Proceedings from the Katz School Symposium on Science, Technology and Health

Edited by Sofia Binioris and Amiya Waldman-Levi

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Foreword

The eleven papers published in these inaugural proceedings—originally presented at the Katz School's 2022 Symposium on Science, Technology and Health—offer a glimpse into the exciting work Katz School graduate students are doing to advance scholarly knowledge, impact industry challenges and transform lives.

The Katz School's 2022 Symposium was held in New York City on May 12, 2022 and was organized by Dr. Rana Khan, Clinical Professor and Founding Director, M.S. in Biotechnology Management and Entrepreneurship, Yeshiva University Katz School of Science and Health.

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Message from the Dean

At the Katz School, we are research scientists, tech builders and patient-centered clinicians working on problems that matter. We take an interdisciplinary approach to research and education, fostering the creativity, collaborative thinking and builder mindset required to take on today's toughest problems. In the lab, classroom and clinic, we lead with integrity, generosity and a commitment to making the world a smarter, safer and healthier place.

Sincerely,

Paul Russo
University Vice Provost
Dean, Katz School of Science and Health
Professor of Data Science and Information Systems
Yeshiva University

Speech Emotion Recognition with Deep Learning and Generative Data

Benjamin Cohen
M.S. in Data Analytics and Visualization
Katz School of Science and Health

Abstract—This project aimed to apply deep learning methods to classify audio data. Traditionally, audio classification has been studied by manually selecting features from spectrograms. However, in the past few years, a lot of interest has been raised about how deep learning can make this process more effective. This project applied deep learning methods to speech emotion recognition (the ability to discern a person's emotions solely from their tone of voice). To do this, we gathered a base dataset of approximately 1400 audio clips, labeled as angry or not angry; augmented the dataset using a generative model; and then used transfer learning with a pre-trained Yamnet model to predict if the speaker′s voice was classified as angry. Ultimately, our predictions achieved accuracy greater than 90 percent. This work demonstrates that deep learning approaches work well with audio data, and even with limited data, as it is possible to augment datasets.

Index Terms—Audio Classification, Deep Learning, Synthetic Data, Transfer Learning

I. Introduction

Speech emotion recognition (SER) is the act of being able to take a segment of speech and categorize that segment as an emotion. There are two disparate approaches to categorizing emotions: 1) discrete, where emotions are broken down into classes such as happy, sad or angry; and 2) continuous, where emotions are broken into different dimensions, such as the dimensional model proposed by Russel in 1977, with axes for arousal and valence. Typically, automating SER has been studied using the discrete model, essen-

tially as a classification problem (Kerkeni et al., 2019).

In the early studies of SER, different feature selection techniques were used to extract features from the audio data, and then a classification algorithm was used to build a model. For example, in one of the first studies on SER, Daelert (1996) extracted features related to pitch and speaking rate, among others, and then used a k-nearest neighbors (KNN) algorithm to classify the segment as either happy, sad, angry or fearful. Later studies built on this work and looked at other classification models, such as a Hidden Markov Model and SVMs (Basharirad, 2017).

In recent years, there has been a growing discussion about using deep learning for audio data analysis. Convolutional neural networks (CNNs) have performed well with extracting features from image data. Although it is not completely intuitive how this can apply to audio data, audio can be transformed into an image by creating a mel spectrogram that can be fed into a CNN (Satt et al., 2017). However, a challenge in these studies has been getting the data. A speaker's emotions can be somewhat subjective, and the data gathering is a very manual process, often done by hiring an actor, which may not generalize well when considering other cultures (Cui et al., 2021). Furthermore, deep learning requires a lot of data.

For this project, we hypothesized that a CNN would be able to extract the meaningful features from a mel spectrogram for speech emotion recognition. We also aimed to solve the problem of a small dataset by generating synthetic data.

II. Methods

To evaluate our hypothesis, the following question was posed: given an audio clip of someone speaking, can we predict if the clip's speaker is angry? There were three main steps used to answer this question: 1) data collection, 2) model creation, and 3) model comparison.

In the data collection phase, data was gathered and processed from the RAVDESS data source. RAVDESS contains 1440 audio clips from North American English speakers and can be downloaded from Kaggle. After retrieving

the data, Python was used to preprocess it. Data was first labeled as either "angry" or "not angry" by mapping the labels the dataset provides. The data was then augmented by speeding up the clips and adding background noise using the Librosa Python library. Next, the text of *Frankenstein* was fed into a generative Tacotron model to create more synthetic data points. Finally, Librosa was used to generate mel spectrograms from the source audio clips. 3000 labeled data points in total were collected to train with.

In the model creation phase, we explored different deep learning architectures, fine-tuned the parameters, and tried to find the optimal model to most accurately predict whether the clips' speakers were angry or not. For model building, we used Keras. We built different models, tuning for the most optimal number of layers, and also used preexisting models with transfer learning available on Tensorflow Hub, such as a Yamnet model.

Finally, the models were evaluated by looking at the performance with the Toronto Emotional Speech Set (TESS). This was a separate dataset, like RAVDESS, that was kept aside to do validation. The key metrics we used were recall and precision. Models with a better f1 score were ranked higher than lower ones.

III. Results

The following results were obtained after evaluating the accuracy of a CNN model and a transfer learning model on the TESS dataset (see Table 1 and Figure 1). This dataset contained 199 non-angry samples and 326 angry samples.

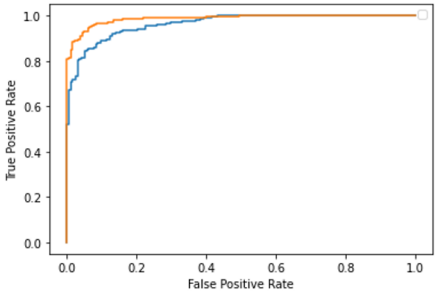
Table 1

Precision and Recall Scores for Created Models

	Precision	**Recall**	**F1-Score**
CNN	.84	.97	.9
Yamnet	.90	.93	.915
VGG16	.98	.58	.73

Figure 1

ROC Curve of Yamnet Model (Yellow) vs. CNN (Blue)



This experiment showed that mel spectrograms are effective for speech emotion recognition. Both the CNN and Yamnet model were able to detect when an angry clip was shown with a recall above 90 percent. The CNN model, however, tended to overpredict anger with a lower precision of .84.

Another finding was that the model trained with the VGG16 significantly overfit the data and predicted anger on almost all the TESS dataset points. Although both Yamnet and VGG16 were trained with transfer learning, the base Yamnet model was trained with image data, which may be why it did not perform as well.

Lastly, this experiment showed that a combination of data augmentation and transfer learning is an effective way to overcome lack of data. With only around 1400 clean samples, we were able to generate enough data points such that our model performed well on another dataset.

V. Conclusion

In this proof-of-concept study, it was found that deep learning methods perform well for speech emotion recognition. Generative models help create enough data for the models to train with so that they generalize well to other datasets. This is important as similar methods could be used for other audio classification tasks without the need for spending a considerable amount of time building a dataset. Additionally, transfer learning works well and allows for utilizing already trained models.

In future analyses, it will be important to explore further how much labeled data is required for this task. We would also like to explore how these generative data translate to speech emotion recognition with other languages and how they can be used for diarization, segmenting longer clips.

VI. Acknowledgment

This project would not have been possible without the expertise of my advisor, Professor Andy Catlin.

VII. References

Basharirad, B., & Moradhaseli, M. (2017). Speech emotion recognition methods: A literature review. *AIP Conference Proceedings, 18*(1). https://aip.scitation.org/doi/abs/10.1063/1.5005438

Cui, C., Ren, Y., Liu, J., Chen, F., Huang, R., Lei, M., & Zhao, Z. (2021). EMOV-IE: A Mandarin Emotion Speech Dataset with a Simple Emotional Text-to-Speech Model. *Interspeech 2021,* 2766–2770. https://doi.org/10.21437/interspeech.2021-1148

Dellaert, F., Polzin, T., & Waibel, A. (1996). Recognizing emotion in speech. *Proceeding of Fourth International Conference on Spoken Language Processing,* 1970–1973. https://doi.org/10.1109/ICSLP.1996.608022

Kerkeni, L., Serrestou, Y., Mbarki, M., Raoof, K., Mahjoub, M. A., & Cleder, C. (2019). Automatic speech emotion recognition using machine learning. In A. Cano (Ed.), *Social Media and Machine Learning.* IntechOpen. https://doi.org/10.5772/intechopen.84856

Satt, A., Rozenberg, S., & Hoory, R. (2017). Efficient emotion recognition from speech using deep learning on spectrograms. *Interspeech 2017,* 1089–1093. http://dx.doi.org/10.21437/Interspeech.2017-200

Force-Indentation Curves of Spheroidal Objects

Benjamin M. Goykadosh
M.A. in Physics
Katz School of Science and Health

Dr. Fredy Zypman
Physics Department Chair
Yeshiva College and Katz School of Science and Health

Abstract—In this project, we propose a theoretical method to recover the energy and force vs. indentation curves produced by deformation using an Atomic Force Microscope. Currently, there are many methods to determine the forces and energy necessary to deform cells. These methods, however, are slow and require testing outside of the body. Our theory depends on parameters such as Young's Modulus, object length and Poisson's ratio, which are obtained by fitting our mathematical expressions to experimental force vs. indentation curves. Our results provide a systematic way to measure those material parameters in general and, in particular, in soft matter where the materials are highly heterogeneous and their properties are often dependent on external stresses.

Index Terms—Atomic Force Microscopy, Cell Contraction, Red Blood Cells, Young's Modulus

I. Introduction

The Atomic Force Microscope (AFM) is one of the favored tools scientists use for micro and nano scale imaging and, of particular interest to the work presented here, soft biological nanoparticles, which include blood plasma, red blood cells and white blood cells (LeClaire et al., 2020). The AFM can achieve accuracies that standard microscopes cannot because of its capabil-

ities of nano-meter spatial resolution and piconewton force sensitivity. In its most common version, the AFM utilizes a sensor made up of a cantilever rod with a tip at its hanging end. When the tip touches or interacts in non-contact with the surface of the particle under observation, the AFM records the tip height and the cantilever's angular deflection; these measurements then enable various quantities to be extracted via mathematical, computational postprocessing. Our main goal in this project was to develop a theoretical model of the sample under study, which in turn would allow us to compute the energy and force versus separation curves produced by the indentation of the sample by the AFM tip. This analysis will help us understand how external forces affect cell deformation. In the end, we hope that this knowledge will elucidate how certain physical parameters such as Young's Modulus and Poisson's Ratio affect the force/deformation connection.

The type of indentation described, and the corresponding force-distance measurement, is uniquely suited for study via AFM. Due to its direct contact with the specimen, the AFM provides mechanical information about the cell that otherwise would not be accessible. We first will provide a general equation that connects the energy and force with the indentation distance and the corresponding cell deformation. In addition, our proposed method would also be useful in determining cell contraction and overall shape. Cellular contraction, an ongoing research subject, is a phenomenon that drives various processes in the body. However, contracting cells only provide a small amount of force and stress, which are difficult to monitor inside the body (Boys & Owens, 2021). Regardless of the progress made in recent years, there are still limitations that prevent the determination of these forces. To solve these issues, studies are often done in vitro, outside the body, which is incredibly difficult, cost prohibitive and slow. Our proposed theoretical method would guide the experiments and thus provide a justification for the deployment of these scarce resources.

II. Methods

The starting point for any theory of elasticity is Hooke's law, a foundational concept used in introductory physics courses as well as in advanced research. Developed by British physicist Robert Hooke in 1678, he described it as *"ut tensio, sic vis"* or "the extension is proportional to the force." In its

simplest form, Hooke's law is known as:

$$\vec{F} = -k\,\vec{x} \qquad (1)$$

where $\vec{F}$ is the force acting on the system, k is a proportionality constant dependent on the spring, and $\vec{x}$ is the displacement from equilibrium. Today's modern theory of elasticity generalizes Hooke's law to three dimensions, such that the strain of an object is proportional to the stresses applied to it. These stresses and strains often are non-uniform fields within the body, and the proportionality factor is no longer a constant; instead, it is a tensor represented by the *elasticity matrix* (Timoshenko & Goodier, 1951). For a three-dimensional system with non-uniform stresses and corresponding strains, Hooke's law is generalized as:

$$\vec{\sigma} = D\,\vec{\varepsilon} \qquad (2)$$

where $\vec{\sigma}$ is the stress vector field, *D* is the elasticity matrix, and ω is the local strain vector.

In order to define the stress and strain vectors, we construct the system of equations to define the body's shape. We model the system as a cardioid (Yates, 1952) with individual components of $\vec{R}(x, y, z)$ such that[1]:

$$\vec{x} = \frac{L\,(2\,a\,Sin[\theta]\{1 + b\,a\,Cos[\theta]\})\,Cos[\phi]}{\sqrt[3]{\frac{32}{3}(1+b^2)L^3\pi}} \qquad (3)$$

$$\vec{y} = \frac{L\,(2\,a\,Sin[\theta]\{1 + b\,a\,Cos[\theta]\})\,Sin[\phi]}{\sqrt[3]{\frac{32}{3}(1+b^2)L^3\pi}} \qquad (4)$$

$$\vec{z} = \frac{L\,(2\,\{1 + b\,a\} - 2\,a\,Cos[\theta]\{1 + b\,a\,Cos[\theta]\} + 2\,b\,a\,(a-1))}{\sqrt[3]{\frac{32}{3}(1+b^2)L^3\pi}} \qquad (5)$$

where L is a constant dependent on the linear dimensions of the object, a is a deformed variable radial coordinate between 0 and 1, and θ and ϕ are variable coordinates in the polar plane with θ having a range between 0 and π and ϕ having a range of 0 and 2π. Most importantly, b is the shape parameter related to the indentation deformation (see Figure 1 and 2 on the following page). The equilibrium is defined as the unstrained starting shape of the body, by a parameter b_0, and its final, strained, position by b that serves to model the indentation of a spheroid by a sharp pin. Therefore, the transformation $b_0 \rightarrow b$ induces the vector displacement for the strain at all points within the body. We propose to build the strains $\vec{\varepsilon}$ from the transformation $b_0 \rightarrow b$ to model how the system deforms and its deformation field, which would then determine the energy and the concomitant force of that deformation. Thus, the parameter b is introduced, in such a way that the deformation of the object will be enhanced at its boundary while not affecting substantially the center of the body.

From this point, we can construct $\vec{\varepsilon}$, and by extension $\vec{\sigma}$ and evaluate the energy of the system. The system of equations modeled by $\vec{R}(\vec{x},\vec{y},\vec{z})$ are used to solve for $\vec{\sigma}$, D, and $\vec{\varepsilon}$ as discussed before. The energy (E_s) relates to these parameters such that:

$$E_s = \frac{1}{2}\iiint_{Volume} (\vec{\sigma} \cdot \vec{\varepsilon})\, dV \qquad (6)$$

The force is determined by the change in E_s over the distance indented, S, which is a variable related to b. Therefore, F would be defined as:

$$F(S) = -\frac{dE_s}{dS} \qquad (7)$$

However, our original conditions (equations 14, 15, 16) are in terms of b, not S. Therefore, we calculate the force required to indent the sphere by:

$$F(S) = -\frac{dE_s}{db}\frac{db}{dS} \qquad (8)$$

where $^{db}/_{ds}$ is solved by solving Equation 23 for b and taking the derivative of the result with respect to S.

