# AP® RESEARCH
# 2016 SCORING GUIDELINES

**AP Research Performance Task Rubric: Academic Paper**

| Content Area | Performance Levels | | |
|---|---|---|---|
| **1 Understand and Analyze Context** | The paper identifies the topic of inquiry.<br>2 | The paper identifies the topic, and describes the purpose and focus of the inquiry.<br>4 | The paper explains the topic, purpose, and focus of the inquiry and why further investigation of the topic is needed by connecting it to the larger discipline, field, and/or scholarly community.<br>6 |
| **2 Understand and Analyze Argument** | The paper identifies or cites previous works and/or summarizes a single perspective on the student's topic of inquiry.<br>2 | The paper summarizes, individually, previous works representing multiple perspectives about the student's topic of inquiry.<br>4 | The paper explains the relationships among multiple works representing multiple perspectives, describing the connection to the student's topic of inquiry.<br>6 |
| **3 Evaluate Sources and Evidence** | The paper uses sources/evidence that are unsubstantiated as relevant and/or credible for the purpose of the inquiry.<br>2 | The paper uses credible and relevant sources/evidence suited to the purpose of the inquiry.<br>4 | The paper explains the relevance and significance of the used sources/cited evidence by connecting them to the student's topic of inquiry.<br>6 |
| **4 Research Design** | The paper presents a summary of the approach, method, or process, but the summary is oversimplified.<br>3 | The paper describes in detail the approach, method, or process.<br>5 | The paper provides a logical rationale by explaining the alignment between the chosen approach, method, or process and the research question/project goal.<br>7 |
| **5 Establish Argument** | The paper presents an argument, conclusion or understanding, but it is simplistic or inconsistent, and/or it provides unsupported or illogical links between the evidence and the claim(s).<br>3 | The paper presents an argument, conclusion, or new understanding that the paper justifies by explaining the links between evidence with claims.<br>5 | The paper presents an argument, conclusion or new understanding that acknowledges and explains the consequences and implications in context.<br>7 |
| **6 Select and Use Evidence** | Evidence is presented, but it is insufficient or sometimes inconsistent in supporting the paper's conclusion or understanding.<br>2 | The paper supports its conclusion through the compilation of relevant and sufficient evidence.<br>4 | The paper demonstrates a compelling argument through effective interpretation and synthesis of the evidence and through describing its relevance and significance.<br>6 |
| **7 Engage Audience** | Organizational and design elements are present, but sometimes distract from communication or are superfluous.<br>1 | Organizational and design elements convey the paper's message.<br>2 | Organizational and design elements engage the audience, effectively emphasize the paper's message and demonstrate the credibility of the writer.<br>3 |
| **8 Apply Conventions** | The paper cites and attributes the work of others, but does so inconsistently and/or incorrectly.<br>2 | The paper consistently and accurately cites and attributes the work of others.<br>4 | The paper effectively integrates the knowledge and ideas of others and consistently distinguishes between the student's voice and that of others.<br>6 |

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| **9 Apply Conventions** | The paper's use of grammar, style and mechanics convey the student's ideas; however, errors interfere with communication and/or credibility.<br>1 | The paper's word choice and syntax adheres to established conventions of grammar, usage and mechanics. There may be some errors, but they do not interfere with the author's meaning.<br>2 | The paper's word choice and syntax enhances communication through variety, emphasis, and precision.<br>3 |

**NOTE:** To receive the highest performance level presumes that the student also achieved the preceding performance levels in that row.

**ADDITIONAL SCORES:** In addition to the scores represented on the rubric, readers can also assign scores of **0** (zero).

- A score of **0** is assigned to a single row of the rubric when the paper displays a below-minimum level of quality as identified in that row of the rubric.

# AP® RESEARCH
# 2016 SCORING COMMENTARY

## Academic Paper

### Overview

This performance task was intended to assess students' ability to conduct scholarly and responsible research and articulate an evidence-based argument that clearly communicates the conclusion, solution, or answer to their stated research question. More specifically, this performance task was intended to assess students' ability to:

- Generate a focused research question that is situated within or connected to a larger scholarly context or community;
- Explore relationships between and among multiple works representing multiple perspectives within the scholarly literature related to the topic of inquiry;
- Articulate what approach, method, or process they have chosen to use to address their research question, why they have chosen that approach, and how they employed it;
- Develop and present their own argument, conclusion, or new understanding;
- Support their conclusion through the compilation, use, and synthesis of relevant and significant evidence;
- Use organizational and design elements to effectively convey the paper's message;
- Consistently and accurately cite, attribute, and integrate the knowledge and work of others, while distinguishing between the student's voice and that of others;
- Generate a paper in which word choice and syntax enhance communication by adhering to established conventions of grammar, usage, and mechanics.

# Testing the Effectiveness of Pulmonary Macrophage Transplantation to treat Pulmonary Alveolar Proteinosis Caused by Niemann-Pick Type C


## Abstract

Niemann-Pick Type C is a lysosomal storage disorder that hinders intracellular trafficking of cholesterol in cells. Poor cholesterol transport in macrophages in the lungs can significantly inhibit macrophage consumption of debris in the alveolar air sacs, interfering with respiration. This condition includes pulmonary alveolar proteinosis (PAP), and is a pathological sign of Niemann-Pick Type C. Research on methods to resolve PAP includes pulmonary macrophage transplantation. Pulmonary macrophage transplantation has been successful in treating PAP resulting from other genetic disorders, but not from Niemann-Pick Type C. In order to determine how well pulmonary macrophage transplantation would treat PAP caused by Niemann-Pick Type C, the quality of cell dispersion in the lungs must be studied. The purpose of this project is to test pulmonary macrophage transplantation on healthy mouse models to determine obstacles that might arise from the treatment. Determining procedural obstacles before testing the effect of the treatment on pulmonary disease, including PAP, caused by Niemann-Pick Type C would provide additional information on the effectiveness of the treatment.

