NEWSLETTER ISSUE 1

SPRING 2024

Message from CEO

Anton Morkin

Dear Members, Supporters and Followers of TBX4Life, In this Issue we would like to reflect on Year 2023 and I am delighted to share some exciting updates from our ongoing efforts in combating TBX4-Syndrome.

Over the past year, our network of connected clinicians and scientists has expanded significantly, now comprising 75 dedicated members. This growth has strengthened our collaboration and knowledge-sharing capabilities, propelling our research forward. In tandem with our network expansion, our community of affected families and individuals has grown by 30%, reaching new countries and corners of the globe. This growth not only strengthens our community bonds but also broadens our impact in advocating for those affected by TBX4-Syndrome.

Our "Tour for Cure" in April 2023 across the US was a great success, fostering personal connections with over 15 scientists and clinicians. These face-to-face interactions are invaluable in advancing our understanding of TBX4-Syndrome, in development of a roadmap to a Cure and in extending our collaboration opportunities. We've also shone a spotlight on our Scientific Team, sharing insights and perspectives through 10 interviews accessible on our YouTube channel. These interviews offer a deeper understanding of the dedication and expertise driving our research forward.

Thanks to the generosity of our supporters, we've raised over $20,000 through various fundraising initiatives, including the "Cure Those Affected by TBX4" and "TBX4Life Apparel" campaigns. Additionally, we've received donations from individual families and companies invested in our cause. Securing two grants totaling $7,700 for awareness and education initiatives further empowers us to spread knowledge and challenge misconceptions about TBX4-Syndrome. You can find our Year 2023 financial report at the end of the Newsletter.

Our inspiring "Family Video" has captured the spirit of our community, serving as a poignant reminder of our shared mission. Furthermore, our efforts to raise awareness have been amplified through the development of multilingual awareness flyers in multiple languages, available now on TBX4Life web portal. On a global scale, we've actively engaged in partnerships with organizations such

as PHA US, PHA Europe, and FCHP, solidifying our presence and impact. Our participation in events like the PAH Workshop by the World Heart Federation in Geneva further underscores our commitment to collaboration and advancement. We're honored to be recognized for our contribution to TBX4-PH research by FCHP during its 15th Anniversary celebration in Madrid last December.

Looking ahead to 2024, we have ambitious plans to continue driving scientific collaboration, extending our partnerships, and supporting our affected community. Together, we will push forward in our mission to find a cure for TBX4-Syndrome.

Thank you for your unwavering support and dedication!

Warm regards,
Anton Morkin

Community Update

LaRae Hacker

The TBX4 family community has been busy this year. We started our first fundraising campaigns and made a great start toward the main goal, funding research for a cure! Several of our amazing community members volunteered to share their story publicly which makes a lasting impact on our ability to spread the word about TBX4. You can find those stories on our TBX4Life social media pages. We are on the ground floor of our fundraising efforts and are looking forward to exciting events in the coming year. Thank you for your support of TBX4Life.

Educational Activities Update

Jayden Swayze

Dr. Laura Southgate (geneticist at St. George University in the United Kingdom) and I are working towards a set of educational videos and brochure packets, intended to inform newly diagnosed patients and their families about TBX4 Syndrome.

These educational videos will begin by answering four general questions: “What is TBX4?”, “What is Pulmonary Hypertension with TBX4 variants?”, “What is CHILD (Childhood Interstitial Lung Disease)?”, and finally “What is TBX4Life?”

We wanted to work carefully in creating these videos with associated animations, illustrations, narration, and other professional workings to make them more accessible for social media, and for sharing among friends and family who are curious in wanting to learn more. As of now, we are in the process of creating scripts for the videos, finding accurate illustrations, and using video editing software for a much more immersive experience for the audience. To add better accessibility for an international community, these videos will also come in the form of pamphlets or brochures translated into different languages.

It has become our goal to have a completed product by June 2024 and to have these videos and pamphlets posted both to the TBX4Life website, and TBX4 associated social media accounts. Ultimately, we wish that families and patients directly involved in TBX4Life will provide us feedback on these videos as well as future questions or topics they would like to see illustrated. With a growing scientific and healthcare community interested in TBX4Life and its

mission, we also hope to provide important introductory education on not only a scientific perspective but a community perspective on the values and missions of TBX4Life. With growing awareness and the resiliency behind the team at TBX4Life we hope these videos can add to a more professional and interactive experience for those interested in our mission!

