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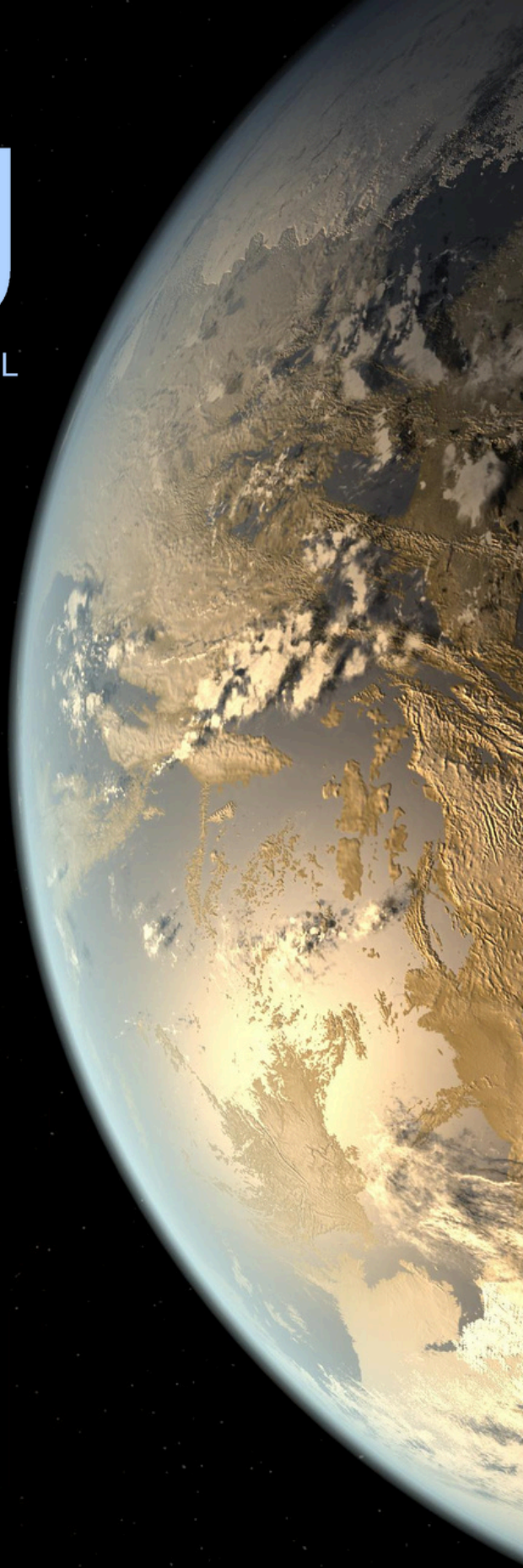
SEPHI of Exoplanets

Kepler-504 b, Kepler-315 b and Kepler 315 c



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About the Journal

Aims and Scope

The Columbia Undergraduate Science Journal (CUSJ) was founded in 2006 by students who were passionate about showcasing undergraduate excellence in scientific research. Since then, CUSJ has remained Columbia's premier publication for original scientific research and scholarly reviews and is managed by an editorial board of undergraduates with a vast scope of interests across all disciplines. The editorial board also manages the Columbia Junior Science Journal (CJSJ), a publication designed to introduce high school students to research, and Columbia Scientist, a publication aimed at increasing scientific engagement and thought at all academic levels. In addition to our publications, the CUSJ team is dedicated to fostering the scientific community, both within Columbia and in the surrounding Morningside Heights and Harlem communities. To this end, the board frequently plans outreach and networking events relevant to young and early career scientists, including an annual Research Symposium poster session each spring.

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Letter from the Editor-in-Chief

Dear Readers

It is with great enthusiasm that I, along with the rest of the CUSJ team, present to you the Winter 2024 edition of our publication. Founded in 2006, CUSJ has continued to remain dedicated to fostering scientific journalism among undergraduates and providing a platform to highlight the brilliance of emerging researchers.

This year's edition continues our tradition of excellence by featuring a diverse array of research spanning multiple disciplines. Each article embodies the intellectual curiosity and determination of its authors, sparking meaningful conversations and inspiring future explorations in science. We hope that these works resonate with you, our readers, and encourage you to engage with the ideas presented by questioning and building upon them.

The publication of this journal represents the tireless efforts of our editorial team, whose commitment to upholding our rigorous standards has been nothing short of extraordinary in a short period of time. I want to extend my deepest gratitude to the editorial board for their reviews, thoughtful feedback, and unwavering dedication throughout the year. I would also like to thank the broader CUSJ committee, whose collaborative spirit and diligence ensured that each article was edited to perfection.

A special acknowledgment goes to the CUSJ executive board, whose leadership and support have been vital in navigating this year's challenges. Your vision and hard work continue to elevate our journal to new heights. Additionally, I am profoundly grateful to the Faculty Advisory Board and Columbia Libraries for their invaluable guidance, which has enabled us to maintain the highest standards of academic excellence.

Serving as Editor-in-Chief for the Winter 2024 edition has been an honor and a privilege. This journal stands as a representation of the innovative spirit of undergraduate scientists and the power of collaboration. We are proud to share these achievements with you all and we look forward to continuing to celebrate and amplify undergraduate research in the years to come.

Thank you for your continued support of CUSJ, and I hope you enjoy this edition as much as we enjoyed creating it.

Sincerely,
Amanda Prashad
Editor-in-Chief, Columbia Undergraduate Science Journal

Letter from the President

Dear Readers

It is with great pride that I present to you the Winter 2024 edition of the Columbia Undergraduate Science Journal. This carefully crafted issue showcases cutting-edge studies made in each author's field. From a study utilizing SEPHI (Statistical-likelihood Exo-Planetary Habitability Index) in the search for habitable exoplanets to a scholarly review of engineered Oncolytic viruses, this edition brings readers a diverse range of novel findings. From its inception, CUSJ has existed to showcase excellence in undergraduate research. These selected pieces exemplify our founding purpose and mission in their rigorous methodology and subsequent innovative findings. To our authors, I commend you for your countless hours of work and unwavering spirit to push the boundaries of knowledge and science. Beyond extraordinary research, the selection of papers reflects a candid desire to make the world a better place through a scientific pursuit.

At CUSJ, we continuously uphold the core value that our work, and science in general, would not be possible without the work and contributions of many. Countless hours of labor made this edition possible, and our work as an organization would be incomplete without the recognition and acknowledgment of such work. Foremost, this edition was only made possible through the incredible work of our editor-in-chief, Amanda Prashad, and her team of editors. From reading, editing, and revising a plethora of submissions, the CUSJ team has thoroughly surpassed their goal of putting together a diverse and meaningful journal edition. Secondly, I would like to extend gratitude to our generous advising faculty board, who ensure that each of our publications remains of high and professional quality. Our work would not be possible without their guidance.

Lastly, to you, our esteemed readers, I extend my sincerest gratitude for your continued support and readership. Your curiosity and dedication to a world made better through science are at the heart of our mission here at CUSJ. Without further ado, I invite you to read our Winter 2024 edition.

Cordially,
Kayla Pham
President of CUSJ

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SEPHI of Exoplanets Kepler-504 b, Kepler-315 b and Kepler-315 c

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KEYWORDS: Habitability, SEPHI, Exoplanets, Life, Liquid water, Temperature, Density, Star, PHI, ESI

ABSTRACT: The search for habitable exoplanets has improved with every passing year. New methods and advanced instrumentation with higher precision help find more habitable exoplanets and refine existing parameters of highly likely habitable exoplanets. This paper presents the Statistical-likelihood Exo-Planetary Habitability Index (SEPHI) values for Kepler-504 b (of star Kepler-504), Kepler-315 b, and Kepler-315 c (both revolving around star Kepler-315).[1,2,3] SEPHI is based on the geometric mean of the likelihood Gaussian estimation of four different comparison criteria with Earth as the only place we know harboring life: Telluricity, Atmosphere and Planet Gravity, Surface Liquid Water, and Magnetic Field.[4,5,6] The seven physical characteristics of exoplanets have been used to calculate those four criteria: planetary mass, planetary radius, orbital period, stellar mass, stellar radius, and stellar effective temperature. This is a follow-up to previously calculated ESI values for the three exoplanets mentioned above, with Kepler-504 b and Kepler-315 b having a high ESI of 71.23% and 69.44%, respectively.[7] It has been found that Kepler-504 b (with a host M-type star (small red dwarf)) has a SEPHI value of 0, Kepler-315 b (with a host G-type star) has a SEPHI value of 0, and Kepler-315 c (with a host G-type star) has a SEPHI value of 0. Thus, more than a combination of host star type, the orbital radius of exoplanet, and the final ESI to determine probable habitability, a further in-depth analysis through SEPHI can help us confirm its actual habitability for Earth-based life.

INTRODUCTION

Astronomers have been observing exoplanets for 32 years and studying their properties. In the last two decades, there has been immense progress in refining existing parameters through multiple missions by Kepler Space Telescope (KST), Transiting Exoplanet Survey Satellite (TESS), which have found 5500+ exoplanets, out of which 247 have an ESI > 0.5. 69 exoplanets are potentially habitable till date as per the Habitability Worlds Catalog.[8] However, calculating Earth Similarity Index, which compares the physical properties of the exoplanet to that of Earth, (ESI) is not enough to determine the likelihood to hold Earth-based life as habitability—an independent criteria of ESI.[9]

The ESI values provide a general idea of possible habitability, but habitability can't solely be concluded based on similar physical properties. Hence to confirm habitability, an independent criterion of the ESI, we need to conduct further in-depth analysis of the exoplanets by comparing more factors that contribute to life's existence, sustenance, and proper environment for it to thrive. Astronomers have used PHI to determine habitability, but certain problems are associated with it. The PHI is defined as the geometric mean of four sub-indexes:

$$PHI = (S.E.C.L)^{1/4}$$

where S is substrate presence, E is available energy, C is the appropriate chemical composition, and L is the presence of a liquid solvent on the particular exoplanet. Each of these variables is subdivided into different parameters (like atmospheric presence, magnetosphere, and redox chemistry) which are considered individually. The problem with PHI is that every parameter is determined through a modulus function (ad-hoc classification) and often combines complementary criteria. For example, the energy received from the star and tidal flexing are both considered together for the determination of E, but these events do not occur simultaneously. Furthermore, the PHI value for Jupiter or Saturn is 0.40 and for Mars, PHI is 0.56. This implies that Mars has a similar planetary habitability potential to that of Jupiter or Saturn, which is not true given the latter two are gas giants while Mars still has a surface. Hence, the SEPHI is used as a new form of PHI calculation that utilizes statistical likelihood. These likelihood functions are determined by Gaussian-like probability profiles (which is the exponential form). Unlike PHI which uses single variables, SEPHI uses comparison criteria (single variable/combination of them) and defines likelihood functions. Additionally, SEPHI does not take into account any free parameter which means that all the comparison criteria in the analysis have equal weight. Adopting SEPHI, with respect to ESI, the results for SEPHI values and ESI values for the exoplanets along with planets from our Solar System have been added to give A reference base for understanding SEPHI's relevance.

