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Official Title of Study: Computer-based Social Skills Training for Autism Spectrum Disorder

NCT Number of Study: NCT03672344

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Document Title: Study Protocol (including Statistical Analysis Plan)

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Study Sponsor: BioStream Technologies, LLC

Note: Supplement to Statistical Analysis Plan Attached as Separate Document

BIOSTREAM TECHNOLOGIES, LLC

Title:	**Computer-based social skills training for autism spectrum disorder**	
Short Title	Computer-based social skills training	
Device Name:	BioStream Computer-Based Social Skills Training	
eIRB Number:		
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Sponsor BioStream Technologies, LLC 1700 Market Street, Suite 1005 Philadelphia, PA 19103 Sponsor Principal Investigator: Dr. Mary Jane Weiss		
Multi-Site Study		

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ABBREVIATIONS AND DEFINITIONS OF TERMS

ADHD	Attention Deficit/Hyperactivity Disorder
AE	Adverse event
ASD	Autism Spectrum Disorder
BioStream	BioStream Technologies, LLC
BioStream Training	BioStream Computer-Based Social Skills Training
CAR	Center for Autism Research
CHOP	The Children's Hospital of Philadelphia
C-JARS	Childhood Joint Attention Rating Scale
DAS-II	Differential Ability Scales, 2nd Edition
EOWPVT	Expressive One-Word Picture Vocabulary Test, 4th Edition
GLM	General Linear Model
IRB	Institutional Review Board
PI	Principal Investigator
PPVT	Peabody Picture Vocabulary Test, 4th Edition
SRS	Social Responsiveness Scale
Subject Computer Setup	Laptop computer with integrated use of a consumer grade eye tracker connected to it via a USB connection
SAE	Serious Adverse Event
TDC	Typically Developing Children
TOVA	Test of Variables of Attention

ABSTRACT

Background:

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized in part by difficulty in social interaction and communication [1], which can affect individuals' employability, educational achievement, and quality of interpersonal relationships [2-4]. Existing therapies targeting the deficits of the school-age population with ASD require significant therapist or educator time and are thus very costly, with estimates of the economic impact of caring for children with ASD ranging from ~$12B [5] to $61B annually [6]. Given this, as well as the fact that ASD affects 1 in 59 children in the United States [7], with identified prevalence rising [5], there is a pressing need for cost-effective, evidence-based interventions to support the ASD community, and BioStream Technologies, LLC (BioStream), in collaboration with the Center for Autism Research (CAR) at the Children's Hospital of Philadelphia (CHOP), has developed one such intervention: the BioStream Computer-Based Social Skills Training (BioStream Training). The intervention to be used in this protocol has been piloted at CHOP as part of an existing IRB protocol (eIRB #15-12594). As part of that protocol, we have run 32 children (18 with ASD and 14 TDC) through procedures that to some degree are overlapping with the present protocol (up to 5 study sessions of gameplay per subject).

Primary and Secondary Aims:

The objective of this study is to evaluate changes in subject performance on social skills assessments after engaging in BioStream Training, as compared to a group of subjects who were trained with an alternate control game, and to evaluate possible correlations in changes in social skills assessments with changes in subject gameplay performance.

Study Design:

This is a comparison group study during which subjects will engage with BioStream Training in a home, school, therapy center, or research organization setting and participate in social skills assessments, or will be exposed to an alternate computer video game in a home, school, therapy center, or research organization setting instead of the BioStream Training.

Setting/Participants:

All participants will participate in the study either in a home, school, therapy center, or research organization setting. The study will involve 150 subjects aged 4-14 with ASD.

Study Interventions and Measures:

BioStream Training is computer-based social skills training that includes interactive game-like applications involving images, video, audio, and animation (e.g. animated characters). The main study outcome measures include both standard and experimental social skills assessments (e.g. a dyadic interaction task with a member of the study team).

