the above person's group participation?

1  2  3  4  5  6  7  8  9  10

This member did 10% of the work expected of them.

This person did 100% of the work expected of them.

3c. Explain your above rating (Required if lower than 8)

4a. Name one person in your group.

4b. How would you rate the above person's group participation?

1  2  3  4  5  6  7  8  9  10
This member did 10% of the work expected of them.

This person did 100% of the work expected of them.

4c. Explain your above rating (Required if lower than 8)

5a. Name one person in your group.

5b. How would you rate the above person’s group participation?

1 2 3 4 5 6 7 8 9 10

This member did 10% of the work expected of them.

This person did 100% of the work expected of them.

5c. Explain your above rating (Required if lower than 8)
Human Papillomavirus-13 Antiviral Project
HUMAN PapillOMAvIRUS
STRAIN 13 (HPV-13)

VIRUS FACTS
• Annually infects 6 million people [4]
• Over 80% of American women contract one or more types of HPV by age 50 [4]
• Can lead to warts, precancerous lesions and cancer [4]

CHARACTERISTICS [5, 6]
• Alphapapillomavirus
• Non-enveloped dsDNA virus
  o Capsid with icosahedral symmetry
• ~8 kb circular genome
• Similar to other PVs in genomic organizations
  o Similar structure and life cycle

Figure: Alphapapillomavirus (J. Shanley, 2009)
ANTI-VIRAL TARGET [7, 8]
- E7 oncoprotein (most important Papillomavirus protein in cells)
- Binds to Retinoblastoma (pRb), a tumor suppressor
- Displaces E2F transcription factors and DNA from pRb
- pRB loses wild-type function and cell proliferates

GENOME/CYCLE [9]
- HPV infects basal epithelial cells through cuts in the skin
- The genome codes for 8 proteins
  - E1, 2, 4, 5, 6, 7 (early) and L1, 2 (late)
- E1, 2, 4, 5 are important to genome replication
- E6, 7 are oncogenes; cause tumorous growth in host cells [8]
- L1, 2 are capsid proteins; expressed only during virion packaging
- Makes 50-100 copies of virion per infected cells
High Throughput Screen

Compound source: compound library from a drug company which will contain thousands of compounds that will be narrowed down.

Transfect cells with a luciferase vector
(Luciferase vector: contains cell-cycle dependent gene (i.e. transcription factor) promoter & firefly luciferase gene)

Cells with the plasmid are introduced to different compounds

Cell are infected with HPV-13

Use luminometer to measure the luminescence of the cells
High Throughput Screen

- We will use the Luciferase Assay to test the luciferase activity of the cells with different compounds.
- The level of luciferase activity will depend on whether the compound(s) will inhibit the E7 viral protein of HPV:
  - ↓ luciferase activity will show unsuccessful inhibition of E7
  - ↑ luciferase activity will show successful inhibition of E7

Figure: Testing luciferase activity based on cytokines TFG-β1 and TNF-α (Lembo, et al 2005)
High Throughput Screen

Compound inhibits:
- Blocked E7
- Cdks function normally
- Cell cycle control
- Less luciferase activity
- Less luminescence

Compound doesn't inhibit:
- E7
- Cdks function abnormally
- No cell cycle control
- Increased luciferase activity
- Increased luminescence
In Vitro Screening of the antiviral Drug

• Northern Blot

• The absence of E7 protein on the gel indicates the antiviral drug inhibits the E7 gene expression.

Figure: Antiviral drug causes repression in E7 transcription in infectious cell (Villanueva, et al, 2006).
In Vitro Screening Cont’…..

RT-PCR

- Cells can be treated with increasing concentration of the antiviral drug
- C33-A is HPV negative cell line
- RT-PCR assay result shows inhibition of E7 as concentration of the antiviral drug increases.

*Figure*: Antiviral drug causes repression in E7 transcription in infectious cell (Villanueva, et al, 2006).
Screening for Compounds Toxicity

- Apoptosis determination
- Control (noninfectious) cells are untreated (top).
- Infectious cells are treated with staurosporine to induce apoptosis (bottom).
- Increased Annexin V staining is seen in infectious cells only in the presence of staurosporine.
- PI (propidium iodide) and Annexin are two widely used dyes.

*Figure*: Anti viral drug does not induce apoptosis (Villanueva, et al, 2006).
Screening for Compounds Toxicity

- Infectious cells are treated with two different concentrations of antiviral drug.
- Percentage of total signal within the quadrant is indicated.

Figure: Anti viral drug does not induce apoptosis (Villanueva, et al, 2006).
Testing lead compound(s) *in vivo*

- Proposed animal model: **Rabbit** (mammalian)
- Why?
  - Genetically similar to humans
  - Can obtain a statistically significant sample size
  - Relatively easy to maintain
  - Generally, at least 3 different species (rodent, non-rodent, and non-human primate) are needed as *in vivo* models before proceeding to clinical trials on humans.

How anti-viral compound(s) will be tested *in vivo*

- Evaluating *toxicity* of compound(s):
  - 5 different concentrations of the compound will be administered to analyze dose-dependent effects.
- Purpose:
  - To ensure that the compound(s) is/are safe

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Treatment Regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical Application</td>
<td>Once daily</td>
</tr>
<tr>
<td>Intra-lesional injection</td>
<td>Once weekly</td>
</tr>
</tbody>
</table>

![Diagram](http://images.google.com/)

*image: courtesy of http://images.google.com/*
How anti-viral compound(s) will be tested *in vivo*

- Evaluating **efficacy** of compound(s):
  - After samples are infected with HPV-13 and are symptomatic (warts), compound(s) (varying in dosage) will be applied to infected region over an 8-10 week period.
  - Geometric Mean Diameters (GMDs) of papillomas will be recorded and compared across both dosage and days post infection.