METHODS

For determining the four comparison criteria for the SEPHI, Mozos et al. (2017) focused on those describing the basic conditions allowing life on Earth and defined four sub-indexes ($\mathcal{L}$) for SEPHI. The final result was the geometric mean of these four sub-indexes, and it represented the statistical likelihood of a planet being potentially habitable with respect to Earth from an astrobiological perspective.[4] This definition was a statistic as the $\mathcal{L}_i$ were statistically independent:

$$SEPHI = \prod_{i=1}^{n} \mathcal{L}_i^{1/n}$$

The following are the four comparison criteria:

(i) Telluricity ($\mathcal{L}_1$): To be a telluric planet, with part of its surface composed of solid silicates similar to the general composition of Earth modeled as 17% Fe and 83% MgSiO3

(ii) Atmosphere and planet gravity ($\mathcal{L}_2$): Existence of a dense atmosphere that can retain the necessary gasses to sustain life, and the presence of a proper planetary gravity that is compatible with life.

(iii) Surface Liquid Water ($\mathcal{L}_3$): Exoplanets having liquid water on its surface which is determined from the calculation of the Habitable Zone for the particular star system.

(iv) Magnetic Field ($\mathcal{L}_4$): Exoplanets having a strong magnetic field that will be able to protect life from solar radiation.

Participants/Organisms:

The data provided by NASA's MAST archive database was used along with the analysis of previous data on ESI for the three exoplanets. The main data used were the previously calculated parameters that were cross-checked for any updates in the parameters from the NASA Exoplanet Archive.[10,11]

Experimental Design:

Quantitative data from the archive database was used in this paper. This data was observed and collected by KST as part of its legacy Kepler and K2 missions. Additional data was also used from TESS. These data packets were already used in the previous ESI calculations. The calculations conducted in this paper were built on the data used previously such as calculation for relative planetary mass (m_p), relative planetary radius (r_p), relative escape velocity (v_e), stellar luminosity (L_n), relative density (ρ_p), Stellar Effective Temperature (T_{eff}), which forms the basis for calculating the four composition criteria used to determine SEPHI values for each of the exoplanets.[4] These particular criteria are essential to understand whether those basic conditions are compatible enough to allow Earth-based life (carbon-based life) on that exoplanet.

Measurements/Calculations:

Python programming language was used to calculate all four sub-indexes which were included in the SEPHI calculation for the exoplanets. Data, such as Habitable Zone coefficients, and coefficients for magnetic field likelihood calculation were taken from previously published papers.[12,13,14] Each of the four criteria used has the following likelihood function:

$\mathcal{L}_1$: Telluricity likelihood function

From Mozos et al. (2017), we know the following conditions for decay of the likelihood function:

$$\mu_{1,m_p} = r_{p,100\%\,MgSiO_3}$$
$$\mu_{2,m_p} = r_{p,[50\%\,MgSiO_3 - 50\%\,H_2O]}$$
$$\sigma_{1,m_p} = \frac{\mu_{2,m_p} - \mu_{1,m_p}}{3}$$

Figure 1: Relationship between a planet's relative radius and composition parameters. The mean radius for a 100% $MgSiO_3$ composition is denoted as μ_1, m_p, and for a 50% $MgSiO_3$ and 50% H_2O composition as μ_2, m_p . The standard deviation of the planet's telluric likelihood function, σ_1, m_p, is calculated from these mean radii.

where μ_1, m_p, is the relative radius (with respect to Earth) of a planet containing 100% $MgSiO_3$ composition, μ_2, m_p, is the relative radius (with respect to Earth) of a planet containing 50% $MgSiO_3$ and 50% H_2O composition, and σ_1, m_p is the standard deviation σ_1 equal to one-third of the

difference of the relative radius of the planet with the above two compositions which limits $\mathcal{L}_1$, m_p between 0 and 1.[4,15,16,17]

The telluricity likelihood function for a given relative planet mass (m_p) and relative radius (r_p) is:

$$\mathcal{L}_{1,m_p}(r_p) = \begin{cases} 1 & \text{for } r_p \le \mu_{1,m_p} \\ e^{-\frac{1}{2}\left(\frac{r_p - \mu_{1,m_p}}{\sigma_{1,m_p}}\right)^2} & \text{for } \mu_{1,m_p} < r_p < \mu_{2,m_p} \\ 0 & \text{for } \mu_{2,m_p} \le r_p \end{cases}$$

Figure 2: The likelihood function $\mathcal{L}_1, m_p(r_p)$ as Gaussian-like profiles for telluricity based on relative radius r_p with respect to Earth. It also shows the pre-condition for each likelihood function under which a planet can be assumed as telluric, with three conditions, one for r_p being less than or equal to μ_1, m_p, another one for a Gaussian decay of exponential form if r_p is between μ_1, m_p and μ_2, m_p , and lastly, if r_p is greater than or equal to μ_2, m_p .

$\mathcal{L}_2$: Atmosphere and planet gravity likelihood function

From Mozos et al. (2017), the likelihood function for planet gravity being compatible with Earth life is:

$$\mathcal{L}_2(v_e) = \begin{cases} e^{-\frac{1}{2}\left(\frac{v_e - 1}{\sigma_{21}}\right)^2} & \text{for } v_e < 1 \\ e^{-\frac{1}{2}\left(\frac{v_e - 1}{\sigma_{22}}\right)^2} & \text{for } v_e \ge 1 \end{cases}$$

Figure 3: The likelihood function $\mathcal{L}_2(v_e)$ for calculating the ability of an exoplanet to maintain an atmosphere on the basis of relative

escape velocity v_e to that of Earth. The functions are based on Gaussian distribution profiles on two conditions of v_e, with $v_e < 1$ and $v_e >= 1$.

where v_e is the relative planet gravity measured by $\sqrt{g r_p}$, $\sigma_{21}=1/3$ and $\sigma_{22}=7.66/3$.

$\mathcal{L}_3$: Surface Liquid Water likelihood function

The Habitable Zone boundaries D_n depend on the following effective stellar flux (S_{eff}) with coefficients a, b, c and d depending on habitable zone and stellar effective temperature (T_{eff}),

$S_{eff} = S_{eff,\odot} + a(T_{eff} - 5780) + b(T_{eff} - 5780)^2 + c(T_{eff} - 5780)^3 + d(T_{eff} - 5780)^4$

and $D_n = \sqrt{\frac{L_n}{S_{eff}}}$ AU, where Ln is the stellar luminosity in solar units.

The likelihood function is defined on the basis of orbital major axis *a* (4). The following are the defined zones with respect to *a*:

Hot Zone: $a < D_1$

Inner-Transition Zone (ITZ): $D_1 \leq a < D_2$

Green Zone: $D_2 \leq a < D_3$

Outer-Transition Zone (OTZ): $D_3 \leq a < D_4$

Cold Zone: $a > D_4$

The likelihood of holding liquid water if it is near (or within) the zone was calculated based on the planet's location with respect to the corresponding habitable zone:

$$\mathcal{L}_3(a) = e^{-\frac{1}{2}\left(\frac{a-\mu_{31}}{\sigma_{31}}\right)^2} \quad \text{for } a < D_2$$
$$\mathcal{L}_3(a) = 1 \quad \text{for } D_2 \leq a \leq D_3$$
$$\mathcal{L}_3(a) = e^{-\frac{1}{2}\left(\frac{a-\mu_{32}}{\sigma_{32}}\right)^2} \quad \text{for } a > D_3$$

Figure 4. The likelihood function calculates the probability of the exoplanet holding water in liquid form on its surface as a Gaussian-like profile distribution function on the basis of the exoplanet's orbital semi-major axis a. The function returns a constant 1 if the semi-major orbital axis is in the Green Zone of D_2 and D_3. The other two conditions give us the probability of containing surface liquid water, one being the Inner Transition Zone, where $a < D_2$ and the other Outer Transition Zone, where $a > D_3$.

where $\mu_{31}= D_2$, $\sigma_{31}= (D_2 - D_1)/3$ for the ITZ and $\mu_{32}= D_3$, $\sigma_{32}= (D_4 - D_3)/3$ for the OTZ (4).

$\mathcal{L}_4$: Magnetic Field

The likelihood function is defined as:

$$M_n = \alpha \rho^{1/2}_{0n} r^{10/3}_{0n} F^{1/3}_n$$

where r_{0n} (normalized radius) and F_n (normalized average convective buoyancy flux) are defined in terms of two corrections $\beta_1= R_p/R_r$ and $\beta_2= \rho_p/\rho_r$ (related to the size and density of the reference planet) and $\alpha= 1$.[14]

In case of Kepler-504 b, a super earth, $\rho_{0n}= 1$, $r_{0n}= \beta_1$, $F_n= \beta_2$ and in case of of Kepler-315 b and Kepler-315 c, two gas giants, $\rho_{0n}= 0.16$, $r_{0n}= 16\beta_1\beta_2$, $F_n= 100\beta_1\beta_2$.[18,19]

The likelihood function of having a strong magnetic field to protect life from strong solar and cosmic radiation depends on M_n:

$$\mathcal{L}(\mathcal{M}_n) = e^{-\frac{1}{2}\left(\frac{\mathcal{M}_n-\mu_4}{\sigma_4}\right)^2} \quad \text{for } \mathcal{M}_n < 1$$
$$\mathcal{L}(\mathcal{M}_n) = 1 \quad \text{for } \mathcal{M}_n \geq 1$$

Figure 5. The likelihood function $\mathcal{L}_4(M_n)$ calculates the probability, using a Gaussian-like distribution, as to whether an exoplanet has a strong dipolar magnetic field, which is required to protect life from cosmic radiation. The function calculates a Gaussian decay value for a relative magnetic moment less than that of Earth and returns a constant 1 for a relative magnetic moment greater than or equal to that of Earth.