Carbonyl iron was used as a marker for macrophages. Macrophages were harvested from healthy C57BL/6J mice and loaded with carbonyl iron. Attempts to transport macrophages via intranasal inhalation into the lungs of three C57BL/6J mice without Niemann-Pick Type C disease were made. These mice were euthanized at differing times after inhalation (15 minutes, 20 hours, and 72 hours). Their lungs were collected, fixed, imbedded in paraffin, and sectioned. Sections were attached to glass slides for histology. These were stained with Turnbull Blue stain and Orange G counterstain. Lungs were examined for macrophage dispersion and quantity. The

results showed dispersion of macrophages across lung tissue, but only a very few cells were found. The lack of cells led to the reasoning for the hypothesis that limited cells reached the lung tissue by intranasal inhalation while a majority may have stuck to the walls of the upper respiratory tract before the lungs. This study proved that pulmonary macrophage transplantation using intranasal inhalation produced inadequate results.

**Introduction**

Niemann-Pick Type C (NPC) disease is an autosomal recessive, neurodegenerative lysosomal storage disorder (Erickson, 2013). Two genes are associated with the disease: *NPC1* and *NPC2*. For this study, *NPC* will be synonymous with *NPC1*, as *NPC2* is only deficient in 5% of cases. Symptoms may first present as soon as the first few months after birth and as late as adulthood. Symptoms of NPC disease include ataxia, declining coordination, dysarthria, and dementia (Erickson, 2013). Niemann-Pick Type C is a very rare disease, with as little as 500 cases diagnosed worldwide. Juvenile Niemann-Pick Type C always results in early death. The earlier the symptoms present, the earlier death supervenes. Children who demonstrate the disease may not live beyond their second decade of life. However, adults as old as 50-60 years may also be diagnosed with late-onset Niemann-Pick Type C disease.

NPC is characterized by impaired cholesterol transport. As a result, cholesterol accumulates in the liver and spleen. Poor lipid transport results in liver disease and cerebellar and central nervous system nerve degeneration. Another symptom of NPC is the lung disease. Excessive storage of cholesterol occurs in macrophages in the lungs, transforming them into "foamy macrophages." A possible pathological sign of this condition is pulmonary alveolar

proteinosis, a condition in which proteins and pulmonary surfactant build-up in the alveoli (Muralidhar et al. 2011). Currently, there exists one study that describes success in treating pulmonary alveolar proteinosis caused by GM-CSF deficiency. This article, published in 2014 in *Nature*, describes success in treating pulmonary alveolar proteinosis using pulmonary macrophage transplantation to successfully remedy the lung disease (Suzuki et al. 2014).

However, this study does not state any relationship with Niemann-Pick Type C disease. Though the lung disease associated with NPC can include pulmonary alveolar proteinosis, further evidence is required to determine if pulmonary macrophage transplantation can ameliorate the pulmonary symptoms of NPC. A study that tests the effect of pulmonary macrophage transplantation treatment on the pulmonary disease of Niemann-Pick Type C would provide enough justification to determine the effectiveness of the treatment. Such a study was the intended project for this paper. However, due to a lack of resources, specifically enough mice born with NPC, a new approach towards understanding the effects of pulmonary macrophage transplantation was pursued.

Pulmonary macrophage transplantation involved tracheal injection of healthy macrophages in a solution of phosphate-buffered saline (PBS) (Suzuki et al. 2014). However, intranasal inhalation was used in this study instead of tracheal injection because the latter required an unavailable level of technical skill. Intranasal inhalation involves the inhalation of a solution dropped in small increments on the nostrils. Understanding how well macrophages disperse in the lungs after the inhalation would provide useful information for assessing the capability of pulmonary macrophage transplantation to treat the lung disease associated with NPC. If the inhaled macrophages congregate in a small, defined area in the lungs, the treatment would not likely be effective. Even dispersion of cells across the inner surface area of the lungs

would indicate that the treatment would likely be successful. Without this information, analysis of the data from the aforementioned potential study would be hindered.

The goal of this study is to determine this information. Carbonyl iron is a compound that can viably be used to mark the location of macrophages within pulmonary tissue. Studies have utilized carbonyl iron in the removal of macrophages from solution, and a similar but modified procedure can be used to mark the location in the lungs macrophages disperse (Lohrman et al. 1974, Mastro et al. 1982). Macrophages can ingest carbonyl iron particles and then be used in pulmonary macrophage transplantation of mice models. The mice's lungs can be dissected, and examined in order to assess macrophage dispersion.

Mouse models were used in this experiment. The mice used were C57BL/6J mice that lack the *Npc1* mutation and, therefore, did not have the disease. These mice were used to answer the following question: how well do healthy macrophages suspended in a phosphate-buffer saline solution disperse in the lungs of C57BL/6J mice after pulmonary macrophage transplantation by intranasal inhalation? The hypothesis for this experiment is that inhaled macrophages will be evenly dispersed across the inner surface area of the lungs. This information would provide preliminary evidence of the technical effectiveness of transplanting macrophages into the lungs.

*Basic Physiology Relevant to Niemann-Pick Disease Type C*

Cholesterol is a class of lipids, one of the four major organic macromolecules. Cholesterol plays an important role in maintaining the structure and fluidity of cell membranes. It is also a major component in the synthesis of steroid hormones. Low-density lipoprotein (LDL) is a protein that transports lipids through the extracellular fluid in the body. One class of

lipids it transports is cholesterol. Liver cells create most of the cholesterol in the body. In liver cells, cholesterol binds with LDL to create LDL-cholesterol. When the LDL-cholesterol reaches tissue, it binds to the membrane proteins of the cells in the tissue. These cells consume the LDL-cholesterol through receptor-mediated endocytosis. Essentially, the cell membrane folds inward, the LDL-cholesterol inside the depression, until a vesicle forms from said membrane. The vesicle carries the LDL-cholesterol into the cell. The *NPC1* gene codes for the protein NPC1, a protein involved in the transport of cholesterol out of the vesicle. *NPC2* is a gene that codes for a much smaller protein, NPC2 which interacts with NPC1 in transferring cholesterol. Transport of cholesterol is a crucial process for biological function. Hindered transport of either prevents proper maintenance of homeostatic conditions (Maxfield et al. 2002).

