# Molecular Pharming to Support Human Life on the Moon, Mars, and Beyond

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## Supplementary Information

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# 1. Supplementary Text

## 1.1 Decision Tree Walkthrough

The decision tree for selecting a molecular farming production strategy is applied to two different test cases of a diagnosed disease state where a pharmaceutical medical countermeasure has been identified as appropriate. The decision tree does not consider alternative or auxiliary non-pharmaceutical countermeasures. As this is the first decision tree to operationalize a molecular medical foundry for space, we chose to develop this first iteration in isolation from other medical systems and aspects of mission architecture. The following is a description of the assumptions and logic applied to navigate the two hypothetical test cases to an appropriate molecular pharming production strategy.

### 1.1.1 Test Case 1: acute radiation syndrome

Acute radiation syndrome is selected as a test case based on NASA's evidence report for risk of acute radiation syndromes due to solar particle events[1]. Here we detail our introduction of the disease state and subsequently detail the progression of the medical response and decision making that ultimately results in transient production of filgrastim in potato leaves, as described in the main text body.

Introduction of disease state

We assume that one crew member develops acute radiation syndrome after receiving a whole body dose of 3.25 Gy ionizing radiation, as recorded by an on-person physical dosimeter, from a major solar particle event (SPE) during extravehicular activity (EVA) outside of low-Earth orbit, where the protective magnetosphere of Earth is absent. The expected frequency of SPEs is highly dependent on the solar cycle, but individual SPEs are not able to be predicted.

Diagnosis of disease state

The illness primarily presents in the hematopoietic syndrome and is diagnosed by a combination of physical dosimeter readings (on the crew member at the time of exposure), clinical manifestations of nausea, vomiting, and fatigue, and a blood test indicating a neutrophil count decreased to less than $1.5 \times 10^9$ per liter of blood (neutropenia).

Identification of pharmaceutical medical countermeasure

The crew physician prescribes the radiation mitigator, filgrastim (NEUPOGEN®, Amgen) to increase circulating neutrophil levels to override the myelosuppressive doses of radiation.

Countermeasure stockpile available?

There is not a countermeasure stockpile of filgrastim (or an effective alternative radioprotectant/mitigator) available, which could be a result of a multitude of root causes (e.g. use-based depletion of medication, spaceflight-induced accelerated drug expiry).

Anticipated disease state?

Acute radiation syndrome is an anticipated disease state, based on the established body of literature on the matter[2].

Time-to-treatment window lower than threshold?

At a dose of 3.25 Gy, there is a 50% mortality rate within 60 days barring appropriate medical intervention[3]. Cause of death is generally due to complications arising from extensive hematopoietic damage. The time-to-treatment threshold in this decision tree is not strictly defined due to the influence of a given reference mission architecture on this value. However, the threshold will be largely based on the response time of transgenic plant production, which would likely be >3 weeks. The time-to-treatment in this test case will have a significant impact on patient outcome, and thus impact mission success through impaired crew member capability, and so is high priority is assigned here to minimize time-to-treatment.

Sufficient transgenic biomass available?

Filgrastim-producing transgenic seeds are not flown as part of the mission, and thus there is not sufficient transgenic biomass available.

Chronic disease state?

Acute radiation syndrome is an acute disease state.

Transgenic seed available?

Filgrastim-producing transgenic seeds are not flown as part of the mission.

### 1.1.2 Test Case 2: microgravity-induced osteopenia

Microgravity-induced osteopenia is selected as a test case based on NASA's evidence report for risk of early onset osteoporosis due to space flight[4]. Here we detail our introduction of the disease state and subsequently detail the progression of the medical response and decision making that ultimately results in transgenic production of teriparatide in lettuce leaves, as described in the main text body.

Introduction of disease state

Microgravity-induced osteopenia is established as a chronic disease state that cannot be completely mitigated through exercise- or nutrition-based countermeasures (e.g. the Advanced Resistive Exercise Device used on the International Space Station) and affects all six crew members. Throughout spaceflight and reduced gravity conditions the crew will experience areal bone mineral density (aBMD) T-scores between -1 and -2.5 (classified as osteopenia by the World Health Organization) but may also report T-scores < -2.5 (classified as osteoporosis). These lower aBMD scores will increase fracture risk.