After this, all the four likelihood function values are taken as a geometric mean to arrive at the SEPHI value = $(\mathcal{L}_1, \mathcal{L}_2, \mathcal{L}_3, \mathcal{L}_4)^{1/4}$.

Data Analysis:

In Table 1, the four criteria for the SEPHI along with the final SEPHI value for the exoplanets are shown.

Exoplanet	$\mathcal{L}_1$ Telluricity (in %)	$\mathcal{L}_2$ Relative Gravity (in %)	$\mathcal{L}_3$ Surface Liquid Water (in %)	$\mathcal{L}_4$ Magnetic Field (in %)	SEPHI (in %)
Kepler-504 b	28.52	1.11101015	0	100	0
Kepler-315 b	0	1.11101032	0	100	0
Kepler-315 c	0	1.11101087	82.186	100	0

Table 1: SEPHI values along with each of the likelihood function values of the comparison criteria for exoplanets Kepler-504 b, Kepler-315 b and Kepler-315 c. The values for $\mathcal{L}$ show the probability of the criteria existing for that exoplanet where $\mathcal{L}_1$ is the exoplanet's telluricity, $\mathcal{L}_2$, is the exoplanet's relative gravity, $\mathcal{L}_3$, is the probability to contain liquid surface water, $\mathcal{L}_4$, is the probability to have a strong dipolar magnetic field.

For comparison, the results of SEPHI values along with the corresponding ESI values, host star type, habitable zone, and orbital radius are shown in Table 2. For the calculation of values, Python's numpy library was used.

Exoplanet	ESI (in %)	ITZ (in AU)	OTZ (in AU)	Orbital Radius (in AU)	SEPHI (in %)
Kepler-504 b	71.23	0.1623	0.3250	0.0646	0
Kepler-315 b	69.44	0.9530	1.6803	0.402	0
Kepler-315 c	35.68	0.9530	1.680	0.791	0

Table 2. Comparison table for exoplanets Kepler-504 b, Kepler-315 b, and Kepler-315 c where the ESI, SEPHI along with the Inner Transition Zone (ITZ), Outer Transition Zone (OTZ), and the exoplanet's orbital radii values are shown. These values indicate the exoplanet's similarity to Earth to hold carbon-based life across multiple parameters.

RESULTS

Kepler-504 b, a super earth, has a telluricity likelihood of about 28.52%, while Kepler-315 b and Kepler-315 c being gas giants have 0 telluricity likelihood. For the exoplanet's relative gravity to that of Earth, all 3 of them had comparable values of 0.011110 or 1.111% that of Earth's gravity. Both Kepler-504 b and Kepler-315 b have 0 surface liquid water likelihood. Kepler-504 b having a surface does not harbor liquid water due to its high temperature of 114.84 Celsius (K), 14.84 degrees above the boiling point of water, and this is also confirmed by the $\mathcal{L}_3$ calculation. This high temperature is due to its close orbit with its host star as its orbital radius is at only 0.0646 AU. It was interesting to see that Kepler-315 c being a gas giant has a high liquid water likelihood of 82.1863%. Its temperature is 51.31 Celsius (K) which allows for water (if present) to exist in liquid form. All the 3 exoplanets were more massive than Earth which means that they have a strong dipole magnetic field which is confirmed by their magnetic field likelihood value of 1 (or 100%) for all. The SEPHI value for super earth Kepler-504 b is 0. Both of the gas giants Kepler-315 b and Kepler-315 c also have 0 SEPHI values. Getting even one value as zero among the four sub-indexes will lead to a 0 SEPHI since the geometric mean of these sub-indexes is taken. Hence, a combination of stellar properties and ESI value cannot be used to completely determine habitability. It is necessary to conduct more in-depth analysis on the particular exoplanet such as SEPHI, analyzing certain characteristics like telluricity, location in the habitable zone, surface temperature, surface liquid water, and strength of magnetic field, etc.

DISCUSSION

Previous papers selected G-type and M-type host stars since most of the discovered habitable exoplanets revolve around those star types.[7] The present data suggests that the chances of finding habitable exoplanets around these stars is extremely high and finding the ESI value confirms that. However, by including more variables and comparison criteria, a more detailed analysis like SEPHI shows us that it is not always the case. Habitability cannot be determined

on the basis of stellar properties and ESI values. Further studies are required to determine habitability. The previous claim does give us a sense of general habitability, but, that is not a confirmation of life-supporting capacity for that exoplanet.

For telluricity, the corresponding relative radius of a planet with 100% $MgSiO_3$ and 50% $MgSiO_3$ - 50% H_2O were used given the exoplanet relative mass in terms of Earth. This was a grid search where corresponding radii can be determined as a function of relative earth mass.[15] For relative planet gravity calculation, 100% Fe was used as the conservative reference to get the calculation within the estimation of the highest relative gravity that can be compatible for life to exist.[20]

The most updated equations for determining habitable zones were used.[12,13,14] For magnetic field calculation $\alpha = 1$ was used since the exoplanets studied were more massive than Earth.[19]

The analysis used Python as the core program for calculations. Therefore, it includes rounding-off limitations floating point numbers. The code returns values only up to certain decimal points.

The study must be extended to a larger group of exoplanets. SEPHI should be checked for exoplanets around other star types as well since SEPHI being characteristic and planet-specific might bring out some interesting observations and conclusions about other host-star type exoplanets. There are also parameter restrictions like Telescope gathered data limitation, which introduces uncertainty in the data with regards to planetary properties like orbital radius, surface temperature, and telluricity.

CONCLUSION

This research wanted to find whether stellar parameters and ESI value is enough for determining habitability. However, upon finding the SEPHI values to be zero for all three exoplanets, it is determined that this is not always the case (even for exoplanets with host stars required for life to thrive, proper surface temperature but lack of surface soil telluricity). Hence, habitability should not be declared once we find the conforming stellar types and high ESI values. There is no doubt that a high ESI value suggests a high probability of finding life-supporting capacity. More comparison criteria must be checked for such that the exoplanet should fulfill for it to support carbon-based life.

For future work, both ESI as well as SEPHI values must be analyzed to completely determine habitability. Future advanced telescopes like the present JWST will be more capable of observing the atmosphere which in turn will make calculations more precise and allow for the inclusion of more comparison criteria in the SEPHI and determine habitability more accurately.

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NOTES

The code used for calculating the SEPHI values and each of the comparison criteria can be found in this Github repository: https://github.com/SattikBhaumik/Analyzing_Exoplanets/blob/main/SEPHI.ipynb

ACKNOWLEDGMENTS

I would like to thank CUSJ for the constructive criticism and feedback to make the paper more accessible for others. I would like to thank my parents for their constant support. The author would like to thank the NASA MAST astronomical archive database which gives open access to all researchers to conduct analysis and study the optical, ultraviolet, and near-

infrared.

ABBREVIATIONS

ESI: Earth Similarity Index

PHI: Planet Habitability Index

SEPHI: Statistical-likelihood Exo-Planetary Habitability Index

KST: Kepler Space Telescope

TESS: Transiting Exoplanet Surveying Satellite

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COLUMBIA UNDERGRADUATE SCIENCE JOURNAL

Ascending Arousal Network Connectivity in Disorders of Consciousness: A Diffusion MRI Study

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KEYWORDS: Diffusion-weighted imaging, neuroimaging, ascending arousal network, disorders of consciousness, MRI

ABSTRACT: The human brain gives rise to a great variety of conscious experiences. Patients with disorders of consciousness, a state characterized by the dissociation of awareness and wakefulness, are particularly noteworthy. This study attempts to find key biomarkers of the disorder of consciousness state and discover key regions of the brain that govern consciousness. The focus is on the ascending arousal network—a network of nodes and edges representing connections from the brainstem to subcortical (thalamus, hypothalamus, basal forebrain) nuclei and reaching the cerebral cortex. Previous studies using animal models have demonstrated a high prognostic value of the ascending arousal network in relation to consciousness. This study conducts a diffusion tensor imaging analysis and generates a tract count plot to illustrate differences in connectivity between (N=6) healthy controls and (N=6) patients with chronic disorders of consciousness. Each region of interest was isolated to investigate its specific role and impact on consciousness. A principal component analysis was performed to assess the separability of the two cohorts. The results found each of the regions of interest to be significantly ($p<0.05$) disrupted in patients with disorders of consciousness. They contributed equally to the linear separability of the two cohorts. This is consistent with previous research and hints at the importance of the ascending arousal network in governing consciousness. These changes are likely associated with the many pathological deteriorations associated with an impaired cognitive state, such as neuronal loss, gliosis, and the degeneration of white matter tracts that connect critical areas of the brain involved in consciousness.

INTRODUCTION

The mechanisms of consciousness are characterized by the dynamically changing interplay of neural activities underpinning two factors: human awareness and wakefulness.[1] A myriad of previous studies have attempted to isolate the precise neurobiology and function of the human brain that governs consciousness. Yet, given the brain is an immensely complex system, this realm of neuroscience remains poorly understood.

An increasingly promising biomarker of the conscious state is the structural connectivity within the ascending arousal network (AAN).