*Basic Pathophysiology of Niemann-Pick Disease Type C*

In NPC disease, the inability to transport cholesterol results from a mutation in either the *NPC1* or *NPC2* genes. The mutated gene codes for either a faulty NPC1 or NPC2 protein, respectively, that cannot transport LDL-cholesterol out of the vesicles. This results in the accumulation of cholesterol in the cell. Blocked transport results in a deficiency of cholesterol available to maintain steroid hormone synthesis and consistency in cell membranes.

In NPC disease, cholesterol accumulates more severely in several types of tissue, including liver and spleen tissue (Erickson 2013). One of the liver's physiological responsibilities is the production and dissemination of cholesterol for the body. Liver cells' role in cholesterol production and transport make them strongly affected by a mutation in either the *NPC1* or *NPC2* genes. Intracellular accumulation in liver cells results in hepatomegaly, or

enlargement of the liver. Cerebellar and central nervous system degeneration is another symptom of the disease.

*Treatments for Niemann-Pick Disease Type C*

The major treatment for NPC disease currently studied is 2-hydroxypropyl-beta-cyclodextrin (HPBCD), a cholesterol-mobilizing compound. Each molecule of the compound is a ring of seven glucose molecules with a hydrophobic (attracted to lipid compounds) center (Muralidhar, 2011). Cholesterol molecules, also hydrophobic, are absorbed inside this ring. The ring promotes the transport of cholesterol as a transporting vehicle.

HPBCD's have not shown to cure the disease, but have been shown to delay the disease's symptoms. HPBCD's have shown to slow the cerebellar neurodegeneration associated with NPC disease. However, HPBCD is a large molecule that cannot pass the blood-brain barrier. However, studies have successfully transported HPBCD into the brain in mice within the first seven days of life, before the blood-brain barrier is formed.

*Pulmonary Pathophysiological Sign of Niemann-Pick Type C Disease*

Niemann-Pick Type C disease can also cause a lung disease. The lungs at the end of the bronchial branches extending into each lung are small air sacks called alveoli. Alveoli are the sites where respiration occurs. Respiration is the oxygen-carbon dioxide gas exchange with the capillary blood vessels. For respiration to transpire, the lungs must be clean and free of debris. The alveoli contain macrophages that "clean-up" inhaled debris. Macrophages are a type of

leukocyte (white blood cell) that consumes pathogens and debris through a process called phagocytosis. Research on the lungs of Niemann-Pick C animal models show elevated levels of cholesterol within these pulmonary macrophages, a pathological presentation continuous with Niemann Pick C. These cholesterol-laden macrophages, dubbed "foamy macrophages," coat the inside of the alveoli. These macrophages lose the ability to consume debris in the alveoli and create a barrier to respiration. This condition is considered a pathological sign of Niemann-Pick Type C and included pulmonary alveolar proteinosis (Muralidhar, 2011).

Research on the effects of HPBCD's on the lung disease show an unchanged prognosis. The reason for the inefficacious results of HPBCD's to treat the lung disease has yet to be determined. Blood circulates through the capillaries adjacent to the alveoli, so transport of the HPBCD's should not be the issue.

*Mouse Models*

Despite vast differences, mouse and human genomes are 95-98% similar. This fact makes mice ideal models for human genetic research. Two distinct mutations in the mouse genome have been recorded to cause lysosomal storage diseases with neurological symptoms (Pentchev et al. 1980). The mouse models with these mutations are $Npc1^{spm}$ and $Npc1^{nih}$. Their respective mutations have been considered to be the murine equivalent to the infantile Niemann-Pick Type C1 mutation (Miyawaki et al. 1982). A better mouse model for Niemann-Pick Type C Disease is $Npc1^{nmf164}$, which possesses a more similar phenotype to the more common juvenile ccounterpart than does the aforementioned two models. An important fact to understand is the difference in

representing human and mouse genes. *NPC1* describes the human gene, whereas *Npc1* represents the mouse gene. The capitalization of the genes differs between humans and mice.

However, the mouse strain used in this study are none of these mouse models and is the C57BL/6J strain. These mice do not possess the *Npc1* mutation. Using mice with the disease is outside of the scope of this study.

*Literature Review of Relevant Sources*

One study published in *Nature* discusses success in treating a hereditary form of pulmonary alveolar proteinosis (PAP) caused by deficiency of the protein GM-CSF. The described treatment was pulmonary macrophage transplantation, a process in which healthy macrophages were transplanted into the lungs of diseased mice. The study describes the current standard to treating pulmonary alveolar proteinosis, a whole-lung lavage. This procedure is invasive and inefficient. In addition, there existed no pharmacological therapy for PAP. Bone-marrow derived macrophages were directly administered into the lungs of Csf2rb-/- mice, a GM-CSF-deficient mouse mutant. One year later, pulmonary macrophage transplantation significantly resolved the abnormal pulmonary symptoms. However, this study does not provide information on how well the macrophages were dispersed in the lungs. (Suzuki et al. 2014). Furthermore, NPC is irrelevant to this study, so its conclusions suggest to a limited degree that pulmonary macrophage transplantation may treat pulmonary alveolar proteinosis associated with NPC.

The aforementioned study directly transplanted healthy macrophages into the lungs of mice using a tube inserted through the trachea and bronchi. However, this procedure requires a

high level of technical skill. The procedure utilized in this study is a simpler, less demanding method: intranasal inhalation. Mice inhaled a macrophage solution in lieu of direct administration of macrophages into their lungs. Evidence supports the effectiveness of this method. An article discussing intranasal inhalation of spores of a ubiquitous fungus known as Alternaria described intranasal inhalation as efficient in dispersing spores in the lungs (Pereira, et al. 2012). The method involved mice inhaling spores applied to the nostrils. The success of intranasal inhalation substantiated utilizing a similar method in this study to transplant macrophages into mouse lungs.