Figure 1

When $\vec{R}(\vec{x},\vec{y},\vec{z})$ *is plotted over the ranges of* $\theta = [-\pi, \pi]$ *and* $\phi = [0, 2\pi]$ *and* $a = 1$, $b = 0$, *we recover a cardioid deformed such that it is a sphere. This sphere can be an accurate model for pre-stressed white blood cells.*

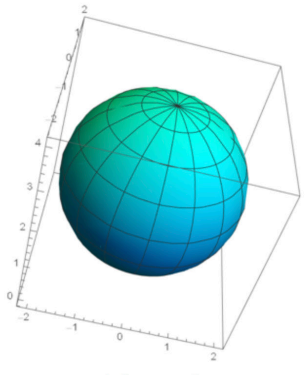
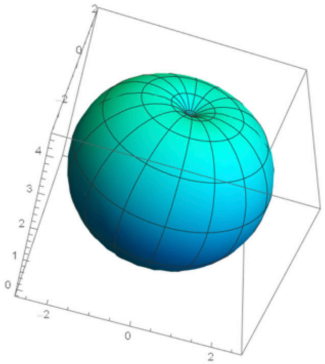


Figure 2

$\vec{R}(\vec{x},\vec{y},\vec{z})$ *is plotted over the ranges of* $\theta = [-\pi, \pi]$ *and* $\phi = [0, 2\pi]$ *and* $a = 1$, $b = 9$, *we recover a standard three-dimensional cardioid. This deformation accurately models a sphere under the influence of a cantliever point stress.*



III. Results

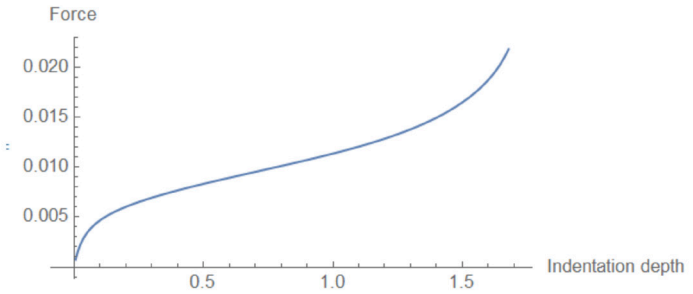
Using the methods described above and using Mathematica in algebra mode, we were able to find a general method for the energy of the system:

$$
\begin{aligned}
E_s = &-\Big(\big(L^3 Y \\
&\Big(8\, b \left(6\, b \left(1+b^2\right)^{2/3} b0 \left(679-121\,\nu\right)+768\, b^5 \left(1+b^2\right)^{1/3} b0 \left(\left(1+b^2\right)^{1/3}-2\left(1+b0^2\right)^{1/3}\right)(1+\nu)+384\, b^6 \left(1+b0^2\right)^{1/3}\left(-2\left(1+b^2\right)^{1/3}+3\left(1+b0^2\right)^{1/3}\right)(1+\nu)+ \\
&16\, b^3 \left(1+b^2\right)^{2/3} b0 \left(59+112\,\nu\right)+3\left(1+b^2\right)^{2/3} b0^2 \left(-679+121\,\nu\right)-b^2\left(1+b^2\right)^{2/3}\left(2037-363\,\nu+8\, b0^2\left(59+112\,\nu\right)\right)+ \\
&8\, b^4 \Big(85\left(1+b^2\right)^{2/3}+16\left(-18\left(1+b^2\right)^{1/3}\left(1+b0^2\right)^{1/3}+9\left(1+b0^2\right)^{2/3}+2\left(1+b^2\right)^{2/3}\nu-18\left(1+b^2\right)^{1/3}\left(1+b0^2\right)^{1/3}\nu+9\left(1+b0^2\right)^{2/3}\nu+ \\
&3\left(1+b^2\right)^{2/3} b0^2 (1+\nu)\right)\Big)\Big)-6\left(1+b^2\right)^{2/3}(b-b0)^2\left(281+768\, b^2\left(-2+b^2\right)(-1+\nu)+889\,\nu\right)\mathrm{ArcTanh}[b]- \\
&3\left(1+b^2\right)^{2/3}(b-b0)^2\left(96\, b^4(-1+\nu)+9\sqrt{9+16\, b^2}\,(37+5\,\nu)-8\, b^2\left(135-14\sqrt{9+16\, b^2}+5\sqrt{9+16\, b^2}\,\nu\right)\right)\mathrm{Log}\left[-1-4\, b+\sqrt{9+16\, b^2}\right]+ \\
&3\left(1+b^2\right)^{2/3}(b-b0)^2\Big(\Big(96\, b^4(-1+\nu)-9\sqrt{9+16\, b^2}\,(37+5\,\nu)+8\, b^2\left(-135-14\sqrt{9+16\, b^2}+5\sqrt{9+16\, b^2}\,\nu\right)\Big)\mathrm{Log}\left[1-4\, b+\sqrt{9+16\, b^2}\right]+ \\
&\Big(96\, b^4(-1+\nu)+9\sqrt{9+16\, b^2}\,(37+5\,\nu)-8\, b^2\left(135-14\sqrt{9+16\, b^2}+5\sqrt{9+16\, b^2}\,\nu\right)\Big)\mathrm{Log}\left[-1+4\, b+\sqrt{9+16\, b^2}\right]+ \\
&\Big(-96\, b^4(-1+\nu)+9\sqrt{9+16\, b^2}\,(37+5\,\nu)+8\, b^2\left(135+14\sqrt{9+16\, b^2}-5\sqrt{9+16\, b^2}\,\nu\right)\Big)\mathrm{Log}\left[1+4\, b+\sqrt{9+16\, b^2}\right]\Big)\Big)\Big)\Big/ \\
&\left(6144\, b^5\left(1+b^2\right)\left(1+b0^2\right)^{2/3}(1+\nu)(-1+2\,\nu)\right)\Big)\Big);
\end{aligned}
$$

While the equation is cumbersome, it can simply be cut and pasted for future use. It saves the user the need to do numerical integrations. This equation allows us to calculate the energy stored in the body, and by extension the force, as a function of the indentation S (connected to the shape parameter b). Y, L, υ and b_0 are all constants that are material dependent. As an example, we calculated the force on an object with parameters b_0=0, υ = 0.25 and arbitrary values of L and Y (Figure 3).

Figure 3

Force-indentation depth curve for initial conditions b_0= 0, υ = 0.25, and arbitrary values of L and Y. Force grows with indentation depth.



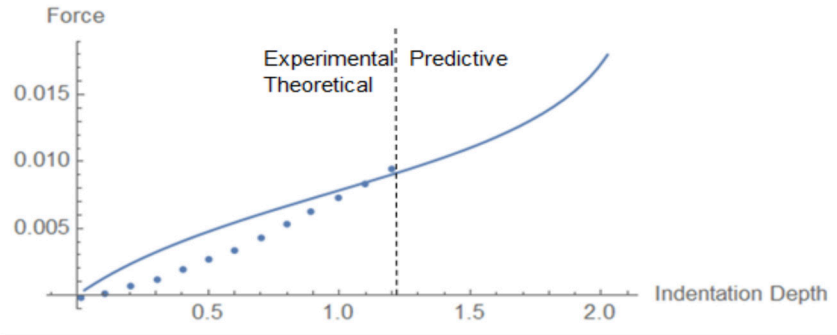
IV. Discussion

To verify the validity of our method, we compared it to published experimental data. Specifically, we compared our data to research done by Yue Ding, Guang-Kui Xu, and Gang-Feng Wang. In a 2017 paper, they note that various studies indicate that the cell is supposed to be linearly elastic within

small ranges of strain based on analysis of AFM indentation data. However, this study does not account for the influences of large deformations and surface tension in cells. Their goal was to determine the scale of these influences on the elasticity of cells (Figure 4). One issue that arose in our comparison is that we did not have access to their values for the Young's Modulus, length or υ. The materials that are of interest are often so complex and small that literature values of Y are not available. Therefore, we obtained these values ourselves by fitting our model to the experimental curves. To do this, we adjusted our model by floating the variables Y, L, υ and b_0 to best approximate experimental conditions. For this experiment, we used Y = .0001 Kpa, L = 8.4 μm, υ = 0.25, and b_0 = 0.4 (Figure 4).

Figure 4

Comparison between the experimental data (dotted line) (Ding et al., 2017) compared to the proposed theoretical model (solid line). Since the original conditions are unknown, we adjusted our model by playing with the vairalbes Y, L, υ *and* b_0*. For this experiment,* Y = .0001 Kpa, L = 84 ×m, υ = 0.25, *and* b_0 *= 0.4*



As shown in Figure 4, the data is not a perfect fit. However, both curves are nearly linear at the onset of the experiment. At large indentation depths, the curves diverge as the theoretical curve grows polynomialy. While it can be assumed the experiment did not reach this point in indenting the material, this is just speculation and can only be determined by conducting physical experiments on objects with known constants.

V. Conclusion

In this project, we were able to determine that our theoretical model aligns closely with independent experimental data reported in the literature. Our theoretical model supports calculating the energy and the force of cell deformations of spheroidal objects. One issue that arose with our comparisons is the speculation necessary, as we did not know the materials other groups used in their experiments. One way we may improve this comparison in the future would be by conducting deformation tests with known materials to determine values for the various constants. This would ensure that we can accurately prove our model by testing it against known data.

VI. Acknowledgment

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VII. References

Boys, A., & Owens, R. (2021). Measuring cellular contraction: Current progress and a future in bioelectronics. *APL Materials, 9*(4). https://doi.org/10.1063/5.0040953

Ding, Y., Xu, G.K., & Wang, G.F. (2017). On the determination of elastic moduli of cells by AFM based indentation. *Scientific Reports, 7*(1). https://doi.org/10.1038/srep45575

Hooke, R. (1678). *Lectures de potentia restitutiva, or, of spring: Explaining the power of springing bodies.* John Martyn.

LeClaire, M., Gimzewski, J., & Sharma, S. (2020). A review of the biomechanical properties of single extracellular vesicles. *Nano Select, 2*(1), 1–15. https://doi.org/10.1002/nano.202000129

Timoshenko, S., & Goodier, J. N. (1951). *Theory of elasticity.* McGraw-Hill.

Yates, R.C. (1947). *A handbook on curves and their properties.* J. W. Edwards.

Financial Impact of Tropical Cyclones on U.S. Real Estate Sector

Brian Livian
M.A. in Mathematics
Katz School of Science and Health

Atreish Ramlakhan and Aishwarya Singh
M.S. in Artificial Intelligence
Katz School of Science and Health

Abstract—This collaborative project between Yeshiva University's Katz School of Science and Health and S&P Global Market Intelligence sought to quantify the financial effects of tropical cyclones on the U.S. real estate market. Using hurricane data from the National Oceanic and Atmospheric Administration (NOAA) and the Intergovernmental Panel on Climate Change (IPCC), and proprietary real estate trust data from S&P databases, we analyzed the growing impact of tropical cyclones in the United States as well as the relationships between climate change and Real Estate Investment Trust (REITs) financial losses. The analysis shows a relationship between the increasing frequency of hurricanes and the financial impact on RIETs, suggesting that REIT owning companies should invest in property risk mitigation expenditures before significant damage is done to their assets by tropical cyclones.

Index Term—Climate Change, IPCC, NOAA, Python, Real Estate

I. Introduction

Both the National Oceanic and Atmospheric Administration (NOAA) and the Intergovernmental Panel on Climate Change (IPCC) verify that hurricanes' increasing frequency can be attributed to global rising tempera-

tures (Knutson et al., 2015; Seneviratne et al., 2012). Climate change and the resulting increase in hurricanes have dire impacts on industries across the globe. However, to date, there has not been extensive study examining the cost in growth inflicted by hurricanes on a company level.

Conducted in collaboration with S & P Global Market Intelligence, this project focused on the impact of the increasing rate of hurricanes on commercial REIT assets in the United States. We sought to investigate the downstream financial impacts, identify risk patterns and quantify the effects of hurricanes for companies that hold real estate assets in the United States.

II. Methods

Using hurricane data from NOAA and IPCC, and proprietary real estate trust data from S&P databases, we analyzed the growing impact of tropical cyclones in the United States and the relationship between climate change and REIT financial losses.

The first step of this analysis required that asset data and hurricane track data from independent sources be joined through geolocation. To achieve this, we acquired the locations of assets along the U.S. eastern seaboard from the S&P Global Market Intelligence platform (now known as Capital IQ). We then fetched the names, categories and coordinates of landfall for North Atlantic hurricanes during the period of observation (2007-present) and completed path data of tropical storms and hurricanes hitting the seaboard from 1950 onwards from the NOAA International Best Track Archive for Climate Stewardship (IBTrACS). Then, using S&P proprietary data, we identified REITs and their assets that were hit by storms (mapping NOAA data by coordinates of assets). We developed an asset mapping algorithm, which utilized known storm paths and wind radii, accounting for the Earth's curvature, to extract impact events to generate severity metrics for each event.

After identifying companies affected by hurricanes, we analyzed the standardized financial statements of said companies from S&P's proprietary database platform. The final quantitative analysis of companies' financial data was performed using Python. Different financial metrics were observed

to determine any trends of hurricane impacts and costs to the company after the fact.

Once assets were identified, we were also able to determine if they are public or private companies. Public companies that operate in the United States are regulated by a federal government agency, the Securities & Exchange Commission (SEC), and the SEC makes company filings available online for the public to view. Using an API, we downloaded and performed textual analysis on 10K reports of affected companies via the SEC's EDGAR site (https://www.sec.gov/edgar/search-and-access) for word counts of 'hurricane,' 'storm,' 'weather,' and storm name.

III. Results

The results show a steady increase in company spending on capital investments and improvements for REIT assets, which correlates with increases in hurricane incidence (see Figure 1).

Figure 1

Company Spending for REIT and Hurricane Incidence

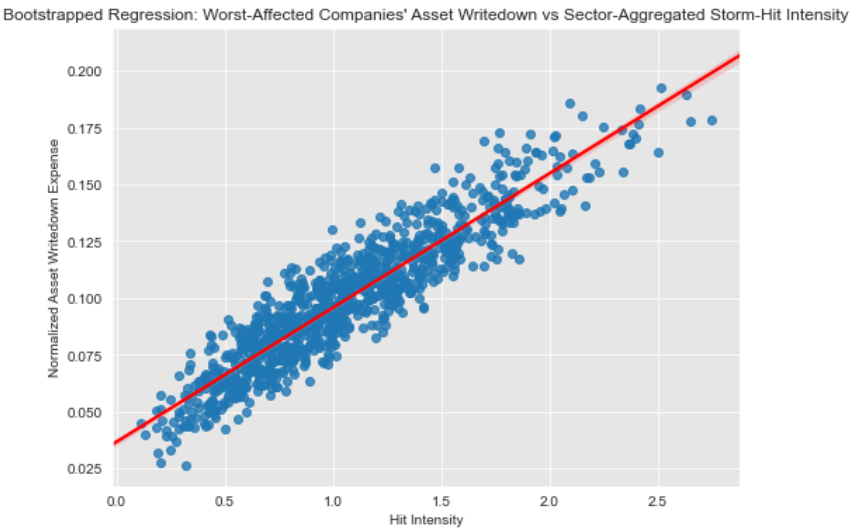


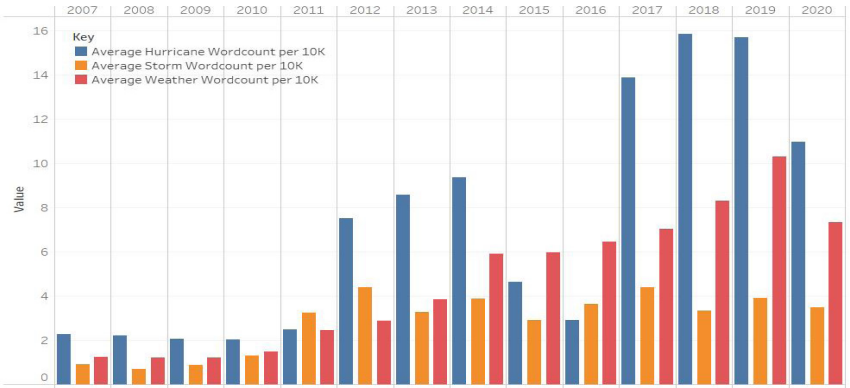
Figure 1 displays the bootstrapped version of expenses of asset write-downs or expenses based on the hit intensity of hurricanes. The higher the hit

intensity, the greater the asset write-downs.

In addition, our textual analysis showed these companies used an increasing number of weather-related terms such as "weather," "storm" and "hurricane" over the period studied (Figure 2).

Figure 2

Use of Weather-Related Terms in SEC 10K Filings for Eastern Seaboard REITs, 2007–2020



We observed a trend where the higher hit intensity implies higher (normalized) asset write-downs for the companies studied. In addition, textual analysis revealed increasing use of weather-related terms such as "weather," "storm" and "hurricane" over the period studied. Together, our analysis demonstrates that over the past 20 years, the frequency and occasionally the severity of tropical cyclones have increased, and so have companies' expenditures in relation to disaster preparedness and capital expenditure.