Update from the Scientific Core Leadership Team

Eric Austin

2023 has been a productive and exciting year for TBX4Life and our TBX4 stakeholders with regard to scientific progress and growth. We witnessed tremendous gains across each of our key priorities, which include:

1. Establish and grow a collaborative international research consortium to facilitate clinical, translational and basic research focused upon TBX4 perturbation and associated genes and pathways.
2. Create and/or support a global infrastructure to support data collection with linked biospecimens.
3. Create and solidify connections among stakeholders to assure sustainability of our efforts.

While much work remains, success was exemplified in many ways, some of which are represented here:

A) Core Research Leadership Team, composed of Eric Austin, Anton Morkin, Matina Prapa, Ripla Arora, Justyna Karolak and Jeff Whitsett, as well as La'Rae Hacker and Jayden Swayze. And, we recently welcomed Olivier Danhaive. We are grateful to these individuals, and all of our members, each of whom have contributed many hours of time and effort.

With contributions from the TBX4Life Scientific Consortium, we introduced several new initiatives including:

- A Working Group focused upon Novel Therapies, anchored upon a roadmap to treat and cure TBX4-associated disease, led by Jeff Whitsett, MD.
- A Working Group devoted to exploration of basic and model systems to explore TBX4 perturbations and related disease, led by Ripla Arora, PhD

In addition, under the leadership of Matina Prapa, PhD, and Olivier Danhaive, our Working Group devoted to Phenotypic Characterization of TBX4 Syndrome and related conditions worked to finalize an overview and consensus statement, which is nearing submission for publication.

Finally, led by Justyna Karolak, PhD, 2023 saw the publication of our first collaborative international paper, in AJRCCM!

In the article, leading researchers and clinicians in the field characterized the developmental, tissue-specific, and pathological TBX4 functions identified through human and animal studies and systematically reviewed the phenotypic spectrum of TBX4 variants. The manuscript also outlines future research directions to fill current gaps in our understanding of TBX4-related disorders. The article can be found here

Karolak JA, Welch CL, Mosimann C, Bzdęga K, West JD, Montani D, Eyries M, Mullen MP, Abman SH, Prapa M, Gräf S, Morrell NW, Hemnes AR, Perros F, Hamid R, Logan MPO, Whitsett J, Galambos C, Stankiewicz P, Chung WK, Austin ED. Molecular Function and Contribution of TBX4 in Development and Disease. Am J Respir Crit Care Med. 2023 Apr 1;207(7):855-864. doi: 10.1164/rccm.202206-1039TR. PMID: 36367783; PMCID: PMC10111992.

B) TBX4Life / TBX4 Syndrome 2nd International Scientific Conference, November 29, November 30, and December 5, 2023. Our annual conference was a huge success, thanks to the hard work of our Core Research Leadership Team, each Scientific/Clinical member of TBX4Life, and our Speakers and Participants.

- Over 80 participants enjoyed 3 days of talks and discussions from world leaders in TBX4 and associated clinical and molecular issues https://tbx4.org/tbx4life-2nd-conference-2023
- Featured Speaker, Plenary Speaker (December 5th): Terry Pirovolakis, Founder of Cure SPG50 and Elpida Therapeutics, inspired the audience with the story of his rapid journey from diagnosis-associated devastation to therapeutic development for his son and other people impacted by their disease of interest.
- TBX4-impacted families inspired all with their stories

C) 2023 also witnessed the sharing of IRB-approved specimens among some TBX4 clinicians and scientists, as we work to share not only knowledge, but reagents to support the important work being conducted around the world.

2023 was a tremendous year for TBX4Life and the TBX4-associated community. But, we know that 2024 will be even more exciting!

TBX4Life Working Group 1

Matina Prapa, Olivier Danhave

Working group 1 focuses on the clinical aspects of TBX4 syndrome integrating radiological, histopathological, and genetic data. We are excited to announce that Professor Olivier Danhaive, Neonatologist with a special interest in developmental lung disorders, will co-lead the working group together with Dr Matina Prapa, Clinical Geneticist, from January 2024 onwards.