Another article focuses on macrophage phenotypes determined that the environment influences their function and physiology (Liddiard et al. 2014). Theoretically, implanting healthy macrophages could have had the effect of sharing their healthy status with the indigenous macrophages in the alveoli. A similar study can be conducted to test the effectiveness of this treatment with the lung disease of Niemann-Pick C. However, information on the dispersion of macrophages in the lungs can be useful to fully understand the effectiveness of the treatment. Finding this information is the purpose of this study.

Marking macrophages before implantation is the method used in this study, and an adequate marker for macrophages is carbonyl iron. In a paper on human T and B lymphocytes, researchers utilized a method to separate macrophages and monocytes (macrophage precursors) from heparinized blood using carbonyl iron, a highly pure iron powder (Lohrman et al. 1974). These researchers stimulated macrophages to ingest carbonyl iron. This project incorporates their procedure and, using mice models, studies where in the lungs macrophages disperse. The mice would inhale the macrophages before they could be euthanized, and then their lungs would be prepared into histology slides and viewed under a microscope.

**Method**

The method used in this study is a mixed, scientific experimental approach. Mice, +/+ for the *Npc1* mutation, will be sacrificed in order to acquire macrophages. +/+ describes the genotype of the mice for the gene, which is *Npc1* in this case. +/+ for the *Npc1* symbolizes that the individual has two dominant alleles of the gene. The mutation is recessive, so two dominant alleles is the healthy genotype. The macrophages will be marked with carbonyl iron. A set of three healthy mice, +/+ for the *Npc1* mutation, will inhale these macrophages while under anesthesia. Each mouse will be sacrificed at different amounts of time after inhalation (15 minutes, 20 hours, 72 hours). Understanding the dispersion of cells at different times after the inhalation can show changes in macrophage dispersion over time. These times were chosen based on the convenience in order to gather results as soon as possible. Lungs will be dissected out and prepared into histology slides for examination. Observations and images of these slides will be recorded and the number of visible macrophages will be counted in order to generate a conclusion about how evenly the macrophages dispersed in the lungs. Because this study requires examination of empirical evidence, it uses a scientific experimental approach.

Using a mixed research method would allow for greater detail to be considered in examining the difference in macrophage dispersion among the three slides. Furthermore, counting the number of macrophages in the slides would ensure that sufficient number of macrophages reach the lungs. Because this study requires examination of empirical evidence, it uses a scientific experimental approach.

Three mice were used to gather data from the inhalation procedure, while additional mice are used to harvest macrophages. More data could not be gathered from extra sets of three mice each due to limited resources. Three mice, each living for different amounts of time before

sacrifice, provided an adequate amount of data to support a conclusion concerning the dissemination of macrophages in the lungs.

*Ethics*

Because this study involves the sacrifice of mice, ethics must be considered. The original project for this study would have tested the effect of pulmonary macrophage transplantation on mice with Niemann-Pick Type C disease. This protocol was IRB approved. The current project is a preliminary study that would provide additional information useful for analysis for the original project, so it is part of the IRB approval.

According to the Institutional Animal Care and Use Committee Guidance 201, the ethical method to euthanize a mouse without anesthesia is cervical dislocation. The method of euthanasia is cervical dislocation and was performed by a properly trained geneticist.

*Sterilize Carbonyl Iron Powder*

With a spatula, 1 mg of carbonyl iron powder was dropped into a tube. About 5 mL of distilled water was poured into the tube. The exact volume did not matter, as long as the powder disperses. The tube was centrifuged to collect the carbonyl iron at the bottom of the tube, and the water supernatant was discarded. This procedure was repeated one more time. This procedure was repeated while replacing the distilled water with 50% ethanol solution. This procedure was repeated with 95% ethanol solution. This procedure was repeated with pure ethanol. The cap of

the tube was removed and the tube was placed into an incubator, which was set to 80 degrees Celsius to evaporate residual water and ethanol and dry the carbonyl iron powder.

*Harvesting Macrophages*

Macrophages were accumulated and harvested from healthy C57BL/6J mice +/+ for the NPC1 gene. These mice did not possess Niemann-Pick C disease. The method used to acquire the macrophages is a peritoneal lavage, the steps of which follow. For each of the three mice in the study, one mouse was sacrificed to acquire macrophages. For one treatment of macrophages, one healthy +/+ mouse was intraperitoneally injected with 3 mL of thioglycolate media. The purpose of the thioglycolate is to stimulate the production of macrophages in the peritoneum, the chamber containing the abdominal organs. After four days, the mouse was euthanized by cervical dislocation. Cervical dislocation is the most ethical and appropriate method. To follow proper protocol, the euthanasia of the mice was supervised by a professional geneticist. Macrophages were collected from the diseased mouse. 5 mL of sterile phosphate-buffered saline (PBS) was injected into the peritoneum of the mouse. The mouse's abdomen was massaged gently. The skin surrounding the abdomen was cut and pulled apart, revealing the wall of the peritoneum. With tweezers, the peritoneum was pulled up and a cut made near the point of tension. With a fire-polished glass pipette, the 5 mL of PBS was withdrawn and drained into a plastic tube. An additional 5 mL of PBS was injected and withdrawn in 1 mL intervals. The plastic tube may not contain all of the 10 mL of PBS. The solution in the tube should be cloudy as a result of the presence of macrophages in solution. Care was taken to not lose the first 5 mL of injected PBS. Otherwise, a large proportion of the macrophages would have been lost.

Macrophages were counted in a hemocytometer and centrifuged for five minutes. All of the supernatant was withdrawn and discarded.