V. Conclusion

This project aimed to put a numeric value on losses in relation to RIETs that are managed by the S&P 500 that have been impacted by hurricanes in the last two decades. Our analysis shows a relationship between the increasing frequency of hurricanes and the financial impact on RIETs. As hurricanes continue to become more frequent, this should serve as an indicator for REIT owning companies to invest in property risk mitigation expenditures

before significant damage is done to their assets by tropical cyclones.

VI. Acknowledgment

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VII. References

Knutson, T., Sirutis, J., Zhao, M., Tuleya, R., Bender, M., Vecchi, G., Villarini, G., & Chavas, D. (2015). Global projections of intense tropical cyclone activity for the late twenty-first century from dynamical downscaling of CMIP5/RCP4.5 scenarios. *Journal of climate, 28*(18), 7203–7224. https://doi.org/10.1175/JCLI-D-15-0129.1

Seneviratne, S. I., Nicholls, N., Easterling, D., Goodess, C. M., Kanae, S., Kossin, J., Luo, Y., Marengo, J., McInnes, K., Rahimi, M., Reichstein, M., Sorteberg, A., Vera, C., & Zhang, X. (2012). Changes in climate extremes and their impacts on the natural physical environment. In C.B. Field, V. Barros, T.F. Stocker, D. Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen, M. Tignor, and P.M. Midgley (Eds.), *Managing the risks of extreme events and disasters to advance climate change adaptation: A special report of working groups I and II of the Intergovernmental Panel on Climate Change (IPCC)* (pp. 109–230). Cambridge University Press.

Two-Step Verification System Using Face Recognition

Mukilan Narayanamoorthy
M.S. in Cybersecurity
Katz School of Science and Health

M. Harish, D. Suresh Kumar and Dr. Angel Latha Mary
Karpagam College of Engineering

Abstract—This project aimed to identify the most secure and efficient approach to user authentication for social networking sites. As technology use increases rapidly across the globe, the number of active social media users has reached 3.7 billion within just the last three years. This increasing demand urges social networking companies to introduce more and better security mechanisms to protect the authenticity of their users. Several companies have introduced two-step verification mechanisms like verification codes and graphical passwords, but these can easily be compromised with simple techniques like sim hijacking. To create a more secure environment, this paper proposes a two-factor facial-recognition authentication engine using a dHash algorithm.

Index Terms—aHash, dHash, Facial Recognition, Grayscale, Hamming Distance, pHash

I. Introduction

The increasing demand for social networking across the globe is urging social networking companies to introduce more and better security mechanisms to protect their users. Social networks store enormous amounts of user information and are extremely vulnerable to attack, particularly because it is difficult to verify the user's identity and prohibit access from unauthorized users. By posing as the user themselves, hackers can use vulner-

abilities in social networks to reveal information about the victim that the victim did not intend to share. As a result, social networking companies have had to introduce more and better security mechanisms to protect the authenticity of their users.

Most companies use one-time password (OTP) based systems, but these systems are liable to attacks like sim-swaps, sim-hijacking and more. Several companies have introduced two-step verification mechanisms like verification codes and graphical passwords, but these can also easily be compromised with techniques like sim-hijacking. For example, Facebook recently introduced a two-factor authentication method in which they send a six-digit passcode to the user's registered email or mobile number. Upon receiving the code, the user must enter the passcode in the area provided to authenticate themselves to use the social network. With this mechanism, Facebook claims that they are protecting people from fifteen kinds of attacks. However, this authentication mechanism may inadvertently give hackers access to the user's mobile phone due to vulnerabilities in the mobile phone or application environment. A hacker can take advantage of this, sniff the authentication code, impersonate the legitimate user and gain access to the account.

To eliminate the multifactor security issue, this project proposes using a facial-recognition based two-factor authentication engine that could easily be integrated with any web application or progressive web app. A two-factor authentication engine stores facial data in a secure manner so that a hacker cannot gain access to it. The engine uses a perpetual hashing algorithm, which works to find the relative gradient between the pixels in an image and calculates the difference in the hashes. In other words, it extracts data from images and converts this data to text. We also tested three different hashing algorithms, aHash, pHash, and dHash, to find the most efficient and reliable method.

II. Methods

First, we developed a facial recognition system and then implemented the system using three different hashing algorithms: aHash, pHash, and dHash. We had 60 students register for our facial recognition system and ran the system using all three algorithms. We asked students to attempt to login to

our system multiple times and also to attempt to login as other users. We then compared the results of each algorithm in terms of run time and accuracy.

III. Results

After testing each hashing algorithm, we found:

1. **aHash:** The main disadvantage is that aHash is slow. It took 3 hours and 45 minutes to run. This is because it took more time to load and scale images. aHash also generated many false positives. In some cases, the sample pictures taken should have matched only 32 times, but aHash returned matches 400 times.

2. **pHash:** pHash was faster than aHash, but it was less accurate. It returned many false positives and false negatives.

3. **dHash:** dHash was the more efficient and reliable algorithm, as it had a very small number of false positives and was quicker to run.

dHash uses the following process to authenticate a user:

1. **Reduce size.** The best way to remove high amounts of details and frequencies is to reduce the size of the image. It is shrunk to 9x8, thus leading to a total of 72 pixels. This is done because we can ignore the aspect ratio and size of the image and it is immaterial for how far the image is stretched or shrunk.

Before reducing size

After reducing the size and extracting the data

2. **Reduce color.** The next step is to convert the image into a grayscale image. With this process we convert 72 pixels into 72 colors, which helps us further in the process.

Color reduced image after converting to grayscale

3. **Compute the difference.** The dHash algorithm works by making a comparison between the adjacent pixels. This calculates the relative gradient between those pixels. Here, nine pixels in each row leads to eight differences between the adjacent pixels, overall having eight rows leading to 64 bits.

4. **Assign bits.** Bits are set by comparing the brightness between the right and the left pixel.

Hash computed for photo above = 3a6c65565898da525

After processing using the dHash algorithm, the hash computed/stored during the registration process and the hash computed during the login pro-

cess are compared and their hamming distance is calculated. If the hamming distance between two hashes is found to be more than 10, it is evident that the two images are different and thus the authentication is denied. If the hamming distance is less than 10, we can conclude that the images are similar and there are only a few variations based on things like lighting conditions. With this, we can then authenticate the user to access their account.

V. Conclusion

This project aimed to find a more secure and easy-to-use two-factor authentication method that could be integrated easily with other web-applications, mainly social networking sites. Though there are multiple advantages of social networks, there are several ways to breach the security and take advantage of the flaws. Social networking companies are trying to patch those flaws, but the main problem is protecting the authenticity of the user. Existing systems are not completely secure; they are vulnerable to breaches with techniques like sim hijacking, malware and others. Our findings show that a facial authentication system using dHash may solve the problem of impersonation and provide greater security to social media users.

VI. Acknowledgment

This research was done with the help of D. Suresh Kumar, M. Harish, and R. Subash Manikandan under the guidance of Dr. Angel Latha Mary, Head of the Department of Computer Science and Engineering at Karpagam College of Engineering in Coimbatore, India. The authors published a longer version of this paper in 2020 under the same title in the *International Journal of Grid and Distributed Computing, 13*(1).

This project builds on the work of Rahman & Adnan, 2017; Bijoy et al., 2017; and Bhanushali et al., 2015.

VII. References

Bhanushali, A., Mange, B., Vyas, H., Bhanushali, H., & Bhogle, P. (2015). Comparison of graphical password authentication techniques. I*nternational Journal of Computer Applications, 116*(1), 11–14.
https://doi.org/10.5120/20299-2332

Bijoy, J. M., Kavitha, V. K., Radhakrishnan, B., Suresh, L. (2017). A graphical password authentication for analyzing legitimate user in online social network and secure social image repository with metadata. 2017 *International* Conference *on Circuit, Power and Computing Technologies,* 1–7. https://doi.org/10.1109/ICCPCT.2017.8074325

Rahman, M. M. & Adnan, M. A. (2017). Two step verification system of highly secure social media: Possible to breach the security. 2017 *International* Conference *on Networking, Systems and Security,* 185–190. https://doi.org/10.1109/NSysS.2017.7885823

Capture the Flag:
Applications for the Cybersecurity Classroom

Kevin Suckiel
M.S. in Cybersecurity
Katz School of Science and Health

Abstract—This study explored the application of Capture the Flag (CTF) events as an instructional approach in cybersecurity and information security (IS) curricula. In a classroom environment, technical skills can sometimes be overshadowed by theoretical knowledge. CTF events remove those barriers by allowing cybersecurity and IS students to use any tools in their arsenal to complete challenges related to the subject matter. CTF challenges range in difficulty and give students a snapshot of problems they may face in the real world. While CTF events are commonly used in the professional arena, they are often overlooked in academic settings because they are considered too gamified. In this project, we hypothesized that incorporating CTF events into the core IS and cybersecurity curriculum could promote students' learning and ultimately increase their chance of employment. The findings of this preliminary study support the use of CTF events as an instructional approach to support cybersecurity and IS students' learning. Further research is warranted.

Index Terms—Analytical Thinking, Hacking, Penetration Testing

I. Introduction

In the high-volume cybersecurity job market, companies often use Capture the Flag (CTF) events to gauge an applicant's skill level, and many businesses host CTF events and list CTF experience as a value added on resumes (Booz Allen Hamilton, n.d.; European Union Agency for Cybersecurity, 2010). A CTF event is a simulated series of cybersecurity challenges. Contestants compete to solve a particular challenge and earn a flag for each

challenge they solve. These events require participants to use key technical skills, such as cracking password hashes, bypassing weak authentication, identifying cloud misconfigurations and actual penetration testing scenarios. CTF events also require an analytical approach—breaking down complex problems into smaller steps—a skillset that applies to cybersecurity, information security (IS) and beyond.

While CTF events are commonly used in the professional arena, they are often overlooked in academic settings because they are considered too gamified. In this project, we hypothesized that incorporating CTF events into the core IS and cybersecurity curricula could promote students' learning and ultimately increase their chance of employment.

II. Methods

This was a pre-and-post quasi-experimental study of the application of CTF events as instructional approach in formal cybersecurity education. The study engaged 41 current cybersecurity and IS students (graduate and undergraduate) in a two-week, fully online CTF event that presented them with increasingly complex, real-world cybersecurity challenges.

To design the CTF challenges, a review of over 100 entry-level cybersecurity job postings was performed to identify in-demand skills expected for new hires. Key skills, such as intelligence gathering from open-source means, encoding web data, cracking password hashes, bypassing weak authentication, identifying cloud misconfigurations and penetration testing became the basis for the challenges that made up the CTF event.

In addition, the challenges required that students: 1) apply knowledge across different domains of cybersecurity; 2) face scenarios at different levels of complexity and difficulty; and 3) use information they gained in a classroom setting to solve real-world challenges. These design principles were intended to ensure that the challenges both reflected real-world cybersecurity scenarios and to assess students' ability to connect theoretical knowledge gained in the classroom to scenarios in the real world. Importantly, challenges could not be easily solved by merely searching for the answer in a database, but rather required the use of analytical skills—such as breaking down a

complex problem into smaller, simpler problems—which are important in the professional world.

To establish a baseline of students' existing knowledge, before the CTF event began, students were questioned about their skills related to the various challenge categories. This was done via an open discussion in a dedicated chat room, where students shared their experience answering related questions from each challenge category. This informal pre-study information gathering also helped to ensure that the CTF challenges were sufficiently difficult given students' existing knowledge.

Once the CTF event began, students were instructed to work through 30-plus challenges across several categories (see Table 1) using any publicly available tool or resource.

Table 1

Challenge Categories

IS Categories	Category of Challenge
Binary Exploitation	Blue Team
Cloud	Cryptography
Forensics	Multiple Choice
Open-Source Intelligence	Red Team
Scripting	Steganography
Web	Real World Example

The CTF event lasted two weeks, and students spent on average four hours per day on the challenges; several students put in significantly more time. Students who found a correct answer submitted a string of text to be verified and were awarded points for their achievements.

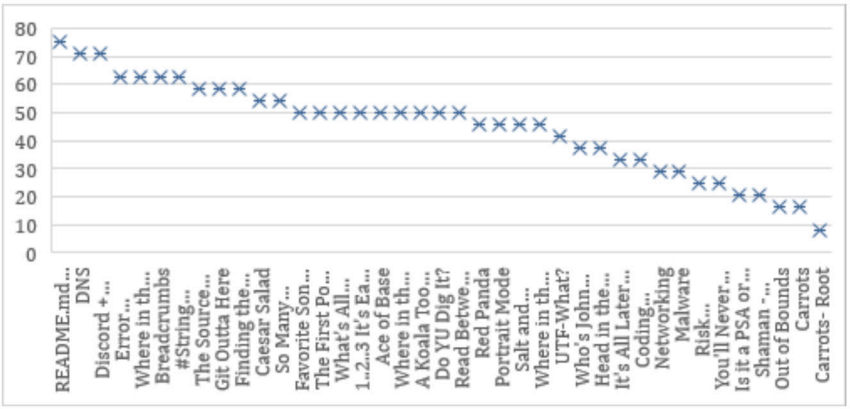
To measure the impact of the CTF event on students' skills development, quantitative and qualitative post-study data was collected via both a questionnaire and by analyzing challenge completion rates. This data was then compared to the pre-study data to determine what, if any, skills development took place as a result of participation in the CTF event.

III. Results

Our preliminary findings suggest that CTF events could play an important role in cybersecurity and IS education. After two weeks of constant hands-on work, all challenges had been solved. Students submitted over 1000 flag submissions, with a solve rate of 40%. The last challenge was only solved the day before the event closed. Table 2 shows the number of challenges solved, in order of complexity (farthest to the right is the final, most complex challenge, with the fewest solves). While all challenges were solved, the most difficult challenges only received two or three completions.

Table 2

Challenges Completed by Students in Order of Complexity



For most CTF events, a solve rate of 15% is considered good. Achieving a solve rate of 40% shows that students were highly engaged in the challenges. Given that every student was not able to solve every challenge, we noted that students were learning to solve problems they had never faced before. Students built skills by engaging with complex problems they did not yet know how to solve, and they were able to apply tools and skills they had learned either in the classroom or from previous challenges in the CTF event to solve increasingly complex challenges. In other words, they were building knowledge over the course of the event.

Before beginning the study, informal chats with students showed that students had more skills than they were aware of, but they did not necessarily

know how to apply those skills in cybersecurity scenarios. For example, most students knew what coding web data was, but they did not know how to apply the principles in an actual challenge. In conversations with students after the event, they expressed that after failing several challenges, they eventually made the connection between what they had learned about coding web data in class and how to apply it to the challenge. This anecdotal information was supported by the post-event questionnaire; 18 of 41 students stated that they learned more doing the challenges than in a typical classroom setting. Of those, six thought they were more prepared for technical job interviews because of their participation in the CTF event.

This study shows that CTF events are an engaging instructional approach, utilizing increasingly complex tasks, as well as an opportunity for students to apply classroom knowledge to actual practice.

V. Conclusion

This study was designed to explore the application of CTF events, a common and important professional tool, as an instructional approach in cybersecurity and IS curricula. While this was a preliminary study with a notably small sample size and lack of control, the results do imply that CTF events could be an important tool for cybersecurity and IS education and training, if not replacing traditional classroom instruction, certainly in conjunction with it.

CTF events exemplify the benefits of experiential learning, helping students to develop and practice the in-demand technical and analytical skills required in the workplace. By combining the theoretical knowledge attained in the classroom with hands-on technical skills developed through CTF challenges, students can feel confident in their ability to tackle real-world problems in a professional setting. It is worth further investigating how CTF events can continue to provide a bridge from theory to application in the cybersecurity and IS curricula. In addition, being able to identify which challenges proved most difficult can provide both students and faculty useful information about where potential knowledge gaps lie, and where further instruction is required.

VI. Acknowledgment

I would like to thank Professor Sivan Tehila for her ongoing support during this project.