Our main goal has been the publication of guidelines on the diagnosis and management of TBX4 disease. Over the past year, we held a series of calls discussing the heterogeneous manifestations of TBX4 syndrome and reached a consensus on the care and management of patients and their at-risk relatives. We are currently in the process of making pre-submission inquiries to suitable Journals.

Following the success of the recent TBX4Life Clinical Workshops as part of our 2023 Scientific Conference, we propose to hold equivalent monthly meetings within our working group with a clinical flavor (e.g. a single center's experience, multidisciplinary discussion of a challenging case/family, treatment strategies for PAH). We have circulated a roster where potential speakers can register their interest.

Alongside the above, we will continue to develop working group 1 projects including the natural history study and phenotypic characterization of the recurrent 17q23 deletion overlapping with TBX4. We are currently in the process of finalizing our patient survey and launching this on the CoRDS international patient registry for a dry run with select members of the TBX4Life community. This will be followed by a pilot study aimed at capturing patient-reported outcomes in addition to deeper phenotypic data submitted by clinicians.

TBX4Life Working Group 3

Jeff Whittsett

We have had an active year in Working Group 3, meeting every other month to present data focused on finding a cure for TBX4-related lung disease. We began by outlining the need for knowledge and potential areas of science that could ameliorate TBX4-related lung disease. This framework seeks to integrate cell, molecular, genetic, and patient findings to identify opportunities to alter TBX4 function and activity as well as to generate knowledge to mitigate parenchymal and pulmonary arterial hypertension. The schematic or scientific outline of the opportunities, as well as both ongoing and needed data to find therapies for TBX4-related disorders, is shown in the schematic (see below).

Dr. Pawel Stankiewicz and collaborators are creating and sharing ever-increasing knowledge regarding the diversity of TBX4 gene variations and their impact on clinical pathological outcomes. Rapid progress in understanding PAH, lung alveolar and acinar development, and the impact of TBX4 on these processes is being made by investigators in the TBX4Life community. In our early meetings, new single-cell RNA data and confocal microscopy from patient lung biopsies were shared, showing sites of TBX4 expression and loss of both alveolar structures and vascular endothelial cells presented by Dr. Minzhe Guo. Drs. Kathryn Wikenheiser-Brokamp, Csaba Galambos, and Gail Deutsch presented data regarding the diversity of pathological changes in lungs from patients affected by TBX4 gene variations. Scientific presentations and TBX4Life scientific workshops in November and December highlighted many discussions among clinicians and science investigators.

In Working Group 3, RNA data from lung tissues from individuals with TBX4 variations demonstrated that RNA is selectively expressed in mesenchymal cells in the lung, in pericytes, fibroblasts, and smooth muscle cells, but not in endothelial cells. Drs Jeffrey Whitsett and Minzhe Guo shared confocal microscopy and single-cell RNA and ATAC-seq data from patient samples with TBX4 lung disorders. Knowledge regarding the heterogeneity and functions of TBX4-expressing cells and their specific roles in lung development is being increasingly understood.

Dr. Anne-Karina Pearl leads a multi-institutional working group to understand the functions of lung fibroblasts, and she summarized the expanding knowledge regarding fibroblast diversity. Dr. Darrell Kotton developed induced pluripotent stem cells from TBX4 patients and the assays that differentiate them into lung-specific mesenchyme. Drs. Marlene Rabinovitch, Mingxia Gu, and Dr. Kotton shared data regarding TBX4 variation-related iPSC cells for the study of lung formation and function. Dr. Rabinovich shared data regarding the pathogenesis and signaling mechanisms involved in pulmonary hypertension and potential molecular approaches to future therapy modeling. She discussed how BMPR4, FOXF1, and TBX4 are likely involved in gene regulatory networks involved in PAH. Plans for expanding iPSC numbers and availability for the TBX4 research community were discussed by Drs. Kotton and Rabinovich.