Knowledge of the AAN used to be largely based on past studies using animals with experimentally induced lesions.[2,3,4] Recent research reveals similarities between the human AAN and that of animals. A mapped neuroanatomic connectivity of the human AAN revealed definite nuclei connections that mediate wakefulness. The AAN is now believed to consist of neuronal projections from the brainstem that extend to subcortical (thalamus, hypothalamus, basal forebrain) nuclei before reaching the cerebral cortex. Thalamic pathways are a key relaying center through which brainstem neurons pass to the cerebral cortex, and the thalamus helps to integrate and modulate these nuclei interactions.[5]

The hypothalamus, along with the basal forebrain further mediates wakefulness by regulating various autonomic functions and circadian sleep-wake cycles. In the end, the AAN is a network of nodes representing gray matter nuclei, beginning with arousal (wakefulness) pathways in the brainstem and connecting to awareness networks in the cerebral cortex.[5,6,7]

The human brain gives rise to a great diversity of conscious experiences based on the varying extents of awareness and wakefulness. In this spectrum, patients in pathological or pharmacological coma experience the least conscious state. They are characterized by a loss of both awareness and wakefulness to the surrounding environment, usually as a result of severe brain injuries.[1] Among those who survive, 44-45% remain in a disorder of consciousness (DoC), such as a vegetative state (VS) or minimally conscious state (MCS).[8] These are unique instances where awareness and wakefulness are disassociated. Patients with DoC have their eyes wide open and are awake but are not aware of and cannot voluntarily interact with their surrounding environment.[1] This is supported by several functional MRI-based approaches, which found preserved cognitive processing and willful modulation of brain activity in DoC patients, indicating that behavioral unresponsiveness might not always equate to a complete absence of cognitive function.[9,10] This unique dissociation of awareness and wakefulness allows studies to isolate awareness from wakefulness and identify specific correlates of each. For instance, there has been a postulation that the default mode network is compromised in DoC patients due to a loss of awareness while structures integral to wakefulness appear to be maintained.[11]

The prognostic importance of the AAN has become particularly evident in comatose patients. Acute damage to the brainstem is a hallmark of these patients, likely a consequence of a lesion centered in the upper pons or in the midbrain which disrupts AAN pathways.[12] Consistent with this, traumatic coma has met the criteria for a disconnection syndrome with a complete disconnection of brainstem arousal nuclei from previously mentioned subcortical nuclei.[7,8] In addition, connectivity between the temporal lobe and the medial thalamus and rostral brainstem seems to be linked to the loss of consciousness associated with temporal lobe epilepsies.[13] A disassociation of awareness and wakefulness is detected during seizures, although this is a much more transient example.[1]

Here, we further test the AAN as a regulator of consciousness using a cohort (n=6) of patients suffering from chronic DoC following traumatic brain injury (TBI) and comparing them with a cohort of (n=12) healthy controls. We use previously published high-angular resolution diffusion imaging (HARDI) data to evaluate AAN connectivity in each cohort.[8] HARDI captures the architecture of white matter fibers in the brain and offers a detailed representation of neural pathways including key intersecting and diverging regions. With the HARDI data, we conducted diffusion tensor imaging (DTI) and tractography analysis to determine differences in AAN connectivity between patients with chronic post-traumatic DoC and healthy controls. Finally, we performed a principal component analysis (PCA) to highlight the axes accounting for the most variance between the cohorts.

METHODS

Patients

Previously published HARDI data of six patients with chronic DoC was obtained from OpenNeuro dataset 003367.[14] Among them, three were diagnosed with VS and three with MCS on the Coma Recovery Scale-Revised (CRS-R, total = 6.5) following TBI. Similarly, HARDI data of 12 healthy controls with no recorded psychiatric or neurological disorders were attained. Details of the HARDI sequence can be found in previous research.[14] Note specifically the 2 mm isotropic resolution, 60 diffusion-encoding directions, and contrast

of b = 2,000 sec/mm2.

Processing

All preprocessing was done using FSL and MRtrix3. Brain extraction (0.2 thresholds), eddy current, and bulkhead motion correction were performed. Linear registration was used to transform each patient's diffusion space to Montreal Neurological Institute (MNI) 152 T1 1 mm space. To guide the tractography, fiber response functions were estimated using the Tournier algorithm, and fiber orientation distributions (FODs) were computed. A white matter mask was also generated to confine the whole-brain tractography. Finally, diffusion tensor imaging (DTI) parameters were calculated specific to the entire brain and AAN regions of interest (ROIs). Metrics such as Fractional Anisotropy (FA), Mean Diffusivity, Axial Diffusivity, and Radial Diffusivity were derived.

Regions of interest

A combination of previously published ROIs, the Harvard Ascending Arousal Network Atlas, and the Harvard-Oxford cortical and subcortical structural atlases included within FSL were used.[14] The thalamus, hypothalamus, and basal forebrain were taken from publicly available ROIs. The cortical lobes were generated from the Harvard-Oxford cortical and subcortical structural atlases, and brainstem ROIs from the Harvard Ascending Arousal Network Atlas. All ROIs matched the MNI 152 T1 1 mm space.

Tractography

Whole brain tractography was first generated, guided by a white matter mask. Three million streamlines were launched and dynamic seeding based on the FODs was used. Then, a second tractography was created only including tracts connecting AAN brainstem ROIs to the thalamus, hypothalamus, and basal forebrain. Here, a threshold of 100 mm was set as the maximum length of streamlines. Otherwise, default parameters were used. Finally, a voxel-wise map representing the number of streamlines passing through each voxel in the AAN tractography was generated, normalized by the total number of streamlines launched. The exact path of the streamlines within each voxel was accounted for and the results were set to unsigned 32-bit integers. Finally, tract-specific analyses of DTI parameters were performed, and the Benjamini-Hochberg method was used to control false discovery rates.

Principal component analysis

To assess the separability of chronic DoC patients from healthy controls, a PCA was conducted in R Studio with connectivity in each AAN ROI as the dimensions. Principal components 1 (PC1) and 2 (PC2) were projected on the same axes along with the eigenvectors for each ROI to explain the major patterns in the data while preserving the maximum variance. The associations of each ROI with PC1 and PC2 were determined by computing their respective loadings using the formula $L = v \cdot \sqrt{\lambda}$, where L is the loading, v is the component of the eigenvector of the ROI on the axis of the PC, and λ the eigenvalue of the PC.

RESULTS

To visualize AAN connectivity between controls and chronic DoC patients, we generated normalized tract plots of streamlines passing between all subcortical AAN structures. The axial and sagittal views are shown (Figure 1).

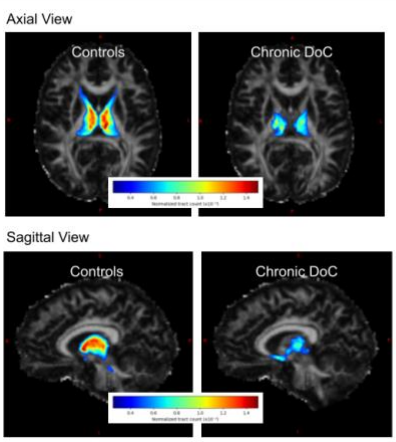

Figure 1. Differences in AAN connectivity between healthy controls and patients with chronic disorders of consciousness.

Connectivity was measured by the group-sum value of the number of streamlines between all subcortical AAN structures (brainstem to thalamus, hypothalamus, and basal forebrain), normalized by the total number of streamlines launched (3M).

With tracts from the brainstem extending fully into the thalamus and hypothalamus regions, controls exhibit much more robust and widespread connectivity than chronic DoC patients. The latter's lack of tracts indicates greatly reduced AAN connectivity, with narrower areas of activation, fewer pathways, and reduced communication between all AAN ROIs.

To determine connectivity in each AAN ROI, a boxplot was created with the tract count of each control and DoC subject (Figure 2). In all of the hypothalamus, basal forebrain, thalamus, and brainstem regions, we observed that the normalized tract counts appear to be significantly ($p<0.05$) lower in the chronic DoC cohort compared to the controls.

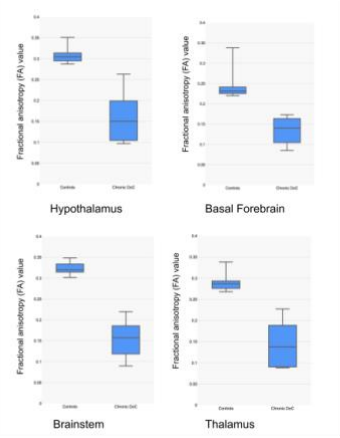

Figure 2. FA values in AAN ROIs of healthy controls and patients with a chronic DoC, $p<0.05$. This whiskers-only box plot shows connectivity, measured by the fractional anisotropy values obtained from diffusion tensor imaging analysis. The differences in all four box plots are significant for the threshold $p<0.05$.

In all four box plots and for both cohorts, the extended whiskers indicate a wide distribution of data. For healthy controls, the variability could reflect natural differences in brain architecture and connectivity that occur within a normal population. On the other hand, the variability in the chronic DoC group likely represents the diverse impacts of various pathologies associated with disorders of neural connectivity.

The box plots for the hypothalamus and thalamus show a notably lower median tract count in chronic DoC patients compared to controls, with the median line of the DoC group visibly closer to the first quartile than the third. This shift in the median indicates that the majority of chronic DoC patients have a lower connectivity profile compared to the median of the control group. Furthermore, the presence of a wider range in the chronic DoC group's data suggests that some patients retain a degree of connectivity closer to healthy norms, while others are significantly more affected.

Finally, we performed PCA with the AAN ROI tract count values to assess the separability of chronic DoC patients from healthy controls (Figure 3).

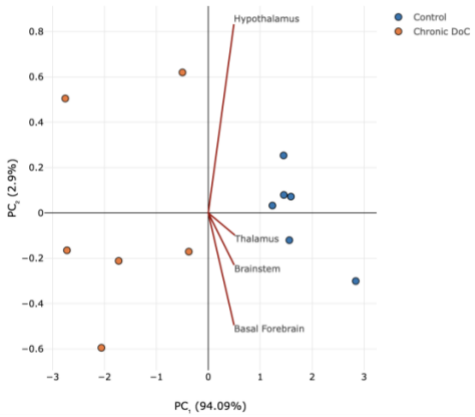

Figure 3. Principal component analysis of structural connectivity among AAN ROIs in healthy controls and patients with disorders of consciousness. The vectors represent the loadings of each AAN ROI onto the principal components. The first principal component explains 94.09% of the variance in the data while the second explains 2.89%. The third and fourth principal components account for 2.43% and 0.58%, respectively. The orange dots represent chronic DoC patients while blue are the controls. PC1 = Principal Component 1, PC2 = Principal Component 2.