PROTOCOL SYNOPSIS

Study Title	Computer-based social skills training for autism spectrum disorder
Funder	BioStream Technologies, LLC
Clinical Phase	N/A
Study Rationale	Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized in part by difficulty in social interaction and communication, which can affect individuals' employability, educational achievement, and quality of interpersonal relationships. Existing therapies targeting the deficits of the school-age population with ASD require significant therapist or educator time and are thus very costly. Given this, as well as the fact that ASD affects 1 in 59 children in the United States according to the Center for Disease Control, there is a pressing need for cost-effective, evidence-based interventions to support the ASD community, and BioStream Technologies, LLC (BioStream), in collaboration with the Center for Autism Research (CAR) at the Children's Hospital of Philadelphia (CHOP), has developed one such intervention: the BioStream Computer-Based Social Skills Training (BioStream Training).
Study Objective(s)	**Primary** To evaluate changes in subject performance on various social skills assessments (including eye-tracking, dyadic interaction, SRS, Ekman 60 Faces Test) after engaging in BioStream Training, including in comparison to an alternate game. **Secondary** To observe changes in subject gaze patterns during social skills assessments after engaging in BioStream Training, including in comparison to an alternate game. To observe possible correlations in changes in social skills assessments with changes in subject gameplay performance.
Test Article(s) *(If Applicable)*	BioStream Training is computer-based social skills training that includes interactive game-like applications involving images, video, audio, and animation (e.g. animated characters).
Study Design	Comparison Group
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Ages 4-14. 2. Meets diagnostic criteria for ASD. 3. Estimated intelligence standard score of at least 40 (can be substituted for a receptive language score such as PPVT or use

of the Differential Ability Scales, 2nd Edition (DAS-II)).

4. Parent reports difficulty with at least one of two social skills (via modified questions from the Social Responsiveness Scale)
5. English as the child's primary/first language (key measures used in the study do not have alternate language versions).
6. Attains a score equal to or less than 75% correct on the Ekman 60 Faces Test.
7. The parent who completes the questionnaires needs to be proficient in English.
8. Has successfully played a video game using a Microsoft Xbox game controller, Sony PlayStation game controller, or other comparable game controller.
9. Parental/guardian permission (informed consent) and if appropriate, child assent.
10. Wi-Fi internet connection at subject's home/school/therapy center/research organization available for use by study laptop computer.

Exclusion Criteria

1. History of seizures.
2. History of traumatic brain injury or other significant medical or neurological abnormality affecting motor or higher cortical functioning.
3. Certain visual, auditory, DSM 5, or conduct disorders (see below).
4. A visual disorder that cannot be corrected through the use of corrective lenses to a level of 20-40 in both eyes.
5. Use of corrective visual lenses that would significantly impede the valid collection of visual attention and gaze pattern data during dyadic interaction tasks.
6. Auditory impairment (that cannot be corrected by a hearing aid) that would significantly impede the valid collection of test measures.
7. Profound intellectual disability or sensory-motor difficulties that would preclude valid use of diagnostic instruments and/or use of a computer or mobile computing device.
8. A DSM 5 disorder or other psychiatric symptoms that would interfere with the participant's ability to participate in the study (e.g., active psychosis), per parent report.
9. History of one or more psychiatric hospitalizations.
10. Presence of significant symptoms of a conduct disorder.

Number Of Subjects	150 subjects with subject participation at home, school, therapy center, or research organization.
Study Duration	The study is comprised of two phases. For Phase I, each subject's participation in BioStream Training or the alternate game will last for up to 6.67 to 33.33 hours over a 28-day period (up to 20 game play sessions, up to 5 days per week for between 20 minutes and 100 minutes per training session or alternate game session) plus approximately 8 hours of assessment and other study related activities. Each parent's participation will last for approximately 4.25 total hours over the 28 day period excluding time spent for oversight of the subject during the BioStream Training or during play of the alternate game. For Phase II, each subject's participation in BioStream Training or the alternate game will last for up to 6.67 to 33.33 hours over a 28 day period (up to 20 game play sessions, up to 5 days per week for between 20 minutes and 100 minutes per training session or alternate game session) plus about 5 hours of assessment and other study related activities. Each parent's participation will last for about 2.5 total hours over the 28 day period excluding time spent for oversight of the subject during the BioStream Training or during play of the alternate game. It is not required that Phase II begin immediately upon completion of Phase I.
Study Phases	**Phase I** • Initial Screening • Visit 1 – Informed Consent, Ekman 60 Faces Test, PPVT • Visit 2 – Assessments and Surveys (Baseline) • Week 1 (following Visit 2) – Gameplay – daily transmission of game data • Week 1 – Survey – data transmission • Visit 3 – Assessments and Surveys • Week 2 – Gameplay – daily transmission of game data • Week 2 – Survey – data transmission • Visit 4 – Assessments and Surveys • Week 3 – Gameplay – daily transmission of game data • Week 3 – Survey – data transmission • Visit 5 – Assessments and Surveys • Week 4 – Gameplay– daily transmission of game data • Week 4 – Survey – data transmission • Visit 6 – Assessments and Surveys (Note: Phase II may not begin immediately following phase I) **Phase II** • Week 1 – Gameplay – daily transmission of game data • Week 1 – Survey – data transmission • Visit 1 – Assessments and Surveys