Dr. Mingxia Gu shared remarkable data demonstrating the co-differentiation of iPSCs to form lung organoids containing blood vessels, epithelial cells, and fibroblasts from normal and TBX4-affected individuals. Plans for perfusing microvessels formed from primary endothelial cells and iPSCs were shared

by Drs. Rabinovich and Gu. Dr. Edward Morrisey shared his findings on how the peripheral lung is formed and emphasized the differences between human and mouse lung structures. His work highlights the importance of cell-cell crosstalk between epithelial cells, fibroblasts, and endothelial cells in the function and formation of the peripheral regions of the lung. He shared extensive single-cell RNA data from the developing human lung, which were compared with those from the recent findings from patients affected by TBX4 gene variations.

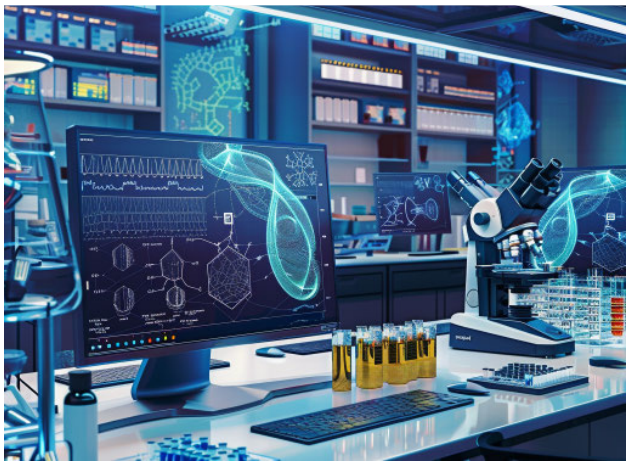
Drs. Kathryn Wikenheiser-Brokamp, Csaba Galambos, and Gail Deutsch, all world experts in pediatric lung pathology, are working together with Working Group 3 investigators to understand the variability of lung pathology in TBX4 lung disorders and how these features are both shared but distinct from those in other lung hypoplasias, including alveolar capillary dysplasia with miss-aligned vessels (ACD/MPV). Recent single-cell RNA data, published by Minzhe Guo and colleagues identify the distinct features of ACD/MPV-related lung disease in comparison with that of TBX4.

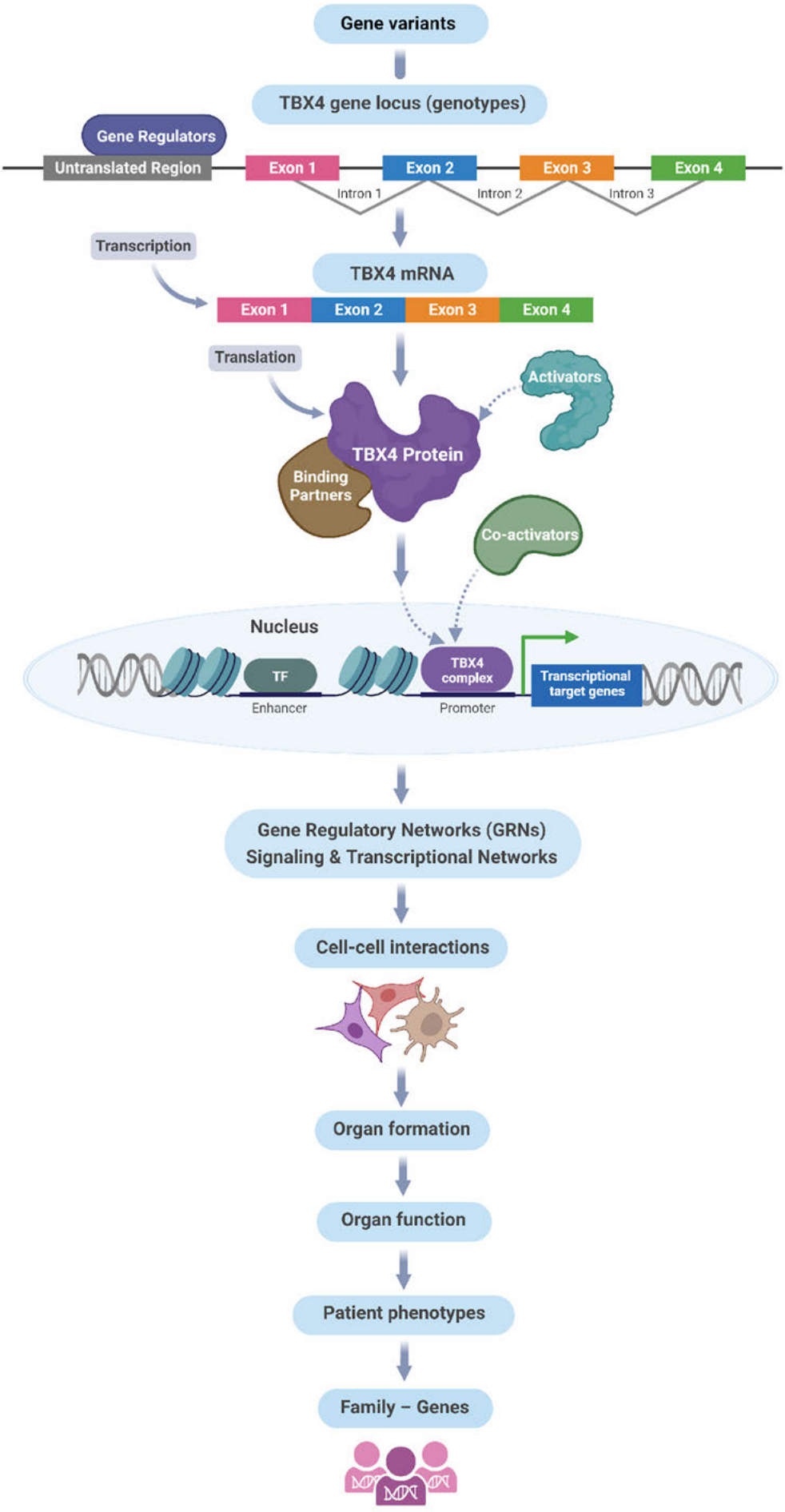
Dr. Vladimir Kalinichenko shared published and ongoing studies using nanoparticles to correct endothelial cell dysfunction in ACD/MPV and his interest in developing similar approaches for cell or gene therapies to improve endothelial function in TBX4-related pulmonary hypertension.