Diagnosis of disease state

Enabling surveillance technology for real-time tracking of space flight-induced bone loss is currently a gap. There will be technological advances that fill this gap. The disease state will be diagnosed through routine monitoring and will additionally present in fragility fractures, most likely during strenuous EVA.

Identification of pharmaceutical medical countermeasure

Reports show that spaceflight suppresses circulatory levels of parathyroid hormone (PTH), which in turn suppresses calcium absorption in the intestines and kidney[5]. The crew physician prescribes teriparatide (FORTEO®, Eli Lily and Company), which is recombinant human PTH residues 1-34, to treat severe bone loss and to facilitate fracture healing.

Countermeasure stockpile available?

There is an available stockpile of teriparatide based on the known risk of spaceflight-induced bone loss.

Stockpile reduced below acceptable limit?

The administration of teriparatide from the stockpile has reduced the available drug quantity to below the acceptable limit. As this disease state is expected to be both highly likely and highly impactful to chances of mission success, the crew will produce additional teriparatide to replenish the stockpile.

Time-to-treatment window lower than threshold?

The time-to-treatment window is not lower than the threshold. The purpose of production is to replenish the drug stockpile for future use.

Chronic disease state?

Microgravity-induced osteopenia is a chronic disease state.

Pharmaceutical storage stability exceeds threshold?

As mentioned in the main body of the manuscript, stability of biologics in spaceflight is completely untested. We do know that biologics are generally less stable than small molecule drugs, which have been shown, in limited capacity, to experience spaceflight-accelerated degradation. We assume that the stability of teriparatide does not exceed the threshold, which we anticipate would be a complex and transient value in practice.

Transgenic seeds available?

Teriparatide-producing transgenic seeds are flown as part of the mission.

Production demand exceeds threshold?

The production demand for replenishment of the teriparatide stockpile does not exceed the threshold. This is consistent with the assumption that storage stability does not exceed the

threshold; a less stable pharmaceutical will need to be produced in smaller quantities and higher frequency to serve as an effective countermeasure to a chronic disease state.

## 1.2 Supporting Production Platform Comparisons

### 1.2.1 Defining Space Exploration Characteristics

The following are definitions of the pharmaceutical production characteristics used to rank the various biological platforms for space exploration utility.

#### *1.2.1.1 In-Situ Resource Utilization*

The capacity of the production platform to make use of resources expected to be available on a space exploration mission to an extraterrestrial planetary body. These resources include sunlight, atmospheric gases, water, and regolith.

#### *1.2.1.2 Just-In-Time Response*

The capacity of the production platform to produce pharmaceuticals in a rapid response manner, whether it be in counteraction to an anticipated or unforeseen threat. This considers the speed of production for scenarios when the gene delivery system, or capable transgenic organism, is ready at hand and also when the gene delivery system or organism must be engineered mid-mission.

#### *1.2.1.3 Operational Simplicity*

A combination of the equipment complexity and the workforce specialization required to manufacture pharmaceuticals using the production platform. This considers control systems, robustness of operation (including multiple states of input material quality and altered gravity), ability to scale-up production as much as for early settlement missions, and specific productivity (production of a given amount of pharmaceutical per unit volume production system per unit time).

#### *1.2.1.4 Product Range*

The capacity of the production platform to generally produce a range of pharmaceutical product (from small molecule to simple peptide to secretory antibodies). The major consideration in this category is glycosylation, an essential post-translational modification where sugar moieties are attached to a therapeutic protein. Ability of the production platform to produce generally cytotoxic pharmaceutical products is also considered.

#### *1.2.1.5 Crew & Planetary Safety*

The likelihood of the production platform contamination and release and the impact on crew safety and planetary protection.

### 1.2.2 Key Production Platform Resources

Here we include relevant resources which, used in conjunction with working process knowledge, were used to rank each generalized biological platform for pharmaceutical production in space exploration – insect cell[6–8], mammalian cell[9–11], plant cell[12–14], autotrophic bacteria[15–17], heterotrophic bacteria[18–20], yeast[21–23], cell-free expression[24–26], transgenic animal[27–29], transgenic plant[30–32], and transient plant[33–35].