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Cidofovir is an antiviral agent that works against the DNA viruses [12].

It is occasionally used for oral lesions [11].

Side effects can be intense such as poisonous, uveitis when used systematically [11].

**Topical Cidofovir Facts** [15]
- Cidofovir is an antiviral agent that works against the DNA viruses [12].
- It is occasionally used for oral lesions [11].
- Side effect can be intense such as poisonous, uveitis when used systematically [11].

**Ethical Issues:**
Safety is the utmost concern when performing the clinical trial with human. Serious ethical issues have been asked.

1. Side effects and other drug interactions.
1. Since most of the patients are teenagers, the effect of their bodies development.
1. Recurrent of diseases due to the failure of trial.

**Clinical Trial II Design**
1. 100 Ethnically different patients.
2. 75% with Cidofovir 25% with Placebo.
3. Cidofovir or placebo to be taken once a day for 5 days in the total of 12 weeks.
4. Check point is designed for drug tolerance and time to best response.
5. Result is pooled after 12 weeks.

**HPV-13 Facts**
- Mostly occurred in small communities or young adults [11].
- HPV-13 causes the focal epithelial hyperplasia or Heck’s disease [11, 13].
- Minority woman are the primary target for testing [14].

The consistent data from a trial phase II of the double blind study, which showed the drug appeared to be effective. The patients that took Cidofovir gel did not show any progressive disease when compared with those who took placebo [16].
HPV resistance to IFN-α [2]

• In vivo and in vitro evidence shows that HPV can avoid anti-viral effects of IFN using the E7 protein.
• The 2fTGH cell line was chosen as it relies on IFN-α for growth signaling.
• If the IFN-α signaling pathway is disturbed (in this case by the E7 protein) there will be a loss of cell growth.

• Different cell lines with various levels of E7 protein expression were treated with SMV (Semliki Forest virus).
• The E7 protein was not found to constrict viral growth in cells untreated with IFN-α (4A).
• Plaque assays were performed to determine virus level reduction when the cells were treated with IFN-α (4B).

Funding

We have chosen to prospect Merck for funding as they have experience in HPV research and have developed the Gardasil HPV vaccine.
Questions

1) What is our anti-viral target and why is the target important for the virus?

2) What in vitro assays will be performed to test compound?

3) What in vivo assay will be performed to test compound as well as what phase was selected for clinical trials?
References

10. Human Papillomavirus infection & replication in cervical epithelial cells (Figure) <http://www.stanford.edu/group/virus/papilloma/2005/papilloma10.html>
Human papillomavirus 13 (Papillomavirus): Specialty 5 (Clinical trial and ethical concerns)

1. Define clinical trial phases I, II, and III.
2. Give one example from primary literature of each of the three phases of clinical trials.
3. List 3 possible ethical issues involved with clinical trials

1. Phase I trials are the first stage of testing in humans. For this, a small group of healthy volunteers is selected (20-100 people). In phase 1, trials are designed to evaluate the safety of the drug, determine a safe dosage range, and identify side effects.
Phase II trials are performed on larger groups of people and are designed to evaluate how well the drug works.
Phase 3 trials are randomized, controlled trials that are done on larger patient groups, 300-3000 people. The purpose of this trial is to assess how effective the drug is as compared to the current treatment. Phase 3 trials are the most expensive, time consuming, and difficult trials.

2. The publication “A Phase 1 study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors” illustrates an example of clinical trial phase 1. It describes a new treatment for tumors, Telomelysin, a human telomerase reverse transcriptase (hTERT) promoter driven modified oncolytic adenovirus. Testing took place with sixteen subjects, with results finding that all doses tested were safe (Nemunaitis J, 2010).
An example of a clinical trial phase 2 is the trial of Praneem. Praneem is a herbal formula for the treatment of reproductive tract infections in women. 20 subjects with HPV were given the treatment or placebo for 30 days and were evaluated at the end. 8 out of 10 of the trials had HPV eliminated (Shukla, S).
The publication “Fda drug approval summary: bevacizumab plus interferon for advanced renal cell carcinoma” is an example of a clinical trial phase 3 trial. It describes the FDA approval of bevacizumab, in conjunction with interferon, in treating renal cancer. The summary details the Phase 3 randomized, double-blind experiment on over 600 subjects (Summers J, 2010).

3. To be ethical, researchers must obtain full consent of the participating subjects. There is also an ethical issue of a person's right to service. There is a group of participants who do not get the treatment that is being studied. But when that treatment may have beneficial effects, persons assigned to the no-treatment control may feel their rights to equal access to the services. Ethical standards also require that researchers not put participants in a situation where they might be at risk of harm as a result of their participation, since some drugs can have adverse reactions. A third issue is confidentiality. Subjects are assured that identifying information will not be made available to anyone who is not directly involved in the study.

References:
Shukla, S. Elimination of high-risk human papillomavirus type HPV16 infection by ‘Praneem’ polyherbal tablet in women with early cervical intraepithelial lesions.