Dr. Jichao Chen joined the working group, sharing an interest in the role of cell crosstalk via Wnt signaling and alveolar development in the application of AI to the ever-expanding multi-omic data being developed by the research community. Working Group 3, with a focus on therapy, is closely connected with Working Group 4, which shares an interest in developing model systems and integrating basic functions of the TBX4 gene and protein. Animal models from zebrafish, mice, and humans are all critical for enabling discoveries leading to therapy of TBX4-related lung disorders.

We hope and expect open scientific discussions in both Working Groups, linking clinical and basic scientists on collaborative projects and grants in the future. It is an exciting time in research as knowledge regarding TBX4 and its cellular physiological functions is rapidly expanding. There is clear momentum in Working Groups 3 and 4 to understand TBX4 and its variations on human health. All of us in the scientific community are inspired by the commitment of families and patients affected by TBX4 and the support provided by TBX4Life that keeps us working together for a cure.

Jeffrey A. Whitsett, MD for WG3 – TBX4LIfe
Co-Director, Perinatal Institute
Chief, Division of Neonatology, Perinatal and Pulmonary Biology




Gene variants
TBX4 gene locus (genotypes)
Gene Regulators
Untranslated Region
Exon 1
Exon 2
Exon 3
Exon 4
Intron 1
Intron 2
Intron 3
Transcription
TBX4 mRNA
Exon 1
Exon 2
Exon 3
Exon 4
Translation
Activators
TBX4 Protein
Binding Partners
Co-activators
Nucleus
TF
TBX4 complex
Transcriptional target genes
Enhancer
Promoter
Gene Regulatory Networks (GRNs)
Signaling & Transcriptional Networks
Cell-cell interactions
Organ formation
Organ function
Patient phenotypes
Family – Genes