With the controls clumped on the right y-axis and DoC patients spread out on the left, the results demonstrate clear linear separability between these two cohorts. The diffuse nature of the DoC patient data, in contrast to

the controls, mirrors the wide range of the box plots. The dominance of PC1, explaining 94.09% of the total variance, suggests structural connectivity differences within the AAN heavily contribute to the total variance in the data. Furthermore, we calculated the absolute loadings of each AAN ROI to determine each ROI's influence in separating chronic DoC patients from healthy controls (Table 1).

ROI	PC_1 Loading	PC_2 Loading
Hypothalamus	0.9580	0.2833
Thalamus	0.9906	-0.0333
Basal Forebrain	0.9634	-0.1686
Brainstem	0.9684	-0.0782

Table 1. Contributions of each AAN ROI to PC1 and PC2. Loadings of PC1 and PC2 for AAN ROIs were extracted from the PCA results and computed using the formula $L = v$, where L is the loading, v is the component of the eigenvector of the ROI in the axis of the PC, and the eigenvalue of the PC. Higher absolute loadings indicate a stronger contribution of the ROI to the respective PC.

The similar loadings on PC1 demonstrate approximately equal contributions from each ROI. It follows that connectivity within the AAN in DoC patients is very distinct from healthy controls, with the former exhibiting significantly fewer tracts and experiencing crucial disruptions in all AAN ROIs. On PC2, the hypothalamus is notably higher than all other ROIs. This holds little weight given PC2 accounts only for 2.89% of the total variance. The importance of PCA is simply its demonstration that there is clear separability between the two cohorts based on AAN connectivity, with each AAN ROI contributing relatively equally to this variance.

DISCUSSION

This study demonstrated that connectivity within the AAN is significantly disrupted in the chronic DoC cohort and that this separates these patients from the healthy controls. Great disruption in all subcortical structures (hypothalamus, basal forebrain, thalamus) and the brainstem was found. These results are consistent with previous research analyzing connectivity differences between these groups, as well as studies aiming to find neural correlates of consciousness.

The disruptions observed in the ROIs within the AAN in patients with DoC in this study can be attributed to the specific pathophysiological changes associated with chronic DoC states. In chronic DoC, the brain often undergoes significant structural changes that can lead to impaired connectivity. These changes may include neuronal loss, gliosis, and the degeneration of white matter tracts that connect critical areas of the brain involved in consciousness.[15]

This disruption is determined by the decrease in the number of intact white matter tracts, which could be attributed to neural damage or degeneration caused by the underlying pathology of chronic DoC. Tract counts refer to the number of neural connections, or white matter tracts, that are identified between the different regions of the brain. These tracts are made up of axons, long, thread-like parts of a nerve cell where impulses are conducted from the cell body to other cells.[16] It follows that tracts are pathways for neural signals to travel and communicate between. Higher tract counts have been found to be correlated with better cognitive and functional outcomes. Furthermore, tract counts are a common biomarker to assess the integrity of the brain's white matter.

The wide distribution of tract counts within both groups, but especially among chronic DoC patients, raises important questions about the heterogeneity of the disorder. In DoC patients, this variability may be due to differences in the etiology of the condition, the extent of brain damage, the duration of the disorder, or individual variations in the capacity for neural plasticity and recovery. Often, a result of cognitive impairments such as DoCs are axonal injuries, which are characterized by widespread damage to the white matter tracts of the brain.[17] Understanding the sources of this

variability is critical for developing personalized approaches to treatment and care for DoC patients.

Moreover, the results suggest that some chronic DoC patients retain a level of connectivity that approaches that of healthy controls. This finding opens several lines of inquiry: it may indicate the potential for recovery in some individuals, or it could reflect the presence of alternate neural pathways that compensate for lost connections. Further research is needed to explore these possibilities and to determine the clinical significance of these retained connections. Potentially, tract counts can be considered biomarkers for the recovery of DoC patients.

The investigation of the AAN has great value given the significant differences in connectivity in each AAN ROI. The hypothalamus is particularly susceptible to disruption in DoC patients due to its central role in maintaining the body's internal balance and arousal states. The hypothalamus regulates autonomic and endocrine functions, including the sleep-wake cycle.[5] Lower tract counts in the hypothalamus may be indicative of damage or dysfunction in this region, which could be a result of traumatic brain injury, or hypoxic events, in addition to a DoC. Such disruptions can directly impair the hypothalamus's ability to contribute to arousal mechanisms, thereby affecting the patient's ability to maintain consciousness. The specific vulnerability of the hypothalamus to disruption in DoC patients could also be attributed to its extensive network of connections with other brain regions involved in consciousness, including the thalamus, basal forebrain, cerebral cortex, and brainstem.

In the thalamus, lower tract counts observed in DoC patients may be related to its role as the primary relay station for sensory information to the cortex. The thalamus's involvement in consciousness extends to its participation in thalamocortical loops, which are essential for cognitive functions, including awareness.[5] Previous research has identified the thalamus as a critical neural correlate of dexmedetomidine-induced unconsciousness, with decreased rates of glucose and cerebral blood flow.[18] In addition, similar studies investigating mild cognitive impairments show a disrupted thalamus white matter anatomy.[19] Damage to the thalamus is quite commonly seen in neuropsychiatric disorders and could stem from a variety of causes, including a direct injury, degeneration of connecting white matter tracts, or secondary effects of cortical damage.[20,21,22] The vulnerability of the thalamus in DoC patients highlights the importance of intact sensory processing pathways for the maintenance of conscious awareness.

The brainstem is critical for its involvement in the reticular activating system, which regulates arousal and wakefulness.[5] Lower tract counts in DoC patients here may be because of its susceptibility to direct injury or as a consequence of secondary brain injury mechanisms such as edema or increased intracranial pressure. The brainstem's role in basic life-sustaining functions also makes its disruption common in diffuse brain injuries.[12]

The first limitation of this study is rooted in the static nature of the box plots and tractography, providing a visualization of connectivity but not capturing dynamic changes over time. Longitudinal studies would be beneficial to understand how connectivity patterns in DoC patients evolve and whether they correlate with changes in clinical status. Secondly, the normalization of tract counts allows for comparison between groups but may mask absolute differences in connectivity that could be clinically relevant. Approaches that involve additional analyses complementing the normalization are recommended as future studies.

The implications of these findings extend beyond the scientific understanding of chronic DoC. These results can inform clinical practices. From this study, the AAN is a potential biomarker for the diagnosis and prognosis of DoC. Given this information and the role each AAN ROI

plays in governing consciousness, therapeutic interventions aimed at specifically enhancing connectivity in the regions of the AAN may arise. Furthermore, there may also be a need for individualized treatment plans, hinted by the great variability in connectivity observed in the DoC cohort. A personalized medicine approach to treat DoC patients targeting specific AAN connections or neural pathways may have strong potential benefits in the path to recovery.

CONCLUSION

The results presented in this study contribute to a growing body of evidence that suggests structural connectivity is fundamentally altered in chronic DoC. These alterations have significant implications for the clinical management of the disorder and for our theoretical understanding of consciousness. Further research should first apply more rigorous statistical techniques and machine learning on a larger dataset to validate the findings of the current study and analyze the connectivity of AAN ROIs in junctions rather than treating them as independent entities.

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Author Contributions

Darwin Li conceptualized the study, collected and analyzed data, and wrote the manuscript.

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COLUMBIA UNDERGRADUATE SCIENCE JOURNAL

Recent Progress in Engineered Oncolytic Viruses: A Thematic Review

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KEYWORDS: Cancer Therapy, Oncolytic Viruses, Medicine, Oncology

ABSTRACT: This review examines recent research in engineered oncolytic viruses and applies a thematic analysis to identify trends in the field. The major themes identified are tumor microenvironment remodeling, enhancing virus efficiency, treating slow-growing tumors, tumor targeting specificity, and combination with chimeric antigen receptor-T cell therapy. The tumor microenvironment plays a significant role in cancer progression through factors like immunosuppression and hypoxia. Engineered oncolytic viruses may be used to remodel aspects of the tumor microenvironment to favor and facilitate an immune response. Another major consideration in the development of oncolytic viruses is their efficiency in inducing antitumor effects. Viral vectors may be engineered with pro-apoptotic or pro-inflammatory signaling molecules to cause cancer cell death or mediate immune cell infiltration, respectively. Slow-growing tumors present a challenge to oncolytic virotherapy since many viruses infiltrate tumors through infected daughter cells arising from cancer cell division. This challenge may be overcome by engineering viruses to maintain a high viral load in infected cancer cells and drawing a sustained immune response. A concern in the development of oncolytic viruses is the issue of viral tropism and infection specificity. Although some viruses have limited tropism, oncolytic viruses may be engineered to specifically target tumors using cancer-specific receptor-ligand mechanisms. Oncolytic viruses may also be used in conjunction with chimeric antigen receptor-T cell therapy to reduce immunosuppression and enhance chimeric antigen receptor-T cell infiltration of tumors. This poses a promising approach in oncology research and treatment.

INTRODUCTION

Cancer is a class of diseases characterized by the uncontrolled proliferation of cells in the body. Affecting approximately 20 million people globally, cancer has one of the highest mortality rates with 10 million deaths per year, posing as a significant issue in the medical field. Cancer incidence is exacerbated by the lack of non-invasive and side effect-free treatment options. While treatments like chemotherapy may be effective at combating cancer, the side effects take a toll on patients.[1] There are various types of cancer, each with its own genetic causes and clinical manifestations, which makes developing treatments more difficult, as an approach that is effective against one type of cancer may not be effective against another type. For this reason, more therapeutics for cancer are vital.