*Stimulating Macrophages to Consume Carbonyl Iron*

1 mL of tissue growth media was vortexed with 5 mg of sterilized carbonyl iron powder. After centrifugation, the cells formed a pellet at the bottom of the tube. These cells were suspended in the vortexed tissue growth media and sterilized carbonyl iron solution. The cell solution was transported into a 1.5 mL tube. The cell solution was incubated at 37 degrees for one hour. Every five minutes, the tube was tipped to re-suspend the iron. After incubation, the supernatant containing the cells and tissue media was withdrawn and pipetted into another 1.5 mL tube, leaving behind a black pellet of iron. The cells, suspended in the supernatant, were counted with a hemocytometer. The cell solution was centrifuged at 3000 rpms for five minutes. The tissue media supernatant was drawn out, and the cells were re-suspended in 1 mL sterile PBS. Centrifugation and re-suspension were repeated for a second wash. Centrifuge again at the same settings. The supernatant was discarded, and the cells were re-suspended in 0.4 mL of sterile PBS and counted with a hemocytometer. The tube of cells was placed next to a magnet for one hour. Building an apparatus in which a tube is set next to an attached magnet would be ideal. After one hour, a black spot on the side of the tube adjacent to the magnet was visible. The supernatant was discarded, and the residual black spot was re-suspended in 0.12 mL of sterile PBS. The black spot should be macrophages that have consumed iron. 0.02 mL of the solution was used to count the cells in a hemocytometer.

*Inhalation of the Iron-laden Macrophages*

The remaining 0.1 mL was used to treat the first of three mice in the study. About 0.05 mL of the solution was dropped with a micropipette on each of the mouse's nostrils at 0.01 mL intervals, alternating between nostrils. Evaporation of the water in the PBS of the solution reduced the available volume for each nostril, so about 0.04 mL per nostril was inhaled for each mouse. This procedure was repeated for two other study mice. Two additional mice were used to for harvesting macrophages. Enough carbonyl iron powder had been sterilized for the entire study.

*Lung Tissue Fixation*

Tissue fixation preserves the tissue's natural state and prevents post-mortem decay. Euthanasia of each mouse follows. The time between macrophage inhalation and euthanasia differed between each of the three studied mice. The following procedure was repeated for each mouse.

The first mouse's lungs were dissected, washed with paraformaldehyde for four hours in a 15 mL tube, washed with PBS solution, and re-set in PBS overnight. The tube containing lungs was set on a tilt table. The lungs were washed with 25% and 50% ethanol for two hours per wash, and then refrigerated in 70% ethanol.

The lungs were sent to a histology core facility for dehydration, embedding, and histological staining. Water must be removed from the tissue and embedded with paraffin wax to chemically and structurally preserve the tissue. The lungs were embedded in a block of paraffin, and thin cross-sections of the block were cut and mounted on slides. Three cross-sections were

cut from each set of lungs and mounted on slides, yielding nine slides in total. Staining is the following step.

*Staining Procedure*

The purpose of histological staining is to increase the contrast between elements within the tissue cross-section on the slide or highlight certain components. Three histology stains were intended for this procedure. Hematoxylin, a general use stain, and a Prussian Blue stain, a stain that specifically stains iron. The Prussian Blue stain was expected to stain carbonyl iron consumed in by macrophages in an earlier step. The Orange G stain acted as a counterstain to contrast with the Prussian Blue and aid in identifying stained macrophages.

The core facility stained one slide from each set of lungs with the Hematoxylin and Eosin (H and E) as a stain and counterstain. Prussian Blue and Orange G staining was conducted outside of the facility. A Turnbull Blue stain was used to replace the Prussian Blue, for this stain did not work as intended. Orange G was reused as a counterstain against the Turnbull Blue Stain.

*Examination of Slides Under Light Microscope*

Each slide was examined under a light microscope. The H and E stained slides were examined to identify components in the tissue. Alveoli, blood vessels, and connective and epithelial tissue. The Prussian Blue stained slides were examined to identify carbonyl iron. Stained carbonyl iron would identify the locations of dispersed macrophages. The Prussian Blue and Orange G stains failed to stain any elements on the first slide, which was of the mouse

euthanized 20 hours after inhalation. Consequently, the Turnbull Blue stain replaced the Prussian Blue stain with greater success. However, further evidence that the Turnbull stained better than the Prussian was needed. As a result, the effectiveness of the Turnbull Blue stain and of the Prussian Blue stain were compared.

*Comparison of Turnbull Blue Stain and Prussian Blue Stain*

Macrophages were acquired and stimulated to ingest carbonyl iron using the aforementioned procedure. Using a carbonyl iron-laden macrophage solution in PBS, nine smears were created on nine slides. One slide was stained using the Turnbull Blue stain and another slide was stained using the Prussian Blue stain. The Orange G counterstain was used for both slides. These two slides were examined under a light microscope and observations of stained artifacts were recorded. Two additional slides were washed in paraformaldehyde for three hours, then in phosphate-buffered saline (PBS) overnight. The aforementioned staining procedure was repeated, one slide with Turnbull Blue stain and the other with Prussian Blue. Turnbull Blue proved to be a superior stain to Prussian Blue.

*Staining Slides of Lung Cross-sections Using Better Stain*

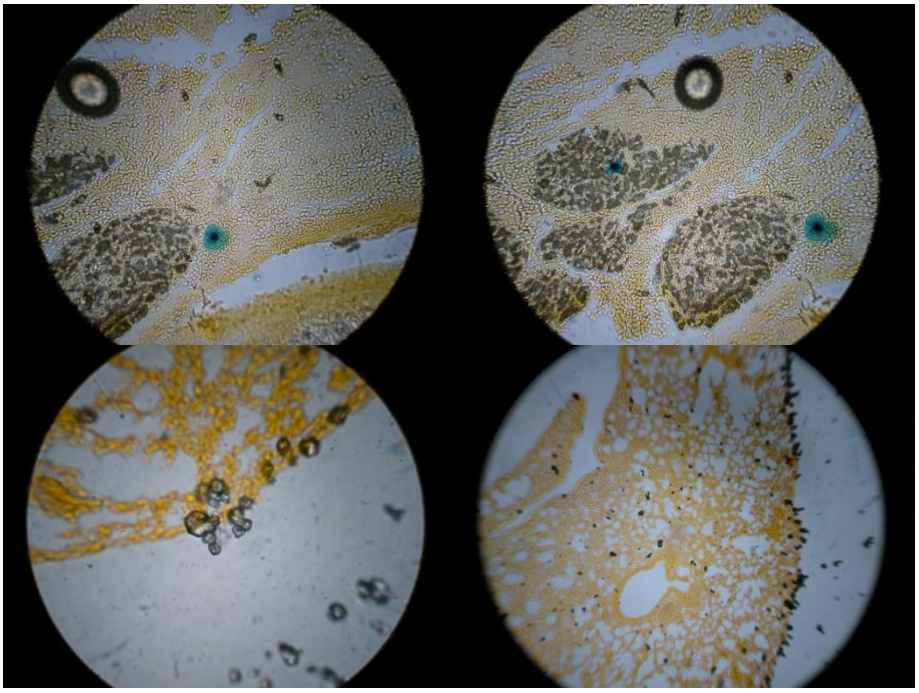
The staining procedure was repeated on unstained slides, one from each of the three study mice, using the Turnbull Blue stain. These slides were examined, and observations of macrophage containing blue-stained carbonyl iron was recorded. These macrophages were also counted. This information served as evidence to draw a conclusion about the quality of macrophage dispersion in the lungs.