VII. References

Booz Allen Hamilton. (n.d.). *Why playing capture the flag will make you a cyber elite.* https://www.boozallen.com/e/culture/capture-the-flag.html

European Union Agency for Cybersecurity. (2021, May 10). *Capture-the-flag competitions: All you ever wanted to know!* https://www.https://www.enisa.europa.eu/news/enisa-news/capture-the-flag-competitions-all-you-ever-wanted-to-know

Strategies for the Development and Commercialization of an Antibody Drug Conjugate as a New Therapy for Solid Tumors of Hypopharyngeal Cancer

Anam Khalid
M.S. in Biotechnology Management and Entrepreneurship
Katz School of Science and Health

Abstract—This project, conducted on behalf of early-stage biotechnology company GritBio, explored the market potential and commercialization feasibility for LIGRECA, GritBio's proposed antibody drug conjugate (ADC) treatment for hypopharyngeal cancer. This paper presents a market analysis, commercialization strategy and financial implications for the successful development and commercialization of GritBio's new treatment. Specific recommendations include that GritBio apply for fast-track FDA approval, partner with a CDMO for manufacturing, prioritize sales and marketing and consider mergers and acquisitions with larger companies to expedite R&D, bypass competition and reduce cost.

Index Terms—Antibody-drug Conjugate, Hypopharyngeal and Laryngeal Squamous Cell Carcinoma

I. Introduction

GritBio, an early-stage biotechnology company, seeks to transform healthcare for oncology patients by developing safer cancer therapeutics, which can keep cancer at bay for the long term without life-threatening side effects. Specifically, GritBio is focused on treating squamous cell carcinoma (SCC), with a priority on a rare type of throat cancer, hypopharyngeal cancer, which is typically diagnosed at a late stage and has a very poor prognosis.

In the United States, the incidence of hypopharyngeal cancer is slightly less than 1 per 100,000 persons. Approximately 3,000 new cases are diagnosed

each year, primarily affecting males and females ages 50 to 60 years and with a 5-year survival rate of 32% (National Cancer Institute, 2012, 2019). The treatment of hypopharyngeal cancer is controversial, in part due to the low incidence and inherent difficulty in conducting adequately powered, prospective, randomized clinical trials (National Cancer Institute, 2012, 2019). Defining the ideal therapy for hypopharyngeal cancer is difficult because hypopharyngeal cancer is typically identified in late stage.

Given this, there is an unmet need for new treatments for hypopharyngeal cancer. GritBio is developing LIGRECA, an antibody drug conjugate (ADC) that would target a specific metabolic process in dormant cancer cells, as a treatment option for hypopharyngeal cancer. The aim of this project was to assist GritBio in understanding the market potential, commercialization feasibility and financial projections for their proposed ADC.

II. Methods

To identify the existing market and determine the potential for LIGRECA, we examined current treatments and current clinical trials for proposed treatments for hypopharyngeal cancer, including ADCs and non-ADCs (e.g., biologics/immunotherapies, senolytic drugs, targeted inhibitors, cell-based therapies and combination therapies). To determine commercialization feasibility and financial projections, we analyzed the websites of competitor treatments as well as their company's 10K financial forms. In addition, we consulted with Contract Development and Manufacturing Companies (CDMOs) to find an organization to upscale ADC development.

III. Results

Market analysis

While there are several therapies for hypopharyngeal cancer on the market—including biologics/immunotherapies, senolytic drugs, targeted inhibitors, cell-based therapies and combination therapies—there are currently no FDA-approved ADCs. The five-year survival rate with current treatments is only 32% (National Cancer Institute, 2012). Non-ADC treatments are not ideal because of non-specific targeting, which causes severe side effects. For example, classical small-molecule chemotherapeutic agents are generally nonspecific, they bind to and affect other physiological targets and may

cause significant side effects.

In contrast, targeted monoclonal antibody (mAb) therapy generally is very selective and has a milder side-effect profile. Antibody-drug conjugates, or ADCs—one type of mAb therapy—are biopharmaceutical drugs that are used as a targeted therapy for cancer. They are complex molecules composed of an antibody: a whole monoclonal antibody (mAb) or an antibody fragment such as a single-chain variable fragment, a stable chemical linker with labile bonds and an active cytotoxic payload. There are currently 10 approved ADCs for various types of cancers, the most well-known being Kadcyla for breast cancer and Padcev for bladder cancer. Collectively, ADCs have been more effective than standard cancer treatments.

There is a high level of competition for head and neck cancer in the ADC market, with several ADCs for head and neck cancer in clinical and pre-clinical trials, mostly driven by large pharmaceutical companies and often by companies outside of the U.S. GritBio's ADC, LIGRECA, remains competitive, since it targets a different antigen and is projected to have a lower cytotoxic impact than its competitors. The payload of GritBio's ADC is a targeted inhibitor, while most other ADCs have a general action payload. LIGRECA's main advantage is that its load molecule is less toxic when compared to other firms' load molecules when targeting solid tumors, and potentially liquid tumors in the future.

Since GritBio is targeting hypopharyngeal cancer, which affects around 3,000 people in the U.S. per year, they are eligible to apply for Fast Track or Accelerated FDA Approval for their ADC. This will help expedite the approval process, potentially allowing GritBio early access to the ADC market.

Commercialization feasibility

There are three primary challenges inherent to commercializing a biotherapeutic: technical challenges, regulatory challenges and market supply challenges. Figure 1 illustrates a general value chain for bringing LIGRECA to market, including the actions GritBio will need to take to develop the product, from the initial reception of materials all the way through its delivery to market.

Figure 1

ACD Market Value Chain

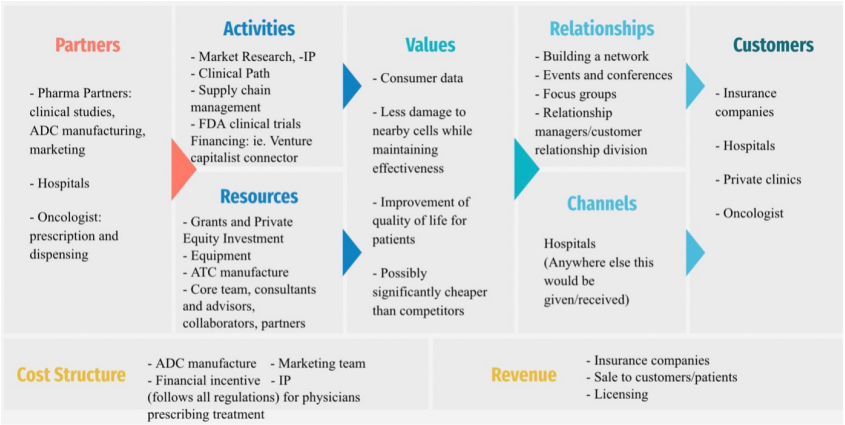
Ensuring that all components in the value chain come together at the right moment requires a variety of skill sets, production capabilities and expertise. Research into the value chains of competitor ADCs revealed that GritBio should focus on building support in two key areas—manufacturing and dispensing—to ensure successful commercialization of LIGRECA.

Manufacturing ADCs is a sophisticated and costly process that requires large capital investment and well-trained operators, as well as strict GMP and high operational problems. Downstream processing challenges for ADCs may limit industry expansion. Launchbio, a company designed to help biotech companies bring products to market, recommends that companies trying to upscale ADCs work with a Contract Development & Manufacturing Organization (CDMO). Specifically, GritBio should look for a CDMO that has an established process development program and that can offer a one-stop shop for bioconjugate research and manufacturing. In addition, GritBio's CDMO must be able to develop an Anti-AC Antibody, which needs to be humanized to be used in their clinical research. Finally, the manufacturer should focus on compliance, particularly for tech transfer across facilities, as well as optimizing the cost of products, selecting the correct equipment and minimizing contamination risks. Lonza and NJ Bio should be considered as potential manufacturers.

GritBio will also need to build a strong business model and marketing and sales team to dispense their ADC. Table 1 shows the proposed business model for GritBio's ADC.

Table 1

Business Model for LIGRECA



GritBio's ADC has a strong value proposition: it is a less toxic ADC, is expected to enhance quality of life and may be less expensive than competitors. GritBio should leverage these benefits in their marketing strategy to dominate the market. This will require that the marketing and sales team builds strong relationships with key stakeholders, such as insurance companies, hospitals, private clinics and hospitals, as they will be the ones directly engaging with patients. GritBio's team should also prioritize attending events and conferences for ADC therapies or head and neck cancer, where they will have access to these stakeholders. To leverage their products' relative affordability, GritBio may want to consider offering financial discounts for low-income patients or developing a payment plan that makes the treatment even more affordable to the average customer.

Financial projections

The global ADC market size was valued at USD 4.3 billion in 2020 and is expected to grow at a compound annual growth rate (CAGR) of 23.7% from 2021 to 2028. With a market value projected to grow from $14.72 billion in 2021 to $44.5 billion by 2026, ADCs are one of the fastest-growing classes of anti-cancer drugs in the industry (*Antibody drug conjugates,* 2022). Increased demand for cost-effective and quality cancer treatment is also expected to

drive the ADC market (Sumant & Phalke, 2017). This suggests a sizeable market for new ADC therapies.

Using the projected number of instances of hypopharyngeal cancer in 2023, which is 6170 total cases, we can calculate for TAM (Total Available Market), SAM (Serviceable Available Market) and SOM (Serviceable Obtainable Market). Because the typical individual requires three to six cycles of therapy, we multiplied the treatment cost by the average number of cycles (4.5). Because each therapy costs about $30,000, the average payment for each participant is $135,000. These calculations estimate TAM to be $833M and SAM to be $484.7 M. In their first year on the market, Grit Bio intends to target the east coast. We estimated that in 2030, Grit Bio will earn a net income of $48.5 million dollars in 2030 if they can capture 10% of that market.

V. Conclusion

The market for ADCs treating head and neck cancer is competitive and potentially profitable. GritBio's ADC, LIGRECA, has a particularly strong value proposition: it is less toxic than other proposed ADCs, is expected to enhance quality of life and may be less expensive than competitors. Applying for fast-track FDA approval, partnering with a CDMO for manufacturing and building a strong marketing and sales team will help GritBio bring this important product to market—potentially ahead of the competitors. If GritBio can demonstrate to stakeholders and potential customers that its ADC is less toxic and more effective than others, they will be able to leverage this in their marketing strategy to dominate the market.

Recommendations for future growth include mergers and acquisitions to enhance market share. In addition, GritBio would benefit from engaging a consultant focused on grant applications and private equity investments to ensure continued funding for their research. This may be an opportunity for GritBio to partner with a large pharmaceutical firm, since larger corporations would want to forego pre-clinical R&D, particularly if they currently have an ADC and are looking to extend their ADC portfolio.

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VII. References

Antibody drug conjugates market size report, 2030. (2022). Grand View Research. www.grandviewresearch.com/industry-analysis/antibody-drug-conjugates-market

National Cancer Institute. (2019, October 4). *Hypopharyngeal cancer treatment (adult) (PDQ®)–health professional version.* https://www.cancer.gov/types/head-and-neck/hp/adult/hypopharyngeal-treatment-pdq

National Cancer Institute. (2012, June 26). *Laryngeal and hypopharyngeal cancer—statistics.* https://www.cancer.net/cancer-types/laryngeal-and-hypopharyngeal-cancer/statistics

Sumant, O., & Phalke, G. (2017) *Antibody drug conjugates market*. Allied Market Research. https://www.alliedmarketresearch.com/antibody-drug-caonjugate-market

A Proposed Novel Two-Drug Combination Topical Treatment of Actinic Keratosis

Jonathan E. Taub
M.S. in Biotechnology Management and Entrepreneurship
Katz School of Science and Health

Abstract—This project, conducted on behalf of therapeutics startup GritBio, aimed to understand the market landscape for a novel topical treatment of Actinic keratosis (AK). Incidence of AK remains underestimated, and AK management may soon become a public health issue due to aging populations and the risks of transformation to squamous cell carcinomas (SCCs). While there are numerous treatment options for AK, no data have been published regarding the overall effectiveness of these therapies. Immunocompromised AK and SCC sufferers, particularly those with transplants, might find even more restricted treatment options in a crowded therapeutic field, as current treatments fail to address their increased vulnerability to toxic side effects. GritBio proposes a combination of two drugs—miltefosine and carmofur—in a novel composition for topical administration for the treatment of AK, which would provide the requisite cytotoxicity without the attendant adverse effects on immune cells. This project provides background on AK classification, existing treatment options and the potential market for GritBio's new treatment option. Findings suggest a large potential market for GritBio's proposed treatment, particularly among transplant and immunosuppressed patients.

Index Terms—Actinic Keratosis, Carmofur, Miltefosine, Squamous Cell Carcinoma, Topical

I. Introduction

Actinic keratosis (AK) is a rough patch on the skin caused by years of sun exposure. Due to the increase in the aging population worldwide as well as

the risks of transformation to squamous cell carcinomas (SCCs), AK management may eventually become a public health issue (Dreno et al., 2014). While there are numerous treatments options, no data have been published regarding the overall effectiveness of AK therapies. Comparisons between topical therapies have been hampered by different outcome measures, the fluidity of diagnostics and the lack of an agreed-upon standardization of system of diagnosis for AK itself. SCCs are markedly increased in individuals with compromised immune function (Veness & Ang, 2012), and treatment for AKs in transplant patients is particularly problematic as current treatments fail to address their increased vulnerability to toxic side effects.

GritBio, a therapeutics startup company focused on the development of novel cancer therapies, proposes a combination of two drugs—miltefosine and carmofur—in a novel composition for topical administration for the treatment of AK, which would provide the requisite cytotoxicity without the attendant adverse effects on immune cells. This project, conducted on behalf of GritBio, aimed to understand the market constraints and opportunities for bringing this novel topical AK treatment to market. The project provides background on AK classification, existing treatment options and the potential market for a new treatment option.

II. Methods

For this project, a market and landscape analysis was conducted, with emphasis on 1) the etiology and classification system for AK and SCC; 2) the size and characteristics of the available market (ethnodemographics and geography); and 3) the performative landscape of existing treatments (cryotherapy and topicals). Additional research was performed on the components of GritBio's novel AK treatment. Most of the scientific and anecdotal data used was procured from scientific literature available online, as well as from websites of pharmaceutical companies that are producing current topical treatments.

III. Results

Etiology and classification system for AK and SCC

AK and SCC incidences have increased exponentially over the last half-century, even as methods of diagnosis and diagnostic definitions remain

malleable. The current gold standard for diagnosis of an AK is classified as an invasive procedure, involving a biopsy of the lesion with subsequent histopathology. There are several clinical and histological classification systems for grading AK lesions and the risk of malignancy. Currently, however, there are no approved objective methods for clinical classification of AKs or approved histopathological grading systems (Rongioletti, 2019). For example, clinically, AKs can be classified according to three grades; in reality, this grading system tends to be reserved for teaching purposes and clinical trials, and in practice, dermatologists talk about thin, thick or hyperkeratotic AKs. These grading systems fall particularly short when attempting to differentiate grade III AKs from SCCs (Fong & Lowe, 2016).

Similarly, a universally accepted staging system for risk stratification of SCC is not yet available; until 2010, SCC was grouped in the American Joint Committee on Cancer (AJCC) staging manual with a multitude of other cutaneous malignancies (AJCC, 2002). Ultimately, a reliable noninvasive diagnostic tool to enable us to determine the difference between a hyperkeratotic AK and an initial SCC in uncertain cases remains elusive (Cramer & Stockfleth, 2020).

The lack of consensus as to whether AK is cancerous or precancerous only increases the urgency and potential market for new AK treatments. For GritBio, this suggests that a new treatment may be particularly marketable, and the fluidity in the classification of AKs could prove to be an effective marketing device.

Size and characteristics of current market

The worldwide incidence of AK is difficult to estimate, given that prevalence varies depending on the population evaluated (Martin, 2010). Given this, it is likely that the incidence of AKs remains underestimated (Atkins et al 2006). Still, AKs had an estimated prevalence of 39.5 million in 2004 alone, 26 million of which were in patients over 65 (Uhlenhake, 2013). AKs are the second most common diagnosis made by dermatologists in their practices, and they account for more than 5.2 million office visits yearly (Uhlenhake, 2013). In the U.S., AK is behind only acne and dermatitis as the primary reason for a dermatological consultation (Reinehr & Bakos, 2019). The U.S.

market for the treatment of AK is expected to grow to from $4.6 billion in 2017 to US $6.1 billion by 2024 (Market Research Future, 2019).