Engineered oncolytic viruses (OVs) are viral vectors designed to damage and destroy cancer cells. OVs can be developed using a number of different theoretical approaches and experimental modifications. Once OVs have been engineered and undergo basic testing, they can advance to animal models and clinical trials. In the past five years, there has been an increasing interest in OVs due to the treatment method's novelty and comparative safety, which has led researchers to perform studies taking different approaches to advancing the knowledge and repertoire of effective OVs for clinical application.[2] It is useful to review recent advances in the development of engineered OVs to determine contemporary research trends. This analysis can be used

to make predictions about possible directions that OV research may take in the future.

In this review, a total of 42 primary research articles were analyzed. The majority of analyzed articles involve the engineering and testing of OVs, but clinical trials and theory papers are also included. Among the virus engineering articles, herpes simplex virus (HSV) was the most represented (Table 1). This is due to the advantages that HSV possesses as a vector: it does not integrate into the host genome since it is a double-stranded DNA virus and possesses a large genome that can serve as a stable base for genetic modifications. Adenovirus and vaccinia virus were also well represented, with approximately half the number of articles as herpes simplex virus. Several other viruses were also utilized in the analyzed studies, including poliovirus and bacteriophage T7.

Viral Vector	Number of Studies
Herpes simplex virus	15
Adenovirus	9
Vaccinia virus	7
Vesicular stomatitis virus	3
Newcastle disease virus	2
Reovirus	1
Measles virus	1
Zika virus	1
Maraba virus	1
Poliovirus	1
Myxovirus	1
Bacteriophage T7	1

*A number of the viruses counted were one of multiple viruses used in a single study.

Table 1. Viral vectors represented in analyzed studies

Five major themes or approaches were identified in the included studies. First, remodeling of the tumor microenvironment led to improvement in immune cell infiltration. This approach allowed the immune system to target and destroy cancer cells with its activated response. Second, additional modification of engineered OVs further improved their antitumor effect. This strategy utilized the variety of OVs already designed by researchers and advanced their work. Third, specialized OVs showed antitumor effects in the treatment of slow-growing tumors. Viral replication or antitumor effects that relied on cancer cell division was ineffective in slow-growing tumors. Fourth, targeting of cancer cells by viruses or immune cells were improved using signaling molecules. Increasing infection specificity improved the effectiveness of OVs, as well as reducing the severity of potential side effects. Fifth, oncolytic virotherapy in conjunction with chimeric antigen receptor-T (CAR-T) cell therapy shows improved antitumor effectiveness. The theme of combinatorial therapy can be applied to other treatments as well, with the hope that the anti-tumor effect will be greater than either treatment alone.

Tumor Microenvironment Remodeling:

When cancer cells accumulate and form tumors, they create a local environment that decreases the effectiveness of the immune system in infiltrating the tumor and destroying the cancer cells.[3] One of the primary effects of this tumor microenvironment is the prevention of natural killer (NK) cells from infiltrating the tumor mass. NK cells are one of the immune system's major cell types, and they can target and destroy cancer cells. In a healthy individual, NK cells will identify and eliminate any abnormally dividing cells. In an individual with cancer, NK cells are unable to overcome the cancer cells. Other components of the immune activity may also become inhibited, allowing cancer cells to proliferate and cause metastasis. Therefore, remodeling and modifying the tumor microenvironment to facilitate targeting of tumor cells by NK cells could be a design goal for engineered OVs. Currently, there is a well-known engineered OV on the market known as talimogene laherparepvec (T-VEC), a clinical treatment that utilizes an engineered herpes simplex virus to combat cancer. It was thought that T-VEC

functioned through direct oncolysis, or the virus infecting cancer cells and causing them to die. Ramelyte and colleagues showed that T-VEC does not directly cause oncolysis after conducting a phase I clinical trial of cutaneous B cell lymphoma patients. Instead, research found that the treatment alters the tumor microenvironment and allows the patient's immune cells to infiltrate and attack the tumor. The researchers examined tumor appearance, sequenced cell genetics, and identified immune cells present in biopsy samples. They confirmed that the treatment reduced tumor scope, both in the tumor at the injection site and in distant tumors. They also found that multiple types of immune cells began to infiltrate the primary tumor as the treatment progressed. Lastly, they found that the viral genetic script was present in both cancer cells and healthy cells, indicating a nonspecific infection mechanism.[4]

These findings provide key insights into the significance of the tumor microenvironment and viral design in engineering effective OVs. Ramelyte and colleagues demonstrated that it was not necessary for an OV to directly destroy cancer cells. Instead, the OV could modify the tumor microenvironment and allow the immune response to take effect. This provides a mechanism for engineering OVs that would not infect healthy cells to a significant degree. One of the major strengths of OVs and modification of the tumor microenvironment is the specificity and efficacy that can be achieved relative to more traditional treatment approaches. Additionally, the tumor microenvironment contains many cell types and active pathways that can be investigated and targeted by future studies, potentially showing anti-tumor effectiveness that further improves on preexisting OVs or targets other cancer types.

The microenvironment remodeling approach has been utilized by a number of research groups addressing various pathways and cell types present in the tumor microenvironment. Cervera-Carrascon and colleagues developed an oncolytic adenovirus that causes infected tumor cells to release two key cytokines into the tumor microenvironment.[5] The cytokines signal local cytotoxic T cells to approach and target the tumor. The antitumor effectiveness of the adenovirus is enhanced by the addition of immune checkpoint inhibitor treatment that activates the immune response. Lin and colleagues engineered an oncolytic herpes virus that releases damage-associated signals to facilitate antigen presentation by dendritic cells and incite an immune response.[6] Rather than counteracting immunosuppressive signals, this approach utilized antigen presentation to incite infiltration of the tumor by immune cells. Gentile and colleagues created an oncolytic herpes virus that causes infected tumor cells to express adenosine deaminase, an enzyme that breaks down extracellular adenosine—an immunosuppressive signaling molecule.[7] The breakdown of adenosine in the tumor microenvironment allows local immune cells to become activated and attack the tumor cells.

Keshavarz and colleagues developed an oncolytic Newcastle disease virus transported by mesenchymal stem cells to deliver apoptosis signaling molecules to tumors.[8] Upon binding to the appropriate receptors in the tumor cells, the apoptosis signaling molecules will cause the cells to undergo cell death. Each of these studies used biochemical pathways as a mechanism of action for OVs to modify the signaling molecule repertoire present in the tumor microenvironment. Other researchers have built on the mechanism behind T-VEC's function, as in the case of the oncolytic herpes virus engineered by Haines and colleagues to not infect healthy cells but still trigger immune recognition of the tumor.[9] This is a significant advance in OV engineering as it improves on a virus in clinical use by increasing infection specificity.

The tumor microenvironment also includes several cell types that are normally found in a healthy body but may be present within a tumor and closely associated with cancer cells. Kurisu and colleagues used an oncolytic reovirus to target and destroy

tumor-associated fibroblast cells, which contribute to the immunosuppressive environment created by tumors.[10] The researchers also found that the OV did not harm healthy fibroblast cells, indicating a level of infection specificity. Infection specificity is important in this case since the OV directly causes fibroblasts, a cell type that also naturally exists in the body, to lyse and die. However, it poses potential risks and side effects since an OV with imprecise action could destroy healthy cell populations.

Another interesting method of approaching tumor microenvironment remodeling is to utilize a viral vector that would not normally infect human cells. Bacteriophages are viruses that infect bacterial cells instead of animal or plant cells. Like other classes of viruses, bacteriophages can also be genetically and structurally engineered. The challenge of using a bacteriophage as the vector for OV engineering is that it does not naturally infect cancer cells. Instead, they must be engineered to target and infect cancer cells through signaling or receptor interactions. Hwang and Myung engineered bacteriophage T7 to express a peptide that contributes to tumor growth inhibition and immune cell infiltration of tumors.[11] This result indicates that bacteriophages are viable bases for OV engineering and that additional studies could explore this class of viruses.

Remodeling of the tumor microenvironment holds massive potential for engineered OV research. T-VEC has already been implemented as a clinical treatment and has shown success in the clinical setting. Microenvironment remodeling also allows immune cells to infiltrate tumors and destroy cancer cells. This approach is generally safe since the direct cause of cancer cell death is the immune response. Additionally, there are a few signaling pathways that are upregulated in cancer cells but not in healthy cells. Upregulating or downregulating these pathways appropriately could disrupt the tumor microenvironment and reduce immune exclusion.

Enhancing Virus Efficiency

Engineered OVs are designed using one or more genetic and structural modifications to cause an antitumor effect. OVs with one or more such improvements can be further improved by making additional modifications that enhance efficacy without compromising safety. These modifications can take the form of increased levels of surface proteins or signaling molecules, as well as directly causing the death of cancer cells.[12] Enhancing modifications could also address different barriers to antitumor effectiveness, such as signaling from tumor cells or the immune system containing and destroying OV particles before they can take effect and destroy cancer cells. Some cancer types, such as acute myeloid leukemia, show limited susceptibility to OVs and require more effective viral vectors and active agents.

One molecule utilized by engineered OVs to cause an antitumor effect is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). This immune factor can be expressed on the surface of OVs and binds to TRAIL receptors on the surface of cancer cells to initiate a signaling pathway that causes cell death. Wang and colleagues aimed to enhance an engineered OV with additional TRAIL to cause a greater antitumor effect.[13] The researchers had previously engineered an OV to express TRAIL on its protein shell that showed antitumor activity. They recognized that additional TRAIL could be bound to their OV through a structural attachment and experimentally achieved this through a zipper domain. This addition of TRAIL improved the antitumor effectiveness of the OV against acute myeloid leukemia when compared to the first iteration of their TRAIL-enhanced OV.