**Results**

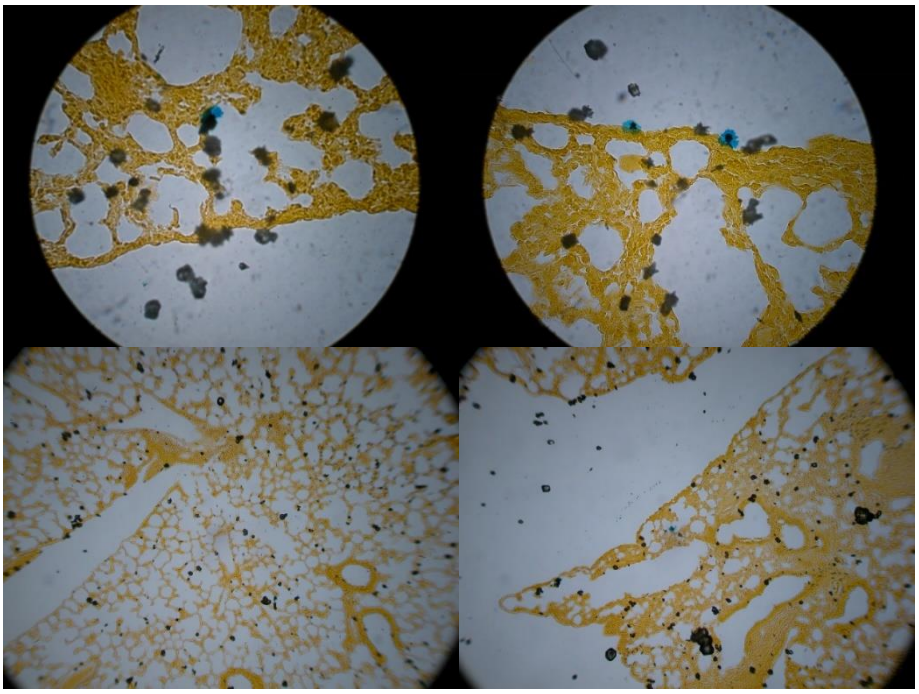
Data on macrophage counts in each step of their preparation before inhalation can be found in the table below. The "first attempt" experimental cell counts were derived from testing the process testing the entirety of this study's method. The "smears" step was the creation of smears of macrophages stained with either Turnbull Blue and Prussian Blue stains to determine which stain better stained macrophages. The cell count for macrophages during this process was not collected after the peritoneal lavage step.

The three, non-test slides used for this study were stained with the Turnbull Blue stain. The lung cross-sections mounted on each of these three slides are of the mice euthanize 15 minutes, 20 hours, and 72 hours after pulmonary macrophage transplantation, respectively. The images below show lung tissue stained orange by the Orange G counter stain. Dark artifacts on the slides are also present. The images also show candidate macrophages containing carbonyl iron stained by the Turnbull Blue stain. The cell counts, found below, prove that the presentation of these macrophages were unexpectedly rare on each slide.

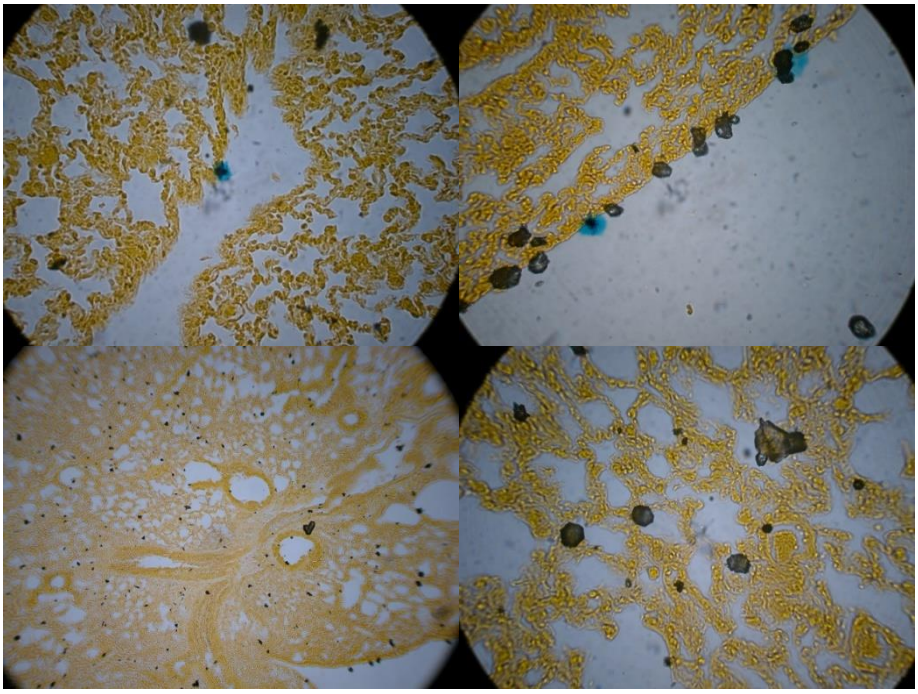
| Experiment Cell Counts | Post-Peritoneal Lavage | Post- Cell Incubation with Carbonyl Iron | Post- Cell Wash | Post-Iron Treatment | Cell Count from Test Slides |
|---|---|---|---|---|---|
| **First Attempt** | 1.28E+06 | 2.33E+06 | 8.33E+05 | 1.62E+05 | n/a |
| **Smears** | n/a | 5.80E+05 | 3.92E+05 | 2.00E+05 | n/a |
| **15 min.** | 5.60E+06 | 3.22E+06 | 2.37E+06 | 7.34E+05 | 10 |
| **20 hr.** | 1.12E+07 | 7.48E+06 | 2.59E+06 | 6.79E+05 | 18 |
| **72hr.** | 1.55E+07 | 5.09E+06 | 2.86E+06 | 8.24E+05 | 6 |
| **Averages** | 8.39E+06 | 3.74E+06 | 1.81E+06 | 5.20E+05 | 11.33 |
| **Standard Deviation** | 6235443.716 | 2648577.127 | 1116974.589 | 314052.0339 | 6.110100927 |
| **Standard Error** | 3.12E+06 | 1.18E+06 | 5.00E+05 | 1.40E+05 | 3.53E+00 |