The prevalence of AK increases with age, ranging from <10% in 20–29-year-olds to 80% in Caucasians ages 60–69 (Reinehr & Bakos, 2019). Initial studies found AKs present in 55% of white men and 37% of white women between the ages of 65 and 74 years who were classified as having high cumulative sun exposure; in contrast, among a similar demographic with low cumulative sun exposure, AKs were present in only 19% of men and 12% of women (Engel et al., 1988). In the southern hemisphere, the prevalence of AK often reached 40-60% in elderly populations (Dreno et al., 2014).

In around 10% of all patients with AK, and approximately 30% of patients with additional immune suppression, an invasive squamous cell carcinoma of the skin is subsequently observed, highlighting that AKs should be identified and treated early (Stockfleth, 2009). Estimates of annual rates of individual transformations from AK to SCC range from 0.03 to 20% (Criscione et al., 2009), though regression rates up to 63 percent per year have been reported (Werner, 2013). Lesions that regress may subsequently reappear; studies with limited follow-up suggest that 15 to 53% of spontaneously regressed AKs recur within one year (Criscione et al., 2009).

The incidence and aggressiveness of SCC and MCC (both immunogenic carcinomas) are markedly increased in individuals with compromised immune function (Veness & Ang, 2012), and patients on chronic immunosuppressive therapy are at increased risk for skin cancers. Organ transplant recipients are reported as having 50-100 times the skin cancer risk of an age- and sex-matched control population (de Graaf et al., 2006), suggesting that treatment for AKs in transplant patients may be less effective than in the general population (Dragieva, 2004). Patients with multiple and confluent AKs are also likely to be at higher risk of nonmelanoma skin cancer (NMSC) than those with single lesions.

These findings suggest that GritBio's primary research target market should focus first on transplant and immunosuppressed patients, where they could both saliently justify an unmet medical need in facilitation of expedited re-

view and for a possible product launch into a crowded but large addressable market. At the outset, GritBio should consider identifying transplant patients from registries who might already be suffering from AK or cSCC and have endured difficult or failed therapy regimens, if their conditions have allowed them to access any available therapies that are not automatically excluded due to adverse effects.

Performative landscape of existing treatments

Cryotherapy, which freezes and destroys single AK lesions with liquid nitrogen, is the first-line standard treatment for isolated or small numbers of AK. Cryotherapy remains the gold standard for individual lesions, with reported cure rates between 75-95% (Nart et al., 2018). However, the main shortcoming of cryotherapy is that it does not treat field cancerization or subclinical lesions. Also, there is a very high recurrence rate of up to 96% within one year (Krawtchenko, 2007). Multiple studies support the need to treat cancer fields rather than individual AKs in the hope of reducing future tumor development (Bickers et al., 2006; Padilla et al., 2010).

Photodyamic therapy (PDT) is another treatment option for AK. In PDT, a photosynthesizing drug, either aminolevunic acid or methyl aminolevulinate, is applied to the field cancerization where it preferentially accumulates in rapidly dividing atypical keratinocytes; on exposure to an external light source in the presence of oxygen, these cells are then eradicated (Dirschka, 2013). The advantages of PDT include a lack of repetitive physician visits, a reduction in pain post-treatment and likely higher patient compliance (Stockfleth, 2012). Limitations of this treatment are that it is not widely available, it is time-consuming, it causes severe pain at time of application and it cannot be self-applied (Reinhold, 2017). A self-applied option might be daylight PDT, where a photosynthesizing cream is applied and then exposed to natural sunlight (Reinhold, 2017). While PDT is more effective than cryotherapy, it is only indicated for thin/non-hyperkeratotic multiple AKs of the face and scalp, which is not the initial treatment target for GritBio's proposed treatment and therefore not in direct competition.

Other treatment options include mechanical curettage of the AK lesions with a curette followed by electrocoagulation, which is reported in the liter-

ature as an alternative to cryotherapy for first-line therapy (Dreno et al., 2014). Among topicals, fluorouracil—the first-line topical—inhibits thymidylate synthetase, a critical enzyme in the synthesis of DNA, resulting in inhibition of cell proliferation and cell death. However, fluorouracil creams cause inflammation and lesion necrosis and in patients with extensive AK, the treated area may become extremely inflamed (Berman, 2020). Often a combination of approaches must be used to successfully control a patient's AK lesions (Stockfleth, 2012). Patients with multiple thin AKs who also have discrete hyperkeratotic lesions may benefit from the sequential use of lesion-directed and field-directed therapies; the success of the treatment is evaluated by the percentage reduction in visible AK lesions (Cramer & Stockfleth, 2020). However, improvements to these treatments must be made. They often must be applied onto the lesions for multiple days and sometimes months at a time, which poses significant challenges to patients, especially those who are elderly (Dirschka et al., 2017).

GritBio's proposed AK treatment

In response to the challenges with existing treatment options, GritBio proposes a combination of two drugs—miltefosine and carmofur—in a novel composition for topical administration for the treatment of AK, which would provide the requisite cytotoxicity without the attendant adverse effects on immune cells.

Miltefosine has been reported to possess similar modes of action in *Leishmania* parasites and human cancer cells; its activity is mainly associated with inhibition of phospholipid turnover and lipid-dependen cell signaling pathways; this inhibition results in apoptosis (Alonso & Alonso, 2016). Early animal studies showed humoral antibodies were not changed by miltefosine pretreatment (Hilgard et al., 1991). In one trial, a mixture of alkylated glycerols of various chain lengths and water was used as the pharmaceutical vehicle to dissolve and to further facilitate tissue penetration of miltefosine. A 6% miltefosine solution was applied once daily in the first week and twice daily in the following weeks. No systemic toxicities were observed (Terwogt et al., 1999).

Carmofur, an antineoplastic that releases 5-fluorouracil-drug intracellularly

and is clinically used as a chemotherapeutic agent (Wu et al., 2019; Hajj et al., 2015) might prove to be the ideal candidate as a second ingredient to alleviate the adverse effects in miltefosine. One clinical trial of Miltex, an antiproliferation drug comprising miltefosine and phospholipid ingredients used for topical treatment of metastatic skin lesions in breast cancer, was conducted in 11 breast cancer patients who were resistant to standard therapy for cutaneous metastasis (Moiseenko et al., 2000). Another study of 72 patients conducted in the same year showed a high response rate and well-tolerated side effects (Koynov et al., 2000). In AK or SCC, there is no pathogen induced adoptive immune response; in cancer, innate immune response is involved (Subbarayan et al., 2019) and thus suppressing adoptive immune response should not interfere with anti-neoplastic effect of the drugs.

As miltefosine and carmofur both lead to accumulation of pro-cell-death lipid ceramide—carmofur inhibiting ceramide conversion into sphingosine, and miltefosine inhibiting its conversion into sphingomyelin (Marco et al., 2009)—on a molecular level, combining the two drugs should have a synergistic effect, further enhancing the prospects of the combination.

V. Conclusion

AK and SCC diagnostic criteria is unclear, and there is no agreed-upon standardized diagnostic system. GritBio's proposed treatment has the potential to benefit the large population of individuals with AK and SCCs, in the US and worldwide, as it suits both diagnoses.

Current project findings suggests that a primary research target would involve a focus first on the transplant and immunosuppressed patients, where GritBio could both saliently justify an unmet medical need in facilitation of expedited FDA review and get approval ahead of the competition in a crowded if large total addressable market. The majority of competing experimental drugs that have been approved are aiming for cancer markets beyond cutaneous malignancies, even beyond the HPV therapies that might share similar etiologies. As opposed to their outside-in approach, GritBio would be taking an inside-out approach by testing in the underserved immunocompromised patient segment, which might eventually lead to a smoother testing path in in the larger cutaneous markets. At that point, GritBio could ex-

pand to a larger target audience of AK patients needing field directed therapy, with the aim of becoming a first line therapy as an enhancement to PDT and eventually replacing other topicals with its superior safety profile. A secondary audience could be patients at risk of AK/SCC, particularly immunosuppressed/transplant patients in need of field therapy—whether for preventative purposes or for patients already taking immunosuppressants.

Recommendations: GritBio will benefit from developing an economic and price point strategy based on current treatments' prices and frequency of use; what insurance will cover/what codes apply to topical chemotherapies; and the potential for an economy of scale based on an expanded patient pool beyond the initial target audience of transplant and immunocompromised patients. Should they achieve expanded access to the total AK market, GritBio could also consider entering into a distribution partnership with a company like Profounda, who has a track regard in producing miltefosine on consignment.

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VII. References

Alonso, L., & Alonso, A. (2016). Hemolytic potential of miltefosine is dependent on cell concentration: Implications for in vitro cell cytotoxicity assays and pharmacokinetic data. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1858*(6), 1160–64. https://doi.org/10.1016/j.bbamem.2016.03.004

American Joint Committee on Cancer. (2002). *AJCC Cancer Staging Manual*. https://doi.org/10.1007/978-1-4757-3656-4

Atkins, D., Bang, R., Sternberg, M., & Chen, S. (2006). Reliable methods to evaluate the burden of actinic keratoses. *J Invest Dermatol, 126*(3), 591–4. https://doi.org/10.1038/sj.jid.5700110

Berman, B. (2020). *Treatment of actinic keratosis.* UpToDate. https://www.uptodate.com/contents/treatment-of-actinic-keratosis?search=actinic%20keratosis&source=search_result&selectedTitle=2~103&usage_type=default&display_rank=2

Bickers, D., Lim, H., Margolis, D., Weinstock, M., Goodman, C., Faulkner, E., Gould, C., Gemmen, E., & Dall, T. (2006). The burden of skin diseases: 2004: A joint project of the American academy of dermatology association and the society for investigative dermatology. *Journal of the American Academy of Dermatology, 55*(3), 490–500. https://doi.org/10.1016/j.jaad.2006.05.048

Cramer, P., & Stockfleth, E. (2020). Actinic keratosis: Where do we stand and where is the future going to take us? *Expert Opinion on Emerging Drugs, 25*(1), 49–58. https://doi.org/10.1080/14728214.2020.1730810

Criscione, V. D., Weinstock, M. A., Naylor, M. F., Luque, C., Eide, M. J., & Bingham, S. F. (2009). Actinic keratoses: Natural history and risk of malignant transformation in the veterans' affairs topical tretinoin chemoprevention trial. *Cancer, 115*(11), 2523–30. https://doi.org/10.1002/cncr.24284

de Berker, D., McGregor, J. M., Mohd Mustapa, M. F., Exton, L. S., & Hughes, B. R. (2017). British association of dermatologists' guidelines for the care of patients with actinic keratosis 2017. *British Journal of Dermatology, 176*(1), 20–43. https://doi.org/10.1111/bjd.15107

de Berker, D., & Pasch, M. (2019). Primary care and actinic keratosis. *British Journal of Dermatology, 181*(1), 13–14. https://doi.org/10.1111/bjd.18102

de Graaf, Y. G. L., Kennedy, C., Wolterbeek, R., Collen, A. F. S., Willemze, R., & Bouwes Bavinck, J. N. (2006). Photodynamic therapy does not prevent cutaneous squamous-cell carcinoma in organ-transplant recipients: Results of a randomized-controlled trial. The Journal of Investigative *Dermatology, 126*(3), 569-574. https://doi.org/10.1038/sj.jid.5700098

Dirschka, T., Gupta, G., Micali, G., Stockfleth, E., Basset-Séguin, N., Del Marmol, V., Dummer, R., Jemec, G. B. E., Malvehy, J., Peris, K., Puig, S., Stratigos, A. J., Zalaudek, I., & Pellacani, G. (2016). Real-world approach to actinic keratosis management: Practical treatment algorithm for office-based dermatology. *Journal of Dermatological Treatment, 28*(5), 431–442. https://doi.org/10.1080/09546634.2016.1254328

Dirschka, T., Radny, P., Dominicus, R., Mensing, H., Brüning, H., Jenne, L., Karl, L., Sebastian, M., Oster-Schmidt, C., Klövekorn, W., Reinhold, U., Tanner, M., Gröne, D., Deichmann, M., Simon, M., Hübinger, F., Hofbauer, G., Krähn-Senftleben, G., Borrosch, F., & Reich, K. (2012). Long-term (6 and 12 months) follow-up of two prospective, randomized, controlled phase III trials of photodynamic therapy with BF-200 ALA and methyl aminolaevulinate for

the treatment of actinic keratosis. *British Journal of Dermatology, 168*(4), 825–836. https://doi.org/10.1111/bjd.12158

Dodds, A., Chia, A., & Shumack, S. (2014). Actinic keratosis: Rationale and management. *Dermatology and Therpay, 4,* 11–31. https://doi.org/10.1007/s13555-014-0049-y

Dragieva, G., Hafner, J., Dummer, R., Schmid-Grendelmeier, P., Roos, M., Prinz, B. M., Burg, G., Binswanger, U., & Kempf, W. (2004). Topical photodynamic therapy in the treatment of actinic keratoses and Bowen's disease in transplant recipients. *Transplantation, 77*(1), 115–121. https://doi.org/10.1097/01.TP.0000107284.04969.5C

Dréno, B., Amici, J. M., Basset-Seguin, N., Cribier, B., Claudel, J. P., & Richard, M. A. (2014). Management of actinic keratosis: A practical report and treatment algorithm from AKTeam TM expert clinicians. *Journal of the European Academy of Dermatology and Venereology, 28*(9), 1141–1149. https://doi.org/10.1111/jdv.12434

Engel, A., Johnson, M. L., & Haynes, S. G. (1988). Health effects of sunlight exposure in the United States. Results from the first National Health and Nutrition Examination Survey, 1971–1974. *Arch Dermatol., 124*(1), 72–9.

Euvrard, S., Kanitakis, J., & Claudy, A. (2003). Skin cancers after organ transplantation. *New England Journal of Medicine, 348*(17), 1681–91. https://doi.org/10.1056/NEJMra022137

Figueras Nart, I., Cerio, R., Dirschka, T., Dréno, B., Lear, J. T., Pellacani, G., Peris, K., & Ruiz de Casas, A. (2017). Defining the actinic keratosis field: A literature review and discussion. *Journal of the European Academy of Dermatology and Venereology, 32*(4), 544–563. https://doi.org/10.1111/jdv.14652

Fong, G. & Lowe, P. (2016). A GP's guide to actinic keratosis. *MedicineToday, 17*(11), 43–53.