Certain types of cancer, including acute myeloid leukemia, may be resistant to the effects of TRAIL signaling. The mechanism behind this resistance is a reduction of TRAIL receptors in the cancer cells, leading to fewer opportunities for TRAIL on the OV to bind and initiate the signaling pathway. Due to the nature of this resistance

mechanism, increasing the availability of TRAIL is not sufficient to induce cell death in cancer cells. Ginsenosides, compounds derived from ginseng, were previously shown to increase the availability of TRAIL receptors in cancer cells.[13] Wang and colleagues investigated the role of the ginsenoside Rh (steroid glycosides) in amplifying the antitumor effect of their TRAIL-enhanced OV. They found that Rh activity led to greater expression of TRAIL receptors on the surface of cancer cells. In conjunction, the inclusion of Rh in the treatment course increased cancer cell death as TRAIL had more receptors to bind to. This demonstrates that OV enhancement can be further improved using synergistic treatment with other compounds, including natural compounds as in the case of ginsenosides.

Other research groups have taken the approach of enhancing viral efficiency by focusing efforts on different structural and genetic targets. Menotti and colleagues modified an engineered herpes simplex virus to target cancer cell-specific receptors by inserting different single chain antibodies into the viral protein structure.[14] This modification increased the number of cancer cells that can be targeted by the OV since different antibodies could be inserted, making that virus more useful across more cancer types. Kim and colleagues increased viral expression of interleukin-12 and granulocyte-macrophage colony-stimulating factor to amplify immune response.[15] More cancer cells will be destroyed if more immune cells are activated and localize to the tumor site. Rivadeneira and colleagues engineered an oncolytic vaccinia virus to express the adipokine leptin in infected tumor cells.[16] Furthermore, Leptin activates a signaling pathway that contributes to an increased T cell response and prolonged immune memory. These studies demonstrate the variety of methods that are available for enhancing OVs.

A major barrier to achieving greater viral efficiency is the containment and destruction of OVs by the human immune system. If the immune response is activated by the presence of viral particles in the body, the OV will not have any significant antitumor effect since the virus will not reach the tumor in sufficient quantity. Atasheva and colleagues addressed this issue through the development of an oncolytic adenovirus that avoids immune response and containment by mutating the region of the viral protein that host antibodies bind to.[17] The resulting OV was able to cause a systemic antitumor effect without being hindered by the immune response since antibodies could no longer bind to the virus. This study highlights the need for OVs engineered to avoid triggering an immune response from the host organism, particularly as OVs become increasingly effective at destroying tumors. In a similar manner, Bah and colleagues engineered an oncolytic measles virus to better avoid targeting by the immune system by replacing key surface glycoproteins with those from another virus.[18] This is another method by which OVs can be engineered to demonstrate stealth against the immune system.

While further laboratory studies are being performed, a few OVs have also moved into the clinical phase. Zhang and colleagues performed phase I and II clinical trials on the antitumor effectiveness of an oncolytic herpes simplex virus designed to express granulocyte-macrophage colony-stimulating factor, which enhances immune response.[19] The researchers found that the OV treatment was effective in metastatic, treatment-resistant esophageal and rectal cancer. The addition of an antibody for a tumor cell receptor that suppresses immune activity enhanced the oncolytic effectiveness of the virus. Side effects observed in the patients were relatively mild, and improved immune infiltration of the tumor was demonstrated. These results are significant because they show that OVs that are not effective against certain types of cancer may be enhanced to become effective. Fares and colleagues found that an oncolytic adenovirus showed antitumor effect in a phase I clinical trial of glioblastoma patients.[20] Glioblastoma is a very aggressive cancer type, and results indicating antitumor activity could be indicative of the potential for clinical

application after additional research is performed.

Enhancing preexisting OVs provides more effective therapeutics. Due to the recent interest in oncolytic virotherapy, many different OVs have been engineered with a few entering clinical trials.[12] These OVs that have already shown antitumor effectiveness could be made more effective through different engineering approaches and antitumor mechanisms. Therefore, future research is not limited to the development of entirely novel OVs. It may also potentially be more efficient to push enhanced OVs into clinical trials if the parent strain was already in clinical trials.

Treating Slow-Growing Tumors

Different cancer types have different pathophysiology and rates of metastasis. Tumors grow at different rates depending on the type of cancer, anti-cancer immune response, and other host-related factors. The growth of tumors are directly the result of cancer cell proliferation and accumulation. Engineered OVs are able to target tumors that grow quickly by spreading through tumors via daughter cells produced by cancer cell division.[21] Slower-growing tumors undergo cell division at a slower rate, and therefore OVs that primarily proliferate in a tumor through cell division are not as effective at combating the cancer cells. Additionally, given that slow-growing tumors usually take an extended period of time before becoming significantly dangerous to patients, there is more time to administer treatments, and treatments that could be very effective in the long-term may have more utility than those of the short term. Slow-growing tumors may also exhibit treatment resistance, which necessitates OV engineering that overcomes resistance mechanisms.

OVs require additional engineering and investigation to move towards the development of novel OVs that utilize mechanisms of action that are effective against slow-growing cancers. Fukuhara and colleagues sought to develop an OV that could be an effective anti-cancer agent in slow-growing tumors. They engineered an oncolytic herpes simplex virus known as T-hTERT which retains the infection specificity of its parent strain while preventing protein synthesis shutdown in infected cancer cells, leading to virus proliferation that does not damage healthy cells. The researchers found that the OV was effective against slow-growing tumors by maintaining a high viral load despite lower cell division rates.[22] Additionally, they demonstrated that the virus was safe for application in brain tissue, which is quite susceptible to impairment due to tissue damage. These findings indicate that OVs utilizing similar mechanisms may be suitable for clinical use in the treatment of slow-growing tumors. OV effectiveness is shown to not be limited to fast-growing tumors and can be effective against slow-growing tumors given the necessary modifications. The researchers also showed that the OV is safe for healthy tissue and may not pose a significant risk to the host organism.

Other researchers have investigated the possibility of OVs that function effectively in slow-growing tumors or have the potential to be useful towards the development of such OVs. Cao and colleagues engineered an oncolytic vaccinia virus that draws macrophages towards infected tumor cells.[23] The involvement of another immune cell type increases the number of possible OV mechanisms that could be utilized by researchers. Liu and colleagues engineered an oncolytic herpes simplex virus to express cytosine deaminase for the treatment of uveal melanoma.[24] This OV was designed to treat a rare and chemotherapy-resistant cancer type, demonstrating the need for OVs for uncommon cancers. Khalique and colleagues created an oncolytic herpes virus that targets immunosuppressive tumors while simultaneously drawing an immune response to cancer cells.[25] Multi-function OVs could be effective against slow-growing tumors, particularly if the tumors grow at higher or lower rates over a period of time. Leoni and colleagues developed a non-cancer cell line for the development of OVs.[26] This cell line could be a useful tool in the engineering of more OVs, particularly OVs designed for slow-growing cancers.

Some research groups have worked towards the development of anti-cancer vaccines which enhance long-term, antitumor immune activity through the activity of viral vectors. Roy and colleagues furthered the approach of engineering OVs that do not rely on division to be effective. The researchers developed an anti-cancer vaccine utilizing a number of viral vectors with antigenic peptides. The separation of these peptide components allowed for anti-cancer immunity enhancement without requiring cell division.[27] Tian and colleagues also developed an anti-cancer vaccine using a herpes simplex virus to carry a monoclonal antibody.[28] This anti-cancer vaccine also downregulates cell adhesion, reducing the ability of a tumor to exclude immune cells. Priming the immune system to combat cancer cells has significant potential in reducing cancer incidence at an earlier stage of disease development. Additionally, increasing the natural immune response to cancer in individuals with a predisposition to cancer development could improve patient outcomes.

Improving immune memory through increasing memory cell counts or extending memory cell survival also contributes to viral effectiveness against slow-growing tumors. As a tumor grows, the immune memory of that cancer type could trigger a response and draw immune cells to the tumor. Thomas and colleagues developed an oncolytic herpes simplex virus that improves immune memory after responding to tumor cells.[29] The researchers achieved this by identifying a virus that demonstrated antitumor activity and expressed a truncated envelope glycoprotein in the virus. They found that the antitumor activity of this OV leads to the activation of signaling molecules that cause immune memory to form via memory cells.

Slow-growing tumors have gained interest as their low division rate causes OVs that rely on cell division to not be as effective. While this is a significant engineering challenge, recent studies have shown that there are still methods of overcoming or circumventing the low division rate. OVs that create high viral loads without quickly lysing cancer cells can gain the most benefit from each cell division in a slow-growing tumor. Progress has also been made on the development of research tools and early-stage mechanisms for treating slow-growing tumors. Anti-cancer vaccines have the potential to develop into clinical treatments. Enhancing immune memory via OV action also holds potential for treating slow-growing tumors through a natural, long-term immune amplification.

Tumor Targeting Specificity

As demonstrated by T-VEC, there is a need to improve the targeting specificity of OVs to infect tumor cells primarily or almost exclusively. This could effectively reduce any possible systemic effect of OV therapy and improve patient safety. One approach that has shown significant potential in facilitating improvements in infection specificity is the utilization of biochemical signaling pathway components as markers for OVs. Biochemical signaling pathways play a significant role in the regulation of tissue maintenance and cell division. Any imbalance or dysregulation in these signaling pathways can lead to the development of cancer. Ligand-receptor binding interactions also provide an opportunity for improving tumor targeting specificity by taking advantage of a signaling pathway that is dysregulated to facilitate localization by OVs.[30] There are many pathways that have the potential to be utilized as the basis of a tumor-targeting system.

One such ligand-receptor interaction that has been studied extensively is that between C-C chemokine ligand 5 (CCL5) and C-C chemokine receptor type 5 (CCR5). In many types of cancer, the CCL5/CCR5 ligand-receptor interaction functions improperly and contributes to tumor growth and metastasis.[31] Li and colleagues developed a pair of OVs that upregulate CCL5 in infected tumor cells and upregulate CCR5 expression in NK cells, respectively. Each of these components individually increased antitumor effectiveness, and the combined treatment system was more effective than either

component alone. The result of this bilateral oncolytic virotherapy was the establishment of a tumor-targeting system which led to an improved antitumor effect. NK cells were also more effectively able to target and destroy the corresponding cancer cells.[32] Additionally, the OV engineered to cause CCL5 expression did not infect NK cells in significant numbers. This is an important finding since NK cells with CCR5 expression would be drawn to NK cells with CCL5 expression, rather than the tumor cells.