## 2. Supplementary Tables

The following tables contain the information used in the crop cultivation calculations and graphical representations for "A Test Case for Molecular Pharming in Space."

### 2.1 Crop Characteristics

Crop characteristics and requirements for lettuce and potato compiled from NASA's Baseline Values and Assumptions Document 2018[36]. DW, dry weight. FW, fresh weight.

| Parameter | | Units | Lettuce | White Potato |
|---|---|---|---|---|
| Harvest Index | | % | 90 | 70 |
| Edible Biomass Productivity | Dry Basis | g DW/$m^2$/day | 6.57 | 21.06 |
| | Fresh Basis | g FW/$m^2$/day | 131.35 | 105.3 |
| | Fresh Basis Water Content | %/100 | 0.95 | 0.8 |
| Inedible Biomass Productivity | Dry Basis | g DW/$m^2$/day | 0.73 | 9.03 |
| | Fresh Basis | g FW/$m^2$/day | 7.3 | 90.25 |
| | Fresh Basis Water Content | % | 0.9 | 0.9 |
| Total Biomass (Edible + Inedible), Dry Basis | Nominal | g DW/$m^2$/day | 7.3 | 30.08 |
| | High | g DW/$m^2$/day | 7.9 | 50 |
| Carbon Content | | % | 40 | 41 |
| Metabolic Reactants & Products | $O_2$ Production | g/$m^2$/day | 7.78 | 32.23 |
| | $CO_2$ Uptake | g/$m^2$/day | 10.7 | 45.23 |
| | $H_2O$ Uptake | kg/$m^2$/day | 2.1 | 4 |
| Support Requirements | Water Use per Dry Biomass | L/g DW | 0.34 | 0.15 |
| | Stock Use per Dry Biomass | L/g DW | 0.034 | 0.022 |
| | Acid Use per Dry Biomass | g acid/g DW | 0.0618 | 0.0428 |
| Light Requirements | Photosynthetic Photon Flux | mol/$m^2$/day | 17 | 28 |
| | Diurnal Photoperiod | hr/day | 16 | 12 |
| Growth Period | | days | 28 | 132 |
| Nominal Planting Density | | plants/$m^2$ | 19.2 | 6.4 |
| Nominal Biomass per Plant at Harvest | | g DW/plant | 10.6 | 620.4 |

## 2.2 Recommended Dietary Allowances

Recommended dietary allowances and adequate intakes for key macro- and micronutrients, as described by the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences[37].

| Nutrient | | Male (31-50 years) | Female (31-50 years) | Average |
|---|---|---|---|---|
| Macronutrient | Water (g) | 3.7 | 2.7 | 3.2 |
| | Carbohydrate (g) | 130 | 130 | 130 |
| | Protein (g) | 56 | 46 | 51 |
| | Fiber (g) | 38 | 25 | 31.5 |
| Micronutrient | Vitamin C (mg) | 90 | 75 | 82.5 |
| | Thiamine (mg) | 1.2 | 1.1 | 1.15 |
| | Vitamin K (µg) | 120 | 90 | 105 |
| | Folate (µg) | 400 | 400 | 400 |

## 2.3 Crop Nutrition

Average nutritional intake from a single serving (100 g FW) of lettuce and potato for key macro- and micronutrients, as described by the U.S. Department of Agriculture in the FoodData Central database[38].

| Nutrient | | Lettuce (lettuce, raw) | Potato (flesh and skin, raw) |
|---|---|---|---|
| Macronutrient | Water (g) | 95.64 | 79.25 |
| | Carbohydrate (g) | 2.97 | 17.49 |
| | Protein (g) | 0.9 | 2.05 |
| | Fiber (g) | 1.2 | 2.1 |
| Micronutrient | Vitamin C (mg) | 2.8 | 19.7 |
| | Thiamine (mg) | 0.041 | 0.081 |
| | Vitamin K (µg) | 24.1 | 2 |
| | Folate (µg) | 29 | 15 |

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