Hajj, C., Becker-Flegler, K. A., & Haimovitz-Friedman, A. (2015). Novel mechanisms of action of classical chemotherapeutic agents on sphingolipid pathways. *Biological Chemistry, 396*(6-7), 669–679. https://doi.org/10.1515/hsz-2014-0302

Harpaz, R., Dahl, R. M., & Dooling, K. L. (2013). Prevalence of immunosuppression among US adults. *JAMA, 316*(23), 2547–2548.

https://jamanetwork.com/journals/jama/fullarticle/2572798

Hilgard, P., Kampherm, E., Nolan, L., Pohl, J., & Reissmann, T. (1991). Investigation into the immunological effects of miltefosine, a new anticancer agent under development. *Journal of Cancer Research and Clinical Oncology, 117*(5), 403–408. https://doi.org/10.1007/BF01612758

Koynov, K., Hristova, R., Shkodrova, S., Tzolova, N., Boydeva, L., Balabanov, V., Ivanova, N., Petkov, V., Davidov, D., Iliev, G., Chilingirov, P., Ilieva, R., Popov, B., Taskova, V., Majdrakova, D., Trifonova, M., Yanchev, A., Spasova, T., & Yancheva, K. (2000). Miltex® in palliative treatment of skin′ metastases of breast cancer. *Bulgarian Medicine, 8*(1), 7–11. https://www.researchgate.net/publication/289033600_MiltexR_in_palliative_treatment_of_skin′_metastases_of_breast_cancer

Krawtchenko, N., Roewert-Huber, J., Ulrich, M., Mann, I., Sterry, W., & Stockfleth, E. (2007). A randomised study of topical 5% imiquimod vs. topical 5-fluorouracil vs. cryosurgery in immunocompetent patients with actinic keratoses: A comparison of clinical and histological outcomes including 1-year follow-up. *British Journal of Dermatology, 157*(s2),34–40. https://doi.org/10.1111/j.1365-2133.2007.08271.x

Marco, C., Jiménez-López, J. M., Ríos-Marco, P., Segovia, J. L., & Carrasco, M. P. (2009). Hexadecylphosphocholine alters nonvesicular cholesterol traffic from the plasma membrane to the endoplasmic reticulum and inhibits the synthesis of sphingomyelin in HepG2 cells. *The International Journal of Biochemistry & Cell Biology, 41*(6), 1296–1303. https://doi.org/10.1016/j.biocel.2008.11.004

Martin G. (2010). The impact of the current United States guidelines on the management of actinic keratosis: Is it time for an update? *The Journal of Clinical and Aesthetic Dermatology, 3*(11), 20–25. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2989810/

Moiseenko, V. M., Orlova, R. V., Ermakova, N. A., & Protsenko, S. A. (2000). [Treatment with Miltex for metastatic skin lesions in breast cancer]. *Voprosy Onkologii, 46*(5), 600–603. https://pubmed.ncbi.nlm.nih.gov/11202195/

Market Research Future. (2019). *Actinic keratosis treatment market: Information by type (medications and procedures), by end user (hospitals & oncology centers, dermatology clinics and ambulatory surgical center)—forecast*

till 2027. https://www.marketresearchfuture.com/reports/actinic-keratosis-treatment- market-2366

Padilla, R. S., Sebastian, S., Jiang, Z., Nindl, I., & Larson, R. (2010). Gene expression patterns of normal human skin, actinic keratosis, and squamous cell carcinoma. *Archives of Dermatology, 146*(3). https://doi.org/10.1001/archdermatol.2009.378

Piquero-Casals, J., Morgado-Carrasco, D., Gilaberte, Y., Del Rio, R., Macaya-Pascual, A., Granger, C., & López-Estebaranz, J. L. (2020). Management pearls on the treatment of actinic keratoses and field cancerization. *Dermatology and Therapy, 10*(5), 903–915. https://doi.org/10.1007/s13555-020-00425-4

Reinehr, C. P. H., & Bakos, R. M. (2019). Actinic keratoses: Review of clinical, dermoscopic, and therapeutic aspects (Corrigir nos altos de página onde tem o título). *Anais Brasileiros de Dermatologia, 94*(6), 637–657. https://doi.org/10.1016/j.abd.2019.10.004

Reinhold, U. (2017). A review of BF-200 ALA for the photodynamic treatment of mild-to-moderate actinic keratosis. *Future Oncology, 13*(27), 2413–2428. https://doi.org/10.2217/fon-2017-0247

Rongioletti, F. (2019, May 23). Actinic keratoses: What classification is useful to predict the risk of progression? PROs and cons. *Journal of the European Academy of Dermatology and Venereology, (33)*6, 983–4. https://doi.org/10.1111/jdv.15649

Scariot, D. B., Britta, E. A., Moreira, A. L., Falzirolli, H., Silva, C. C., Ueda-Nakamura, T., Dias-Filho, B. P., & Nakamura, C. V. (2017). Induction of early autophagic process on leishmania amazonensis by synergistic effect of miltefosine and innovative semi-synthetic thiosemicarbazone. *Frontiers in Microbiology, 8*(255). https://doi.org/10.3389/fmicb.2017.00255

Stockfleth E. (2017). The importance of treating the field in actinic keratosis. *Journal of the European Academy of Dermatology and Venereology, 31*(S2), 8–11. https://doi.org/10.1111/jdv.14092

Stockfleth E. (2012). The paradigm shift in treating actinic keratosis: a comprehensive strategy. J Drugs *Dermatol., 11*(12), 1462–1470. https://pubmed.ncbi.nlm.nih.gov/23377517/

Stockfleth E. (2009). Topical management of actinic keratosis and field cancerisation. *G Ital Dermatol Venereol., 144*(4), 459–62. https://pubmed.ncbi.nlm.nih.gov/19755950/

Subbarayan R. S, Arnold, L., Gomez, J. P., & Thomas, S. M. (2019). The role of the innate and adaptive immune response in HPV-associated oropharyngeal squamous cell carcinoma. *Laryngoscope Investig Otolaryngol., 4*(5), 508–512. https://doi.org/10.1002/lio2.300

Sunyoto, T., Potet, J., & Boelaert, M. (2018). Why miltefosine—a life-saving drug for leishmaniasis—is unavailable to people who need it the most. *BMJ Global Health, 3*(3). https://doi.org/10.1136/bmjgh-2018-000709

Terwogt, J. M., Mandjes, I. A., Sindermann, H., Beijnen, J. H., & ten Bokkel Huinink, W. W. (1999). Phase II trial of topically applied miltefosine solution in patients with skin-metastasized breast cancer. *British Journal of Cancer, 79*(7-8), 1158–1161. https://doi.org/10.1038/sj.bjc.6690184

Uhlenhake, E. (2013). Optimal treatment of actinic keratoses. *Clinical Interventions in Aging, 8,* 29–35. https://doi.org/10.2147/cia.s31930

Veness, M. J., & Kian Ang, K. (2012). Chapter 39: Cutaneous carcinoma. In L. L. Gunderson & J. E. Tepper (Eds.), *Clinical Radiation Oncology (Third Edition)* (pp. 757–769). W.B. Saunders. https://doi.org/10.1016/B978-1-4377-1637-5.00039-0

Werner, R. N., Sammain, A., Erdmann, R., Hartmann, V., Stockfleth, E., & Nast, A. (2013). The natural history of actinic keratosis: A systematic review. *British Journal of Dermatology, 169*(3), 502–518. https://doi.org/10.1111/bjd.12420

Wu, K., Xiu, Y., Zhou, P., Qiu, Y., & Li, Y. (2019). A new use for an old drug: Carmofur attenuates lipopolysaccharide (LPS)-induced acute lung injury via inhibition of FAAH and NAAA activities. *Frontiers in Pharmacology, 10.* https://doi.org/10.3389/fphar.2019.00818

Regulation of Testicular Sertoli Cells by SUMOylation

Manveet Singh Nanda and Shanza Baseer Tariq
M.S. in Biotechnology Management and Entrepreneurship
Katz School of Science and Health

Tania Kiesel and Kayla Perlmutter
Stern College for Women

Dr. Amitabha Sengupta and Dr. Margarita Vigodner
Department of Biology, Stern College for Women

Abstract—This study aimed to show that KAP1 regulates SUMOylation in testicular Sertoli cells. The molecular regulation of Sertoli cells and their crosstalk with germ cells has not been fully characterized. Small-ubiquitin-related modifier (SUMO) proteins are essential for normal sperm development and are expressed in mouse and human Sertoli cells. However, the cell-specific role of SUMOylation in those cells has only started to be elucidated. In other cell types, including granulosa cells, SUMOylation is regulated by a SUMO ligase KAP1/Trim28. Deletion of KAP1 in Sertoli cells causes testicular degeneration. However, the role of KAP1 in those cells has not been identified. The results of this study show that both murine and human Sertoli cells undergo apoptosis upon inhibition of SUMOylation with a chemical inhibitor or via a siRNA technology, coupled with changes in the Sertoli cell proteome. Findings suggest that, among others, the expression of ER/stress-related proteins is highly affected by this inhibition. SUMOylation may also regulate the NOTCH signaling, which is important for the maintenance of the developing germ cells. Furthermore, siRNA-down-regulation of KAP1 in a Sertoli-derived cell line causes an almost complete inactivation of SUMOylation. In conclusion, SUMOylation regulates

important survival and signaling pathways in Sertoli cells, and KAP1 can be a major regulator of SUMOylation in these cells.

Index Terms—KAP1, NOTCH Signaling, Sertoli Cells, SUMO Ligases, SUMOylation

I. Introduction

Infertility affects 8-12% of couples in the U.S. Half of these cases can be attributed to the male partner, and 30-50% of these cases are idiopathic (occurring for an unknown reason). One major cause is damage to spermatogenesis, the process of formation of sperms. New insights into the regulation of spermatogenesis can shed light on specific cases of male infertility. Sperm formation is supported by testicular somatic cells, known as Sertoli cells. They are essential for spermatogenesis by producing important hormones and growth factors. Spermatogenesis and functions of Sertoli cells are supported by post-translational modifications (PTMs). The focus of this research is a PTM known as SUMOylation, which is mediated by addition of small-ubiquitin-related modifiers (SUMO) to the target proteins, and which supports spermatogenesis (Vigodner, 2011; Vigodner et al., 2020).

SUMOylation is the process in which SUMO proteins covalently attach to cellular proteins and modify their functions. SUMOylation is implicated in cellular events such as transcription regulation, protein-protein interactions, cell division and nuclear-cytoplasmic transport. SUMOylation requires activating enzymes (E1), conjugating enzymes (E2) and cell specific ligating enzymes (E3) (Chymkowitch et al., 2015). One of the enzymes regulating SUMOylation in other cell types is a SUMO-ligating enzyme called KAP1 (KRAB-associated protein-1). This SUMO ligase was specifically chosen to study in Sertoli cells because it is a major regulator of SUMOylation in granulosa cells, the female equivalent of Sertoli cells (Rossitto et al., 2021). Additionally, KAP1 is highly expressed in testis, and it colocalizes SUMO in Sertoli cells (Xiao et al., 2016). We hypothesized that KAP1 regulates SUMOylation in Sertoli cells.

II. Methods

Cell lines, primary mouse cells, and human Sertoli cells

The mouse Sertoli cell line 15P-1 (ATCC®, CRL-2618) was purchased from ATCC (Manassas, VA) and grown in DMEM media with 5% fetal bovine serum (FBS, Life Technologies, 16,140e071), 5% bovine growth serum (Fisher Scientific, SH30541.03), 1% penicillin/ streptomycin (Life Technologies, 15,140e122) and 0.5% Fungizone (Life Technologies, 15,290e018) at 32°C with 5% CO_2. The primary human Sertoli cell comprehensive kit was purchased from ScienCell Research Laboratories (Carlsbad, CA), and the cells were cultured at 32°C with 5% CO_2. The human Sertoli were isolated from the human testis and characterized by immunofluorescence with an antibody specific to GATA-4 and Sox-9. C57BL/6NCrl mice were purchased from Charles River (Kingston, NY). The Animal Committee of Albert Einstein College of Medicine approved all animal protocols that followed the National Institutes of Health guide for the care and use of laboratory animals. The cells were cultured on the FBS-coated dishes at the concentration of 1e5x106 cells/100 mm dish for a duration of 2–3 h. Sertoli cells continued to grow for several days and were used for the experiments at 70–80% confluency.

Ginkgolic acid and si-RNA treatments

SUMOylation inhibitor Ginkgolic acid (GA) was diluted with DMSO and used at the concentration of 25e150mM for 2 h. The concentration for each cell type was chosen based on our previous studies and at the range that would not cause massive cell death and detachment. For the proteomic experiments, FSH (F4021, Sigma Aldrich) was added at the concentration of 20 IU/ml 4 h before the addition of the GA (50mMfor 2 h) to some FSH and control wells. Eighty pmols of UBC9 (the only conjugating enzyme for all SUMO isoforms), KAP1, or control siRNAs (Santa Cruz Biotechnology; sc-36773, sc-38551, and sc-36869, respectively) were used for the transfection of the Sertoli-derived cell line using siRNA transfection reagent (sc-29528) and siRNA transfection medium (sc-36868). The cells were subjected to 6 h of transfection followed by a 48h recovery period before analysis. The dose of the siRNAs and the transfection time were determined in pilot experiments, and the down-regulation was assessed using western blot analysis.

Viability and apoptotic assays

WST-1 cell proliferation assay (Cayman Chemical, Ann Arbor, MI) and The Caspase-Glo® 3/7 assay (Promega, WI, USA) were performed according to the manufacturer's instructions. The measurements were collected using a Promega Glomax microplate reader. Each experiment was repeated three times in triplicates. To calculate the difference between the samples, students' paired t-test was used. $P < 0.05$ was considered statistically significant.

Whole-cell protein lysate preparation

Whole-cell protein lysates were prepared using the whole-cell extraction kit and protease inhibitor from Millipore (2910, Sigma-Aldrich) complemented with 2.5 mg/ml of NEM (a de-SUMOylation inhibitor; E3876-100G, Sigma-Aldrich) according to the manufacturer's instructions. Protein concentrations were determined via bicinchoninic acid (BCA) protein assay (23,225, Thermo Fisher) using bovine serum albumin (BSA) as the standard.

Gel electrophoresis and Western blot

Gel electrophoresis and western blotting were performed using NuPAGE 4%e12% gradient bis-tris polyacrylamide gels (Thermo Fisher) and MOPS running buffer (Thermo Fisher). The membrane was incubated with primary antibodies in PBS containing 2% BSA and 0.1% sodium azide for either 2 h at room temperature or overnight at 4°C". Rabbit polyclonal anti-SUMO1 antibody (Abcam, ab32058) was used at a 1:500 dilution; a rabbit polyclonal antibody against KAP1 (Bethyl, Montgomery, TX, A300-274A) was used at a 1:1000 dilution; a mouse anti-ubiquitin antibody from Millipore (MAB1510) was used at a 1:100 dilution. Equal loading was ensured with monoclonal anti-b-actin (sc-1615, Santa Cruz) at a 1:1000 dilution. Following three washes with PBS-T, the membrane was further incubated with secondary antibodies that were diluted to 1:5000 in PBS-T for 1 h at room temperature. The secondary antibodies used in this study included the following: anti-rabbit IgG horseradish peroxidase (HRP) linked (NA934V, GE Healthcare UK Limited) and goat anti-mouse IgG (H þ L) HRP (AP308P, EMD Millipore Corporation, Sigma Aldrich). Western blot detection was performed using Luminata™ Forte (Sigma-Aldrich), following the manufacturer's instructions.

III. Results

To test whether SUMOylation is important for Sertoli cell survival and functioning, we performed a treatment of a mouse Sertoli-derived cell line and human primary Sertoli cells with an increasing concentration of the SUMOylation inhibitor GA. The inhibition of SUMOylation caused a significant decrease in cell viability and an increase in apoptosis. Similarly, the use of anti-UBC-9 (the only SUMO conjugating enzyme) siRNAs caused a consistent and statistically significant decrease in cell viability and an increase in cell apoptosis. Immunoblot with anti-SUMO antibody was used to confirm the decrease in SUMOylation after its inhibition by the GA (the dose-response effect is seen) and by UBC-9 siRNAs.

Our data suggests that SUMOylation may regulate ER stress and Notch signaling in Sertoli cells. However, what proteins regulate SUMOylation in Sertoli cells is not currently known. A recent study in granulosa cells (female counterparts of the Sertoli) identified KAP1 as a major SUMO ligase. KAP1 is highly expressed in the testes, including Sertoli cells. Notably, a Sertoli-specific inactivation of KAP1 causes testicular degeneration (Rossitto et al., 2021). Indeed, upon down-regulation of KAP1 using siRNAs, a dramatic decrease in the overall SUMOylation was observed. Almost all high molecular weight (HMW) bands disappeared while the level of the free SUMO was significantly increased. These results support the possible specific role of KAP1 as SUMO ligase.

IV. Discussion

SUMO proteins are highly expressed in all testicular cells where they were implicated in cell-specific functions during spermatogenesis. Sertoli cells provide support and regulation of developing germ cells. However, the molecular regulation of Sertoli cells and their crosstalk with germ cells has not been fully characterized. In this study, we have shown that both mouse and human Sertoli cells undergo apoptosis upon inhibition of SUMOylation with a chemical inhibitor or employing siRNA technology.