TBX4Life Working Group 4

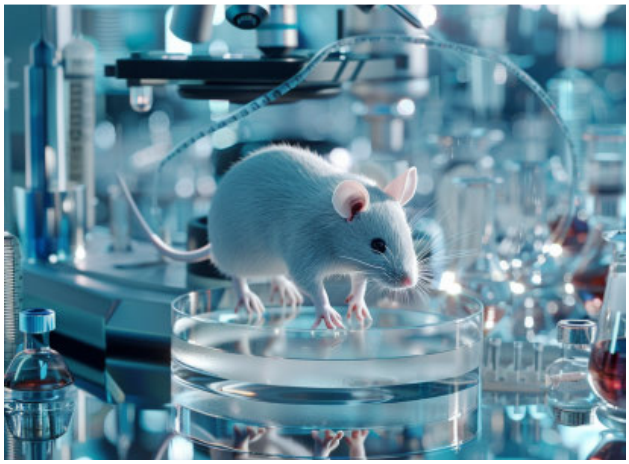
Ripla Arora

Working group 4 comprises of researchers using animal models, human tissues, organoids and cell lines to study mechanisms underlying lung development. Working group 4 was established in July 2023 as the TBX4Life community sensed a need for basic scientists to come together to share published and unpublished data and brainstorm ideas for collaborations with a common goal to accelerate the understanding of mechanisms of TBX4 regulated lung development. This working group operates under the philosophy that free exchange of data will accelerate the research and expedite discovery of mechanisms that underlie TBX4 syndrome and eventually aid in identification of targets for therapeutic purposes.

The working group is led by Dr. Ripla Arora. In 2012, Dr. Arora generated the first mouse models with conditional deletion of Tbx4 in the mouse suggesting a key role in fetal lung branching morphogenesis. Her group has now generated a new TBX4-deficient animal model to study the embryonic and early postnatal function of TBX4 in establishing lung mesenchymal lineages. Dr. Jeffery Whitsett presented large scale omics data obtained from patient lungs with TBX4 mutations. His group has identified abnormal AT1 and AT2 epithelial differentiation in these lungs. Single cell genomics suggest dysregulation of both epithelial and endothelial differentiation in the patient lung samples.

Dr. Csaba Galambos's research uses histopathology of lungs with TBX4 mutations. He described proximalization of TBX4-deficient lungs and an organization suggesting arrest pseudoglandular or saccular stage of lung development. Dr. Mauro Lago DoCampo a postdoctoral fellow in Dr. Marlene Rabinovitch's lab is using TBX4-deficient induced pluripotent stem cell lines to uncover interactions between smooth muscle cells and endothelial cells that underlie the etiology of pulmonary hypertension in TBX4 syndrome.

Discussions in this working group has already led to establishment of new collaborations and will lead to submission of joint proposals to generate grant funding to expand research investigating the mechanistic basis of TBX4 syndrome.



Closing Message & Outlook 2024

TBX4Life Core

As we come to the end of another chapter in our fight against TBX4 Syndrome, we want to extend our heartfelt thanks to all who have been part of this journey. Our dream of finding a cure for TBX4 Syndrome is alive and well, thanks to the hard work and dedication of our scientific consortium and staff. Together, we've seen a growing interest in TBX4 research, which gives us hope for the future.

As we look ahead to 2024, we're excited about what's to come. We encourage everyone to get involved in our workgroups and share your thoughts and ideas. Every bit of data and insight brings us closer to our goal. While we're focused on TBX4 Syndrome, we're also eager to expand our reach and collaborate with others in the field. Whether you're a scientist or a patient, we welcome you with open arms.

And finally, we're thrilled to announce plans for our first-ever Scientific Conference in 2024/2025. It will be a chance for us to come together, share knowledge, and renew our commitment to finding a cure. With your support, we know that anything is possible.

Warm regards, and THANK YOU!

Financial Report 2023

Anton Morkin

Profit and Loss

TBX4Life

Date Range: Jan 01, 2023 to Dec 31, 2023

ACCOUNTS	Jan 01, 2023 to Dec 31, 2023
Income	
Donations - Through Benevity	$3,413.32
Donations - private	$16,695.64
Non-Government Grants	$5,000.00
Total Income	**$25,108.96**
Total Cost of Goods Sold	**$0.00**
Gross Profit	**$25,108.96**
As a percentage of Total Income	100.00%
Operating Expenses	
Banking Fees	$94.43
Government and tax fees	$275.00
IT Services Expenses	$1,142.45
Marketing Expenses	$526.99
Total Operating Expenses	**$2,038.87**