Researchers have explored additional avenues to improve target specificity. Vijayakumar and colleagues engineered an oncolytic Newcastle disease virus to deliver immunotherapeutic molecules to tumors, eliciting a targeted antitumor response.[33] Zuo and colleagues developed an oncolytic vaccinia virus that encodes for a single-chain variable fragment that targets immune cells.[34] Wang and colleagues created an oncolytic vaccinia virus that inhibits PD-1, an immunosuppressive signaling molecule, and elicits a cancer-specific immune response.[35] Wu and colleagues engineered an oncolytic vesicular stomatitis virus that also inhibits PD-1, causing an antitumor effect.[36] Walton and colleagues developed an oncolytic poliovirus that proliferates rapidly, evades immune response, and is cytotoxic to infected cancer cells to treat glioblastoma, an aggressive brain cancer.[37] These studies utilize different structural interactions and signaling pathways to effectively target tumors and combat cancer cells.

Triple-negative breast cancer is very aggressive and difficult to treat. OVs engineered to take advantage of signaling pathways specific to triple-negative breast cancer can demonstrate infection specificity while also being more effective. Rodriguez Stewart and colleagues developed an oncolytic reovirus that activated the caspase-mediated apoptosis signaling pathway in a novel manner.[38] The caspase pathway involves a series of caspase signals that end with caspase-3, triggering apoptosis of the cell. The researchers found that the caspase pathway was activated but caspase-3 was not, indicating that some other signaling mechanism was responsible for apoptosis of tumor cells.[38] This difference in signaling pathway activity may explain why the tumor cells were affected with specificity while also causing apoptosis. Additionally, this study indicates that variations of previously understood signaling pathways may potentially facilitate the discovery or design of other OVs with specific tumor targeting and antitumor effectiveness.

Other types of cancer, such as pancreatic ductal adenocarcinoma (PDAC), have also demonstrated resistance to treatment. Seegers and colleagues engineered an oncolytic vesicular stomatitis virus that effectively infects treatment-resistant PDAC cells due to enhanced viral attachment. The researchers used directed viral evolution to create mutations in the viral genes and identified two mutations that appeared in more than one evolved virus. OVs with these mutations were advanced to testing and demonstrated increased replication in treatment-resistant PDAC cells.[39] This finding indicates that directed evolution could be used to identify other beneficial mutations in OVs. Additionally, other treatment-resistant cancers could potentially be treated more effectively by OVs which are designed to utilize targeting mechanisms that are not involved with the cancer's resistance to treatment. An OV that relies on a different targeting mechanism could identify and infect cancer cells more effectively than an OV that relies on a mechanism which is made ineffective by a mutation in the cancer cells.

Significant improvements have been made in tumor targeting specificity by OVs and immune cells. This approach includes a number of different specific mechanisms that have been utilized to improve oncolytic virotherapy. OVs can infect cancer cells and cause the expression of different signaling molecules and other proteins on their surfaces that aid immune cells in identifying them. OVs can also be engineered to improve their infection specificity and more selectively infect cancer cells. Engineering of OVs can also be specialized to different cancer types in order to take advantage of

certain receptor-ligand interactions that may be upregulated in different cancers. This could lead to the development of an OV repertoire that demonstrates a significant degree of precision in its mechanism and cytotoxicity.

Oncolytic Viruses and CAR-T Cell Therapy

Certain types of cancer, particularly blood cancers, can be treated using chimeric antigen receptor T (CAR-T) cell therapy. This treatment utilizes immune cells taken from the patient and engineered to carry receptors that better allow them to target cancer cells. This method is beneficial because it relies on the patient's own cells and reduces the likelihood of severe side effects. The immunosuppressive nature of the tumor microenvironment decreases the efficiency of immune cell-based therapies in the same manner as it decreases the efficiency of natural immune responses. This means that CAR-T cell therapy could potentially be ineffective against tumor growths that express high levels of immunosuppression.[40] Combining CAR-T cell therapy with another form of treatment could potentially overcome the challenge of immunosuppression and improve the antitumor effectiveness of the therapeutic approach.

Oncolytic virotherapy can be utilized to counteract immunosuppression in the tumor microenvironment. After OVs have been implemented to cause the tumor region to become immunologically active, CAR-T cells can more easily infiltrate the tumor and cause cancer cell death. McKenna and colleagues developed a multi-component oncolytic therapeutic delivered by mesenchymal stromal cells combined with CAR-T cell therapy to achieve a greater antitumor effect. The first stage of this therapeutic strategy is the delivery of oncolytic viruses by the mesenchymal stromal cells. After the viruses have been delivered, they infect the tumor and reduce immunosuppression in the microenvironment. The final stage involves infiltration of the tumor by CAR-T cells and the death of cancer cells. This combined treatment was more effective than the engineered OVs alone.[41] The researchers also found that T cells were identified in larger numbers within the tumor mass. This result indicates that the immune response is amplified in addition to the CAR-T cell activity.

The concept of utilizing chimeric receptors and combination therapy for anti-cancer activity has been investigated by researchers developing OVs as well. Froechlich and colleagues engineered an OV targeted to mesothelin, a molecule expressed by cancer cells.[42] They achieved this by creating a chimeric receptor that included an immune component and the binding site for the cancer ligand target. Huang and colleagues developed an oncolytic adenovirus that contains a gene circuit for the control of viral replication after infecting cancer cells.[43] Simulation of the population dynamics of the OV revealed that controlling immune signaling at the tumor site would be more effective than combination therapy using an OV with immune signaling. This finding is interesting but was not experimentally determined to a sufficient degree.

Combination therapy using engineered OVs and CAR-T cell therapy can also be utilized to overcome the challenge of homogenous expression of CAR targets. Tumor cells and healthy cells both express certain CAR targets, which causes CAR-T cell activity to be nonspecific to tumors. Park and colleagues developed an OV that causes infected tumor cells to express truncated CD19 on their surface before lysing due to viral activity. CAR-T cells engineered with CD19 were drawn to infected cancer cells due to the binding mechanism of CD19. Native T cells were also signaled and drawn towards the tumor. These findings indicate that CAR-T cell activity can be made specific through the use of OVs to express chimeric antigen receptor targets in tumors.[44] The researchers concluded that they had developed a method of improving the tumor targeting of CAR-T cells using OVs and that this interaction had potential therapeutic applications in the future.

Combination therapy utilizing oncolytic

virotherapy and other cancer therapeutic strategies, such as CAR-T cell therapy, holds significant potential for improving patient outcomes. Multiple therapeutic strategies that can function effectively could have additive antitumor effects and prolong life or destroy tumors. Additionally, therapeutics that do not cause severe side effects could be implemented in the clinical setting without causing great discomfort or additional risks for patients. Currently, oncolytic viruses are entering clinical use and CAR-T cell therapy has been in use for treating patients suffering from blood cancers. It is reasonable that two clinically approved treatment strategies could be utilized as a combined treatment in the future.

CONCLUSION

Significant advances have been made in research on engineered oncolytic viruses in the last five years. Several studies have led to the development of novel OVs which have demonstrated antitumor effectiveness, including in vivo. Researchers have also taken many different approaches to developing engineered oncolytic viruses. Tumor microenvironment remodeling has been investigated as a method of improving immune cell infiltration of tumors. Genetic and structural modifications have been utilized to enhance the antitumor effectiveness of OVs. Specialized OVs have entered development for the treatment of slow-growing tumors and rare cancers. The targeting and infection of tumor cells has been improved through signaling and receptor upregulation. Oncolytic virotherapy is effective in combined treatment with CAR-T cell therapy. These approaches address a variety of issues and challenges in the development and refinement of OVs for potential clinical treatment.

Outside the direct development of OVs with genetic and structural modifications, researchers have approached the question of engineered OV implementation in a number of ways. Engineering experts have applied their expertise to the delivery of OVs, as in the application of control theory for the regulation of OV dosage.[45] Theoretical biologists have also approached the question of implementation through mathematical models, such as a Voronoi cell-based model for viral spread in tumors.[46] These types of studies contribute additional information towards the larger effort to optimize and implement OVs in the clinical setting. Some researchers have also published the specific methods they used to engineer OVs and quantify them, allowing others to pursue their own virus engineering projects.[47,48,49] Clinical trials in veterinary medicine are also being conducted, with the same types of cancers being treated in animals as in humans.[50] This variety of research approaches is additional evidence for the interest engineered OVs are gaining.

In conclusion, OVs demonstrate significant potential for treating cancers with efficacy and safety. OVs can be engineered to deliver signaling molecules to cancer cells, facilitate immune cell infiltration of tumors, and upregulate immune response to tumor growth. There are many viruses and viral modifications that are suitable candidates for engineering into OVs. Cancer treatment strategies such as chemotherapy often cause systemic symptoms and side effects that are very unpleasant for patients. These symptoms may be mitigated by OVs since the viruses can be engineered to selectively target tumors and utilize safe molecular components. As more OVs are engineered and move into clinical trials, there will likely be a greater number of OV therapeutics available for medical use in the next decade. The future of oncolytic virus research appears very promising as it provides hope for the development of meaningful cancer therapeutics.

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ACKNOWLEDGEMENTS

The author expresses his gratitude to Professor Phoebe Stewart (Department of Pharmacology, Case Western Reserve University School of Medicine) for guidance and feedback in the preparation of the article.

ABBREVIATIONS

OV: Oncolytic virotherapy
HSV: Herpes simplex virus
CAR-T: Chimeric antigen receptor T
NK: Natural killer
T-VEC: Talimogene laherparepvec
TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand
hTERT: Human telomerase reverse transcriptase
CCL5: Chemokine ligand 5
CCR5: Chemokine receptor type 5
PD1: Programmed cell death protein 1
PDAC: Pancreatic ductal adenocarcinoma
CD19: B-lymphocyte antigen CD19

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