E3s (SUMO-ligases) show tissue- and cell-specific distribution. In other cell types, including granulosa cells, SUMOylation is regulated by a SUMO ligase KAP1/Trim28. KAP1 is expressed in mouse and human Sertoli cells, and

previous studies have shown that the inactivation of KAP1 in Sertoli cells causes testicular degeneration. KAP1 interacts and co-localizes with SUMO in mouse and human Sertoli. The results herein show that a downregulation of KAP1 in the Sertoli-derived cell line causes an almost complete inactivation of SUMOylation in these cells. Therefore, KAP1 may be a major regulator of SUMOylation in Sertoli cells. These results should be confirmed by in-vivo experiments, since the transfection procedure affected the viability of the primary Sertoli cells.

These studies lay a foundation for future studies *in vivo* that would include a generation of transgenic animals with inactivated SUMOylation machinery and a mutation abolishing the SUMO-ligase activity of KAP1 specifically in Sertoli cells. Analysis and comparison of these phenotypes among themselves and to the one reported for the KAP1 Sertoli knockout will be important in dissecting the molecular events regulated by SUMOylation and its corresponding KAP1-regulating activity in Sertoli cells.

V. Conclusion

The inhibition of KAP-1 causes down-regulation of SUMO conjugation to its target proteins. KAP-1 regulates SUMOylation in Sertoli cells. Additionally, the down-regulation demonstrates that KAP-1 may act as a SUMO ligase. Down-regulation of KAP-1 affects the viability of Sertoli cells and initiates certain apoptosis. This study provides a foundation for further in-vivo studies in mice where SUMOylation and KAP-1 activity will be inactivated in Sertoli cells. Testicular biopsies from infertile patients can be used to check the level of SUMOylation and screen for mutations in the KAP-1 SUMO ligase activity site.

VI. Acknowledgment

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VII. References

Chymkowitch, P., Nguéa P. A., & Enserink, J. M. (2015). SUMO-regulated transcription: Challenging the dogma. *BioEssays, 37*(10), 1095–1105. https://onlinelibrary.wiley.com/doi/10.1002/bies.201500065

Rossitto, M., Déjardin, S., Rands, C., Le Gras, S., Migale, R., Rafiee, M. R., Neirijnck, Y., Pruvost, A., Nguyen, A., Bossis, G., Cammas, F., Le Gallic, L., Wilhelm, D., Lovell-Badge, R., Boizet-Bonhoure, B., Nef, S., & Poulat, F. (2021). TRIM28-dependent SUMOylation protects the adult ovary from the male pathway. *HAL*. https://doi.org/10.1101/2021.03.24.436749

Vigodner, M. (2011). Roles of small ubiquitin-related modifiers in male reproductive function. *International Review of Cell and Molecular Biology, 288,* 227–259. https://doi.org/10.1016/B978-0-12-386041-5.00006-6

Vigodner, M., Lucas, B., Kemeny, S., Schwartz, T., & Levy, R. (2020). Identification of sumoylated targets in proliferating mouse spermatogonia and human testicular seminomas. *Asian Journal of Andrology, 22*(6), 569–577. https://doi.org/10.4103/aja.aja_11_20

Xiao, Y., Pollack, D., Andrusier, M., Levy, A., Callaway, M., Nieves, E., Reddi, P., & Vigodner, M. (2016). Identification of cell-specific targets of sumoylation during mouse spermatogenesis. *Reproduction (Cambridge, England), 151*(2), 149–166. https://doi.org/10.1530/REP-15-0239

Adbot: Integrated Communications Go-to-Market Plan

Hannah Kraitberg, Tomer Mendler, Jingyuan Wang, Panxinyue Zhang and Linyu Zheng
M.S. in Digital Marketing and Media
Katz School of Science and Health

Abstract—In this collaborative project, the Katz School's digital marketing and media team developed a comprehensive brand and marketing strategy for Adbot, a startup company aiming to change the way YouTube creators monetize. The resulting strategy leverages deep customer and competitor insights as well as the company's business purpose and differentiators. The strategy also combines all relevant marketing channels. It provides a roadmap to steer Adbot's successful brand development and a clear framework for reaching Adbot's target audience, including details on planned activity and the relevant methods of communication.

Index Terms—Financial Freedom, Gen Z Demands, Influencers, Passive Income, Social Media Monetization, Social Platforms

I. Introduction

Adbot is a startup company looking to change the way YouTube creators monetize. With over 2 billion monthly users, Adbot recognized that YouTube was the ideal platform to convert views into customers. Adbot processes thousands of branded deals to find the best match. Then, using curate personal storefronts, YouTubers can offer their fans exciting discounts while simultaneously earning with each purchase.

Adbot hired the Katz School digital marketing and media team as consultants with specific goals in mind: to help solidify their brand strategy; pinpoint and segment their audience; create a marketing and communications strategy; draft a creative, content, and channel strategy; plan their

editorial/content calendar; develop collateral samples; and design a go-to-market roadmap. The objective of this project was to find the most effective ways to reach YouTubers and convince them to join Adbot. This was done by leveraging deep customer and competitor insights to create specific messaging and content strategies to attract the target audience.

II. Methods

First, to establish background on Adbot's target audience, the research team generated a deep listening report. This consisted of visiting 50,000 websites, including Reddit, YouTube, LinkedIn, Facebook, Instagram, Twitter, TikTok, and various message boards, to eavesdrop and search for insights about how YouTubers view monetization, what YouTubers desire, who can be a YouTuber, and the motivations behind these types of influencers. Verbatims were gathered from these sites, which provided insight into Adbot's potential audience and how best to reach them.

Next, relevant data about YouTube usage was gathered from YouTube Storage Usage Statistics (Omnicore, 2022). This allowed the research team to "count" and monitor how frequently certain suggestions or concerns were brought up by users. Finally, an omnichannel exploration approach was initiated using Adbot's Google Analytics to collect data about how existing users interact with Adbot's existing landing page, ads, videos and posts. Together, this background research informed strategy integration and proposal discussions, leading to the development of a 2022 creative brief, as well as content and seeding calendars for Adbot.

III. Results

The deep listening report revealed these key insights:

- GenZ demands financial freedom
- YouTubers demand passive income
- High importance of micro-influencers for small brands
- YouTube partner program is not for everyone
- Influencers on TikTok and Instagram are more effective
- Anyone has the potential to be a YouTuber

Using the information from the deep listening report as well as YouTube statistics and the omnichannel exploration, a 2022 creative brief (Figure 1),

content calendar (Figure 2), and seeding calendar (Figure 3) were developed. Collateral samples and a go-to-market roadmap (Figure 4) were also developed.

Figure 1

Creative Brief

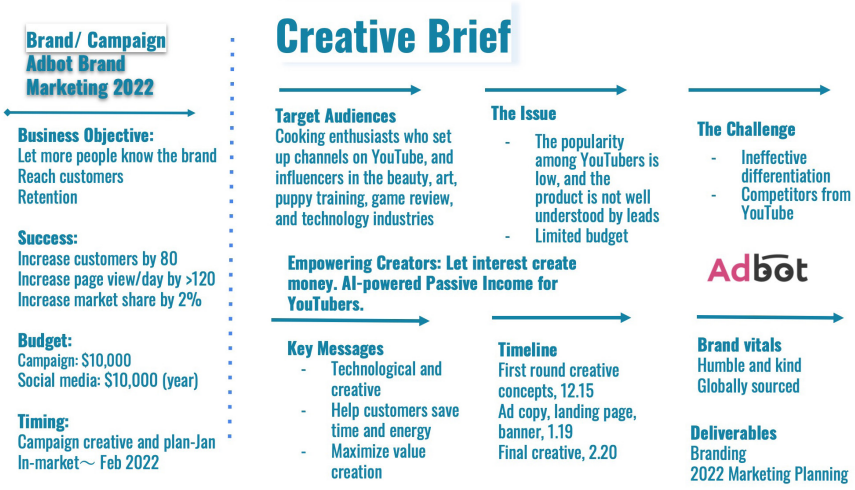


Figure 2

Content Calendar

For Creators, For You

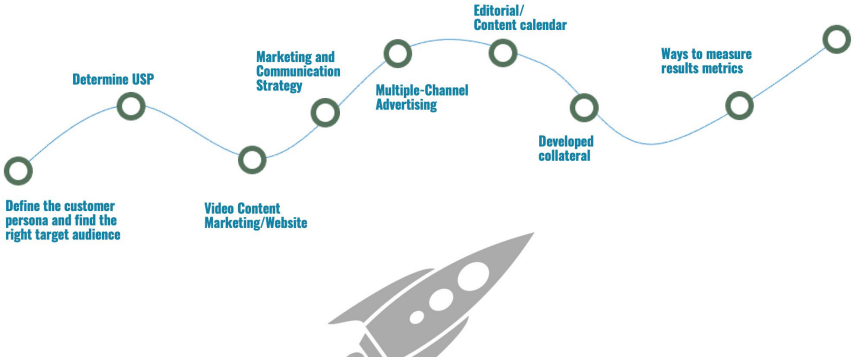
STAGE	PROJECT	SHARING CONTENT	CONNECTION CHANNELS	BUDGET
Q1	LET INTEREST CREAT MONEY	Cooking demonstration, puppy training, creative home furnishing, plant management	Community housewives groups, local cooking associations, community household goods member groups	20%
Q2	GROW.GROW.GROW.	Beauty design, makeup skills, game review, entertainment	Small and medium-sized beauty companies and entertainment studios	20%
Q3	KNOW MORE, GET MORE	Knowledge-based information sharing (business, marketing, art, animation production, language learning, data analysis, games)	Paid knowledge institutions, educational institutions	30%
Q4	SOCIAL MEDIA STRATEGY	Trend, newsjacking, tag, event, marketing, customer successful case, WOM	Facebook, Twitter, Instagram, TikTok, Linkedin, other(service fee, KOL)	30%

Figure 3

Seeding Calendar

Figure 4

Marketing Strategy Roadmap



IV. Discussion

The objective of this collaborative project was to find the most effective ways to reach YouTubers and convince them to join Adbot. Despite other monetization methods being available, our goal was to show how Adbot was the exemplary choice for YouTubers in terms of customer service, personalization and support. We also wanted to show YouTubers that they have the

power to turn their hobby into a business with Adbot as their guide.

By researching websites, blogs, and social media channels; reading YouTube comments; and scrolling through Reddit message boards, we were able to gain a deeper understanding of what Adbot's target audience—YouTubers—want. This gave us the ability to formulate our campaigns and creative strategies with their particular desires in mind. Through this project, we found that the biggest limitations to Adbot's success were a lack of understanding about the product among leads, a limited budget and stronger competitors. Therefore, our marketing plan included several strategies for Adbot to solidify its brand, better engage and understand its audience, and differentiate its products and services. Some of our key recommendations included:

- Focusing marketing efforts on micro-influencers.
- Implementing a brand awareness strategy using various methods, including influencer webinars, product and service training videos, self-nurturing landing pages, free customer experiences and organic social media promotion.
- Using the latest online marketing and social media techniques to strengthen Adbot's marketing funnel, drive web visits and build awareness.
- Creating potential "customer personas" and a customer journey for Adbot to pursue improved engagement, which could also be used as a starting point for potential emails, videos and other messaging techniques to attract users.
- Investigating the benefits of a personalized loyalty program, referral program, and re-engaged email program to keep users engrossed and enhance Adbot's word-of-mouth and public image.

One of the key takeaways from our listening research was that micro-influencers are a critical component of Adbot's target audience—as they are both potential customers and potential brand ambassadors and collaborators. Therefore, one of our key recommendations was to focus marketing efforts on micro-influencers, subdivided into different fields and communities. This would allow Adbot to both reach and collaborate with potential customers, to develop content and to build its reputation.

Social media marketing will also be critical to Adbot's success, providing the

most impact with minimal initial effort. Facebook, Instagram, YouTube and Reddit are some of the most cost-efficient digital marketing methods used to syndicate content and increase Adbot′ visibility. By implementing a social media strategy, Adbot will be able to engage with a broad customer base, ultimately increasing its brand recognition. Getting started only requires creating social media profiles for their business and beginning to interact with others by getting employees, business partners and sponsors to ″like″ and ″share″ Adbot pages. Simply having people interact with Adbot content will increase brand awareness and begin building Adbot's reputation.

V. Conclusion

The purpose of this project was to develop a comprehensive brand and marketing strategy for Adbot, a startup company aiming to change the way YouTube creators monetize. Through "deep listening" and detailed research, we discovered relevant, crucial and interesting insights regarding Adbot's target audience and what they truly want. These insights informed a comprehensive brand and marketing strategy. We believe that by implementing our strategic proposals, Adbot will raise awareness, strengthen their go to market plan and ultimately grow their market share.

This work contributes to the entire field of research in digital marketing and communications. Through deep listening, we can better understand consumers′ needs, motivations and pain points. These insights allow us to better tailor and personalize products, services and messaging so that we can more effectively reach and engage potential customers.

VI. Acknowledgment

We would like to express our gratitude to our program director, Joseph Panzarella, who guided us throughout this project. We also wish to acknowledge the help provided by the Founder and CEO of Adbot, Vladimir Davidov, and co-Founder of Adbot, Anna Davidov. We also thank Professor Thomas R. Kennon, who supported us and offered deep insight into the study. Finally, we extend our appreciation to our classmates for their input and inspiration in completing this work.

VII. References

Omnicore. (2022). *YouTube by the numbers: Stats, demographics & fun facts.* https://www.omnicoreagency.com/youtube-statistics/

Lost in Translation: Dual Language Exposure & Central Auditory Processing Disorder

Marsha Firmin
M.S. in Speech Language Pathology
Katz School of Science and Health

Abstract—This paper provides a review of scholarly sources aiming to identify the possible effects of dual language exposure for individuals with central auditory processing disorder (CAPD). CAPD is a cognitively and auditorily demanding deficit affecting a person's ability to internally process sounds. It becomes increasingly difficult for an individual with CAPD to isolate, process, and/or comprehend sounds in overwhelming listening situations. Dual language exposure is also cognitively and auditorily demanding; however, research has shown it to be advantageous, stimulating auditory processing and increasing processing effectiveness in demanding listening situations. Based on a review of literature, it was found that for individuals with CAPD, dual language exposure may be more of a hindrance than a benefit, potentially leading to confusion in either language. In addition to specifying potential consequences and benefits, this paper provides implications for speech-language pathologists (SLPs) working with individuals concerned.

Keywords—Auditory Processing, Bilingualism, Dual Language Exposure

I. Introduction

Dual-language exposure occurs when an individual is exposed to two languages either sequentially or simultaneously. Sequential bilingualism may be seen in a child who speaks one language at home and learns English as they enter preschool or elementary school. Simultaneous bilingualism occurs when a child develops both their home language and English at the same time with comparable levels at an early age. More and more, children

are being raised in bilingual homes, and research has shown that bilingualism can have a positive impact on children's language and literacy development. In fact, research has shown that dual language exposure does not confuse children; instead, it has been proven that children who are exposed to two languages can distinguish those languages from infancy and can learn two phonological systems, two vocabularies and two grammars simultaneously (Hoff et al., 2012). In addition, bilingualism provides an advantage for metalinguistic tasks due to children's enhanced control of processing skills, and the overall effect of bilingualism is an increase in the performance of the executive function system, in both linguistic and nonverbal processing (Barac et al., 2014).

CAPD is a cognitively and auditorily demanding deficit in a person's ability to internally process and/or comprehend sounds (ASHA, n.d.). Despite its impact on a person's ability to interpret sound, CAPD is not due to hearing loss. Instead, CAPD may be caused by genetic determinants, age-related changes in central auditory nervous system function, neurological disorder or prenatal/neonatal factors. While bilingualism has been shown to benefit children generally, in an individual with central auditory processing disorder (CAPD), this may not be the case, as it becomes increasingly difficult for an individual with CAPD to isolate, process and/or comprehend sounds in overwhelming listening situations (ASHA, n.d.). Given this, a child with CAPD may be unable to learn or distinguish differing sounds in multiple languages, as well as interpret the information in either language. As a result, in individuals with CAPD, dual language exposure may be more of a hindrance than a benefit, leading to confusion in either language.

This literature review aimed to identify the possible effects of dual language exposure for an individual with CAPD. In addition to specifying potential consequences and benefits, this paper also suggests tools that a speech-language pathologist (SLP) may use to support the individuals concerned.