15 minute post-inhalation slides: Few blue stained macrophages are visible (top left, top right). Much non-cellular debris appeared on the slide (bottom left, bottom right).



20 hours post-inhalation slides: Few blue stained macrophages are visible (top left, top right). Much non-cellular debris appeared on the slide (bottom left, bottom right).



72 hours post-inhalation slides: Few blue stained macrophages are visible (top left, top right). Much non-cellular debris appeared on the slide (bottom left, bottom right).

**Discussion**

The images of each stained slide show candidate macrophages containing carbonyl iron stained by the Turnbull Blue stain. The orange stained tissue augmented the presentation of the blue-stained carbonyl iron in the macrophages. The presence of macrophages in the lung tissue provides partial evidence of success of pulmonary macrophage transplantation.

Each set of four images is associated with a slide, and each slide is associated with one of the three mice euthanized at different times after the inhalation treatment. The top two images of each set show evidence of macrophages with blue-stained carbonyl iron. The bottom two images of each set demonstrates the rarity of these macrophages by showing a dearth of macrophages on the slides. In each slide, macrophages did not congregate in one or a few areas of the lung tissue. Instead, they were discovered across the entire slide. However, the observed macrophages were

fewer in number than expected. Less than twenty macrophages could be found in each slide despite the fact that the mice studied inhaled tens of thousands of these cells. Only thin cross-sections of each set of lungs were mounted on the slides, so fewer cells should be visible. However, only less than twenty cells were visible. There is an astronomical difference between the number of macrophages inhaled and the number visible. Due to the lack of cells, concluding that cell dispersed evenly across the lung tissue would be inaccurate. More cells should have presented on the slides had a significant percentage of these cells been inhaled into the bronchioles and alveoli of the lungs. Because fewer cells appear to have reached these spaces in the lungs, a majority of the cells must have been lost since inhalation.

One hypothesis for the loss of these cells is that macrophages stuck to the pharynx, trachea, or other section of the tract between the nostrils and the bronchiole and alveolar cavities inside the lungs. Macrophages may have been more adhesive than anticipated and adhered to these canals. This reasoning would explain the limited cell counts of cells that reached the bronchioles and alveoli.

The technique to transplant macrophages into the lungs chosen for this study was intranasal inhalation. In this process, mice inhaled small quantities of phosphate-buffered saline containing macrophages. However, in the method utilized by the a previously mentioned study testing pulmonary macrophage transplantation, macrophages were directly administered into the alveolar spaces in the lungs using tracheal injection (Suzuki et al. 2014). That this technique would have ensured that all macrophages dispersed evenly and profusely is a likely possibility.

There are two reasons this method was not used in this study. The technical skill required to directly administer the macrophages into the lungs was not available. Lack of such skill would have risked damaging lung tissue during inhalation and injuring the mice. In addition, a

previously mentioned study testing the immune response to the fungal spore Alternaria successfully used intranasal inhalation to disperse spores inside the lungs (Pereira, et al. 2012). However, the adhesive quality of macrophages may have been significantly greater than that of spores. This possibility would address why intranasal inhalation failed to disperse macrophages into the lungs.

In order to conclude that macrophages adhered to a respiratory canal before the lungs, a study comparing the effectiveness between intranasal inhalation of macrophages and direct administration of macrophages into the lungs should be conducted. The results of this potential study would determine whether the intranasal inhalation was a significantly less effective method to administer macrophages into the mice's lungs.

**Conclusion and Future Directions**

The results of this study point to the minimal effectiveness of pulmonary macrophage transplantation using intranasal inhalation. Macrophages appeared dispersed within the lungs reasonably well. However, too few of them reached the lungs to substantiate this claim. These limited cell counts suggest that intranasal inhalation should not be used to successfully transplant macrophages into the lungs. Therefore, future research related to pulmonary macrophage transplantation should refrain from transplanting macrophages via this method in order to potentially save resources and time. Furthermore, these results may have broader significance. Any method involving transplanting cells into the lungs may should not be conducted by intranasal inhalation, for the adhesive quality of macrophages may be shared by other cells.

However, this statement is a mere suggestion and cannot be categorically justified due to the vast variety of cells.

This study yields some future directions. Further research comparing the quality of cell administration using a direct method and this inhalation procedure would provide more information on the effective way to transplant macrophages. Another avenue of research would be testing the effectiveness of macrophage transplantation on pulmonary alveolar proteinosis that may result from Niemann-Pick Type C mice. However, intranasal inhalation should not be used to transplant macrophages into the lungs. This potential study was the original goal of this project, but do to unforeseen circumstances, it was not pursued.