II. Methods

To identify the possible effects of dual language exposure for an individual with CAPD, this study examined research published in peer-reviewed journals over the last ten years. Since there are no studies directly linking dual

language exposure and CAPD, this review focused on studies that explored 1) the impacts of dual language exposure on children's language development, cognition and executive functioning and 2) the effects of CAPD on auditory, cognitive and language functioning. Together, these studies help to illustrate the potential effect of dual language exposure for individuals with CAPD.

III. Results

Dual language exposure, language development and cognition

While it is commonly assumed that exposure to multiple languages in childhood leads to language confusion, the majority of research on this topic suggests that dual language exposure is actually advantageous in the areas of cognition, language development, executive functioning, speech and listening. For example, Barac et al. (2014) found that exposure to and speaking two languages changes the non-verbal executive control skills and theory of mind abilities of typically bilingual children. Children exposed to two languages showed more advanced skills than their monolingual peers and outperformed them in tasks that carry relatively high executive demands. Children exposed to two languages also showed different brain responses to processing linguistic stimuli. Advantages of bilingualism were also documented in theory of mind and executive control processing, regardless of the language combinations children were exposed to or spoke (Barac et al., 2014).

Bilingualism also impacts the brain networks that support language and cognition (Kroll et al., 2014). Kuipers and Thierry (2015) found that bilingualism increases overall attention during speech perception and that bilingual language processing is associated with cognitive advantages rather than disadvantages. Bilingual experiences also seem to positively influence dichotic listening, which is the process of receiving different auditory messages presented simultaneously to each ear (Gresele et al., 2013). Similarly, Sharifinik et al. (2021) found that dual-language exposure could provoke autonomic sound processing, making it more efficient in challenging listening conditions. Finally, it has also been shown that bilingualism has advantages in cognitive processing for tasks that require ignoring irrelevant information, task switching and resolving conflict (Kroll & Bialystok, 2012; Sharifinik et al., 2021).

The effects of CAPD on auditory, cognitive and language functioning

CAPD may lead to difficulties in listening, higher order language processing, communication and academics. As a result, CAPD is typically diagnosed by a multidisciplinary team, with the ultimate diagnosis being made by an audiologist (Bellis, n.d.). The audiologist administers multiple tests in a sound-treated room that requires listeners to attend to a variety of signals and to respond to them via repetition, pushing a button or in another way (Bellis, n.d.). In addition, a teacher may shed light on academic difficulties; a psychologist may evaluate cognitive functioning; and a speech-language pathologist (SLP) may investigate written and oral language, speech and related capabilities, which may contribute to a CAPD diagnosis.

According to ASHA (n.d.), processing speech is a very complex task involving simultaneous auditory, cognitive and language mechanisms. Given this, individuals with CAPD may have particular difficulty understanding speech in noisy environments, following directions and discriminating similar-sounding speech sounds (Bellis, n.d.). It also becomes increasingly difficult for an individual with CAPD to isolate, process and/or comprehend sounds in overwhelming listening situations (Bellis, n.d.)—thus taxing many of the same cognitive and linguistic functions that are enhanced by (and required for) dual language exposure and bilingualism.

Possible impact of dual language exposure on individuals with CAPD

While there is no research directly examining the impact of dual language exposure on individuals with CAPD, the effects of CAPD on auditory, cognitive and language functioning suggest that bilingualism is likely to be a hurdle rather than a benefit. Individuals with CAPD exhibit difficulties with theory of mind and other meta-linguistic tasks due to decreased executive control processing (Tomlin et al., 2015), functions generally seen as necessary for bilingualism. Since bilingualism requires a level of processing that individuals with CAPD may have challenges with, exposure to two languages may overwhelm individuals with CAPD and their ability to comprehend in both languages. The individual may confuse both languages and/or may need support to process either.

V. Conclusion

This paper sought to identify any effects dual-language exposure may have on an individual with CAPD. Based on the reviewed research, it is likely that dual-language exposure may be a hinderance to an individual with CAPD. This has implications for speech-language pathologists (SLPs) who work with children. Dual-language exposure usually begins before school age, but CAPD is generally not noticed until children are school-aged. This means that young children with undiagnosed CAPD in dual language homes may be exhibiting confusion in both languages, and while this is often thought to be the result of bilingualism, it may in fact be an early sign of CAPD.

Treatment of CAPD by SLPs generally focuses on three primary areas: changing the learning or communication environment, recruiting higher-order skills to help compensate for the disorder and remediation of the auditory deficit itself (Bellis, n.d.). Treatment of CAPD is individualized, and therefore an SLP needs to take into consideration if the individual is exposed to multiple languages and may be better supported in their primary language. In addition, SLPs can work with children on compensatory strategies that strengthen cognitive processes (language, problem-solving, memory, attention and other cognitive skills), which can assist in the treatment of the auditory disorder.

VI. Acknowledgment

This literature review would not have been possible without the support of many people. Many thanks to Dr. Troy Dargin, who supported me from the infancy to completion of this review of literature. I would also like to thank all the speech-language pathology professors in Yeshiva University's Katz School of Science & Health, who provided me with the knowledge and passion to study this topic and many others.

VII. References

American Speech-Language-Hearing Association. (n.d.). *Central auditory processing disorder.* https://www.asha.org/practice-portal/clinical-topics central- auditory-processing-disorder/

Bellis, T. J. (n.d.). *Auditory processing disorders (APD) in children*. American Speech-Language-Hearing Association. https://www.asha.org/public/hearing/understanding-auditory-processing-disorders-in-children/

Barac, R., Bialystok, E., Castro, D. C., & Sanchez, M. (2014). The cognitive development of young dual language learners: A critical review. *Early Childhood Research Quarterly, 29*(4), 699–714. https://doi.org/10.1016/j.ecresq.2014.02. 003

Gresele, A. D., Garcia, M. V., Torres, E. M., Santos, S. N., & Costa, M. J. (2013). Bilingualism and auditory processing abilities: Performance of adults in dichotic listening tests. *CoDAS, 25*(6), 506–512. https://doi.org/10.1590/s2317-17822014000100003

Hoff, E., Core, C., Place, S., Rumiche, R., Señor, M., & Parra, M. (2012). Dual language exposure and early bilingual development. *Journal of Child Language,* 39(1), 1–27. https://doi.org/10.1017/S0305000910000759

Kroll, J. F., Bobb, S. C., & Hoshino, N. (2014). Two languages in mind: Bilingualism as a tool to investigate language, cognition, and the brain. *Current Directions in Psychological Science, 23*(3), 159–163. https://doi.org/10.1177/ 0963721414528511

Kuipers, J. R., & Thierry, G. (2015). Bilingualism and increased attention to speech: Evidence from event-related potentials. *Brain and Language, 149,* 27–32. https://doi.org/10.1016/j.bandl.2015.07.004

Sharifinik, M., Ahadi, M., & Rahimi, V. (2021). Bilingualism and cognitive and auditory processing: A comprehensive review. Iranian *Rehabilitation Journal, 19*(3), 231-240. https://doi.org/10.32598/irj.19.3.1408.1

Skoe, E., & Karayanidi, K. (2019). Bilingualism and speech understanding in noise: Auditory and linguistic factors. *Journal of the American Academy of Audiology, 30*(02), 115–130. https://doi.org/10.3766/jaaa.17082

Tomlin, D., Dillon, H., Sharma, M., & Rance, G. (2015). The impact of auditory processing and cognitive abilities in children. *Ear & Hearing, 36*(5), 527–542. https://doi.org/10.1097/aud.0000000000000172

COVID-19 and the Effects on Expressive Language Abilities in School-Age Children

Morgan Rosman
M.S. in Speech-Language Pathology
Katz School of Science and Health

Abstract—The purpose of this literature review was to explore studies discussing the effects of societal changes due to the COVID-19 pandemic on children's expressive language development. Relevant studies addressed the effects of mask wearing on communication; the effects of providing speech-language pathology services remotely via tele-practice; and revised CDC milestones for children's speech, language and literacy development. These societal changes have implications for the diagnosis and treatment of speech and language disorders. Further research is warranted to determine the extent of these effects as well as implications on children's expressive language abilities after the COVID-19 pandemic.

Index Terms—COVID-19, Expressive Language, School-age Children, Speech-language Development

I. Introduction

The COVID-19 pandemic introduced numerous changes to the way we interact, communicate and learn. Mask wearing and social distancing became normal parts of our daily lives, and workplaces, schools and other services, including speech-language pathology, transitioned to tele-health platforms. This project aimed to explore the effect of these COVID-19 related societal changes on children's expressive language development. While there is limited research on this topic, studies related to the impacts of mask wearing on communication as well as the effectiveness of tele-health for speech-language pathology (SLP) services suggest that children exhibited impairments in expressive language development during the COVID-19 pandemic. Addi-

tionally, revised CDC guidelines suggest that children are now expected to underperform in communication-related skills across several age brackets, which may lead to the underdiagnosis of speech and language disorders.

II. Methods

This study consisted of a review of the literature using peer-reviewed articles from 2019-present. The review focused on studies of adjacent topics from which inferences about the impact of societal changes due to the COVID-19 pandemic on children's communication and language development could be made. Specific topics included 1) the transition to tele-health for speech-language pathology; 2) the impact of mask mandates on communication and education; and 3) changes in the CDC's language milestones. Each of these areas directly impacts school aged children's speech and language development.

III. Results

Transition to tele-health

The COVID-19 pandemic introduced several barriers to the delivery of speech-language pathology (SLP) services for children, particularly in the areas of evaluation and treatment. Parents were scared to take their children to locations to be screened and evaluated for speech and language difficulties due to fear of contracting COVID-19. Without evaluation, children remain undiagnosed and do not receive much needed services.

At the same time, speech-language pathologists were expected to deliver interventions remotely via tele-health, adhering to the American Speech-Language-Hearing Association's (ASHA's) Code of Ethics (ASHA, 2020). Web-based platforms such as Zoom became the norm for speech-language pathologists. However, Tambyraja et al. (2021) found that only 16% of SLPs received training in tele-health, and 25% received no support from their districts to deliver those services.

Limited training on virtual platforms was not the only barrier that speech-language pathologists faced. They also had to ensure children were attending their virtual therapy sessions. With parents and caregivers working from home, they were less able to ensure their children were attending

their therapy sessions with sustained engagement (Tambyraja et al., 2021). Additional barriers to tele-health delivery, such as poor Wi-Fi connections, limited access to technology and multiple family members in the home further impacted speech and language service delivery.

Parents' and students' perception of tele-health also impacted its effectiveness. Lam et al. (2021) found that both parents and students felt tele-health was less effective than in-person; they also found an association between students' preference for tele-health and its overall effectiveness. Campbell and Goldstein (2022) studied treatment effectiveness across tele-health platforms and found that certain treatment areas were less likely to show improvement. Areas that showed limited improvement included utilizing augmentative and alternative communication (AAC) devices (66.2% effective treatment rate), pragmatics (68% effective treatment rate), hearing (74% effective treatment rate) and speech sound production (74% effective treatment rate). Speech and language goals set criteria for 80% accuracy or above. When the effective treatment rate is less than 80%, this means students are not mastering their individual goals for speech and language.

Mask mandates

As a precaution to stop the spread of COVID-19, children and teachers have been required to wear a mask in schools, often for 6-8 hours per day. In a 2020 study, Nobrega et al. found that face masks in schools posed two serious communication-related problems for children. The first problem is that typical masks (cloth or KN95) present a visual barrier to the mouth, lips, teeth, tongue and cheeks, rendering it impossible for a child to receive visual cues from the speaker (teachers or classmates). The second problem is a teacher's voice becomes distorted behind a mask, which means that children are not receiving the full frequency range of sounds.

Visual cues are what allow individuals to determine the pragmatics, phonology and semantic meaning of messages conveyed by the speaker. Children, who are still developing their communication abilities, are often left to "fill in the gaps" of what a speaker has stated. The loss of visual cues results in children not picking up on pragmatic cues, as half the face is covered. The lips, teeth and tongue aid in the listener's ability to differentiate speech

sounds to determine the difference between words, such as 'thank' and 'tank" or 'friend' and 'bend.' Together, these factors make it difficult for listeners to understand speech, which is especially problematic for children who are developing speech, language and literacy skills (Nobrega et al., 2020).

Milestone implications

In 2022, the CDC introduced new developmental milestones for speech and language development which, coupled with the impacts of mask wearing and the move to tele-health, may cause further delays in identifying early developmental problems. ASHA released statements in response to the revised CDC milestones expressing their concern. ASHA remarked that 67.7% of the milestones that remained were moved to older brackets (ASHA, 2022), pushing the expectations of a 12-month-old to a 15-month-old, a 24-month-old to a 30-month-old, and so on (Solis-Moreira, 2022). While these new milestone checklists can be followed to aid in the observation of children's speech, language or motor development, ASHA warns that these milestones should not be used during development screening and assessment to aid in clinical decisions (ASHA, 2022), as the new guidelines may lead to the underdiagnosis of speech and language abilities.

IV. Conclusion

Research has already begun to show a relationship between societal changes due to the COVID-19 pandemic and children's expressive language development. Specifically, the move to tele-health for speech-language pathology as well as mask mandates in schools can be linked to impairments in children's overall literacy, language and speech development. Updated CDC milestone guidelines for children's speech and language development, and the potential for delayed or underdiagnosis, further complicate this issue.

Future studies should focus on the direct areas of language development that will see lasting deficits as well as the diagnosis rate of speech and language disorders from the present to the future. If specific areas of language development can be determined and targeted during therapy, that will aid in the regression of speech and language disorders or delays. In addition, educators, SLPs and parents must advocate for their students, clients and chil-

dren. Research indicates that the regression and stagnation of speech and language abilities will persist into older ages if change is not addressed. First, it is critical to educate SLPs on the use of web-based platforms, such as Zoom, to conduct tele-health. However, if a child can attend speech therapy in person, that is highly recommended. Second, it is important to ensure that children continue to attend school in person, as safely as possible, to be able to develop their language skills. If this means continued mask wearing, then to reduce signal to noise ratio disrupted by mask wearing, teachers should utilize FM systems to allow for messages to directly enter the child's ear. Finally, it is imperative that parents and SLPs continue to work collaboratively to ensure their children's speech and language development.

V. References

American Speech-Language-Hearing Association. (2022). *ASHA comments on CDC and AAP developmental milestones updates*. https://www.asha.org/practice/asha-comments-on-aap-and-cdc-developmental-milestones-updates/

American Speech-Language-Hearing Association. (n.d.). *Issues in ethics: Ethics and delivery of care in public health and safety emergencies*. https://www.asha.org/practice/ethics/ethics-public-health-and-safetyemergencies/#:~:text=Principle%20of%20Ethics%20I%20of,in%20their%20approach%20to%20any

Campbell, D. R., & Goldstein, H. (2022). Evolution of telehealth technology, evaluations, and therapy: Effects of the COVID-19 pandemic on pediatric speech-language pathology services. *American Journal of Speech-Language Pathology, 31*(1), 271–286. https://doi.org/10.1044/2021_ajslp-21-00069

Solis-Moreira, J. (2022). *The CDC updated their developmental milestones for kids-here's what parents need to know. Parents.* https://www.parents.com/toddlers-preschoolers/development/behavioral/everything-parents-need-to-know-about-the-updated-cdc-guidelines-on-developmental-milestones/

Lam, J. H., Lee, S. M., & Tong, X. (2021). Parents' and students' perceptions of telepractice services for speech-language therapy during the COVID-19 pandemic: Survey study. *JMIR Pediatrics and Parenting, 4*(1). https://doi.org/10.2196/25675

Nobrega, M., Opice, R., Lauletta, M. M., & Nobrega, C. A. (2020). How face masks can affect school performance. International *Journal of Pediatric Otorhinolaryngology, 138*. https://doi.org/10.1016/j.ijporl.2020.110328

Tambyraja, S. R., Farquharson, K., & Coleman, J. (2021). Speech-language teletherapy services for school-aged children in the United States during the COVID-19 pandemic. *Journal of Education for Students Placed at Risk (JESPAR), 26*(2), 91–111.
https://doi.org/10.1080/10824669.2021.1906249

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of Science and Health