Despite discouraging results on the effectiveness of the pulmonary macrophage transplantation procedure in this study, evidence of some macrophages reaching the lung tissue is a hopeful sign of progress.

**Acknowledgements**

I thank the Erickson Lab in the Banner-University Medical Center Tucson Campus for providing a major contribution to this study. I thank Dr. Robert P. Erickson for providing the mentorship and advice imperative for my completing this study, Craig Marshal for providing instruction in various lab techniques, and the Burness Lab of the University of Arizona for donating the mice used in this study.

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# AP® RESEARCH
# 2016 SCORING COMMENTARY

## Academic Paper

**Sample: K**
**Content Area: Understand and Analyze Context — Row 1 Score: 6**
**Content Area: Understand and Analyze Argument — Row 2 Score: 6**
**Content Area: Evaluate Sources and Evidence — Row 3 Score: 6**
**Content Area: Research Design — Row 4 Score: 7**
**Content Area: Establish Argument — Row 5 Score: 7**
**Content Area: Select and Use Evidence — Row 6 Score: 6**
**Content Area: Engage Audience — Row 7 Score: 3**
**Content Area: Apply Conventions — Row 8 Score: 4**
**Content Area: Apply Conventions — Row 9 Score: 3**

**HIGH SAMPLE RESPONSE**

"Testing the Effectiveness of Pulmonary Macrophage Transplantation to treat Pulmonary Alveolar Proteinosis Caused by Niemann-Pick Type C"

**Content Area: Understand and Analyze Context — Row 1**
The response earned 6 points for this row because it clearly articulates a topic, focus, and purpose, as well as the need for the inquiry. The topic of lung disease is identified and then focused down to the narrow area of potential treatment of a specific variant of lung disease, pulmonary alveolar proteinosis (PAP) caused by Niemann-Pick Type C. Further, the paper identifies a need and offers a rationale for the inquiry: to explore the potential for improved treatment options for PAP caused by Niemann-Pick Type C by examining the process in mice — with a goal of eventual application to human protocols (p. 4 and p. 7).

**Content Area: Understand and Analyze Argument — Row 2**
The response earned 6 points for this row because extensive research was conducted to examine current, existing work in the field involving similar protocols (see Literature Review beginning on p. 8, as well as sections of the paper that explain the specifics of the condition and the treatment, such as the first and second full paragraphs on p. 3). Further, the paper explores research on alternatives to current work being done on confirming/addressing the presence of PAP caused by Niemann-Pick Type C. The paper uses a synthesis of the current understanding in the field as a means of guiding its own research and informing the decisions made for experimentation on mice to test the efficacy of nasal inhalation: "This information would provide preliminary evidence of the technical effectiveness of transplanting macrophages into the lung" (p. 4).

**Content Area: Evaluate Sources and Evidence — Row 3**
The response earned 6 points for this row because the sources are included as context and rationale for the project (see the discussion in the Literature Review), they are clearly tied to the student's work, and they form a strong foundation for the paper's inquiry into methods for introducing macrophages into the lungs. While there are areas where examining the relevance and significance of the sources could be more explicit, (e.g., "...another article focuses on..." p. 9, paragraph 2), overall the sources are credible, relevant, and clearly tied to the topic.

**Content Area: Research Design — Row 4**
The response earned 7 points for this row because it offers an explicit and clearly articulated approach to the inquiry by examining the current status of the understanding of the disease and then referencing appropriate protocols from the field. The approach is precisely identified, and a rationale for the procedures is included. Additionally, there is a connection between the method

employed and what is appropriate to the discipline for such diagnostic protocols, as corroborated by the provided evidence (see Methods section, pp. 10–11). Ethical considerations of using mice as research subjects and "sacrificing" them are acknowledged.

**Content Area: Establish Argument — Row 5**
The response earned 7 points for this row because it supports its approach and findings with literature from the field of medical research and recent investigations into lung disease, and it references appropriate foundational work from the discipline. The project itself is appropriate for AP Research, and the paper arrived at a conclusion that is credible: the procedure as attempted was not successful, but it shed light on next steps and alternatives for introducing macrophages into the lungs. The response articulates how the conclusion is reached through the experimentation on mice and considers the shortcomings of the findings — namely, that the method of administration did not allow the macrophages to move past the nostril/trachea tract and on to the lungs (p. 20). It discusses implications for future exploration of the approach (p. 21).

**Content Area: Select and Use Evidence — Row 6**
The response earned 6 points for this row because it includes scholarly literature from the field (see both the bibliography and the references throughout the Introduction section) and refers to similar diagnostic protocols for other diseases analogous to this particular variant of lung disease, offering strong evidence for the appropriateness of the inquiry and the conclusion that was reached. The use of evidence from other sources serves as a foundation for the inquiry before moving forward with the planned mouse inhalation/sacrifice/dissection. In other words, the paper provides interpretation and synthesis of evidence to move toward the experiment, and then analyzes the student's own process and findings to arrive at the eventual, compelling conclusion.

**Content Area: Engage Audience — Row 7**
The response earned 3 points for this row because the paper's organization is clear and assists the reader in understanding a technical subject. The paper is divided into aptly labeled sections and subsections which aid navigation of its components. This organization enhances communication with explicit description of procedures and added graphics (pp. 17–19) to improve the reader's ability to follow the inquiry and findings.

**Content Area: Apply Conventions — Row 8**
The response earned 4 points for this row because attributions and citations are accurate throughout, and it is evident when the work of others is included, especially in the Introduction section, which includes a review of the literature. The paper did not earn 6 points on this row because the research was not effectively integrated or embedded; other scholars' research was definitely separated out from the student's work — simply cited without explication. The separation of other scholars' work from the student's research does not distract the reader; it just does not represent smooth integration of sources.

**Content Area: Apply Conventions — Row 9**
The response earned 3 points for this row because the writing is clear, precise, and readable. This paper serves as a good example of an author taking a highly technical scientific subject and making it understandable for an educated but non-expert audience.