	• Week 2 – Gameplay – daily transmission of game data • Week 2 – Survey – data transmission • Visit 2 – Assessments and Surveys • Week 3 – Gameplay – daily transmission of game data • Week 3 – Survey – data transmission • Visit 3 – Assessments and Surveys • Week 4 – Gameplay– daily transmission of game data • Week 4 – Survey – data transmission • Visit 4 – Assessments and Surveys
Efficacy Evaluations	The primary efficacy endpoints will be: The mean change from baseline score on the following assessments based on post-final game play session assessment compared to the alternate game group: a. Subject gaze patterns as measured by video eye tracking assessment. b. Subject gaze patterns as measured by the dyadic interaction task. c. Mean change in baseline score on the Ekman 60 Faces Test. d. Mean change in baseline score on the TOVA. e. Mean change in baseline score on the SRS. Secondary endpoints: The mean change from baseline score on the following assessments based on post-final game play session assessment compared to the alternate game group: a. Subject gaze patterns as measured on the videos of emotional expression assessment. b. Subject responses to the CJARS survey.
Pharmacokinetic Evaluations	N/A
Safety Evaluations	Subjects will always be supervised by a parent, teacher, or therapist (therapist includes Registered Behavior Technicians, assistant speech pathologists, assistant behavior analysts (BCABA), and teaching assistants) during use of the BioStream training and alternative game. Clinical adverse events (AEs) will be monitored throughout the study through parent reporting in the case of at home use and through teacher reporting in the case of in school use. Since the study procedures are not greater than minimal risk, AEs are not expected.
Statistical And Analytic Plan	Paired-sample t-tests will be used to measure changes from baseline within each group, and independent sample t-tests will be used to measure changes from baseline relative to the alternate game group for all endpoints.

	For all endpoints, we will determine whether there were observable changes in scores from baseline or relative to the alternate game group and whether these changes are statistically significant. Significance will be set at $p<0.05$ Multiplicity of primary endpoints will be managed via statistical corrections.
DATA AND SAFETY MONITORING PLAN	The PI will periodically review the data collection and storage practices associated with this study and determine whether changes to enhance confidentiality and privacy are required. The PI will frequently monitor the quality control of the data with staff and research assistants. All written or electronic forms (except Informed Consent Forms and Assent Forms) will employ a unique alphanumeric identifier; in no case will the participant's name or other identifying information appear on these forms. The user ID for the BioStream Training and alternate game shall use the same unique alphanumeric identifier. The study team will accomplish this on all parent/guardian or teacher or therapist questionnaires (as applicable) which have lines for child and parent name by applying a label with unique alphanumeric identifier and instructing parents/guardians or teacher or therapists to use generic labels (e.g., biological mother) rather than writing their names. The PI will keep a master list containing PHI and subject ID number separate from coded data forms, which will be maintained in a locked file drawer, cabinet, or lock box in the PI's office. Only the PI will have access to this location. The master list will be maintained on a separate password-protected computer and be stored in an encrypted password protected file. Video recordings will stored on password-protected and/or encrypted data storage devices including computers, external hard drives, and/or flash drives only accessible by members of the study team.

TABLE 1: SCHEDULE OF STUDY PROCEDURES (INITIAL SCREENING)

	Estimated time	Parent or subject?
Informed Consent	15 minutes	Parent
Review of Inclusion/Exclusion Criteria (Initial Screening Interview)	15 minutes	Parent
Video Game Interview Form (Parent-Report)	15 minutes	Parent

TABLE 2: PHASE I SCHEDULE OF STUDY PROCEDURES (HOME, SCHOOL, THERAPY CENTER, OR RESEARCH ORGANIZATION)

	Estimated time (total over all visits)	Parent or subject?	Visits applicable (Total 6 Visits)
Informed Consent/Assent (if applicable)	30 minutes	Parent and subject	Visit 1

Peabody Picture Vocabulary Test (PPVT)	20 minutes	Subject	Visit 1
Ekman 60 Faces Test	10 minutes	Subject	Visit 1
SRS (Parent-Report)	50 minutes	Parent	Visits 2-6
C-JARS (Parent-Report)	50 minutes	Parent	Visits 2-6
Dyadic Interaction Task	50 minutes	Subject	Visits 2-6
Eye Tracking Video Assessment	50 minutes	Subject	Visits 2-6
Ekman 60 Faces Test	50 minutes	Subject	Visits 2-6
Emotional Expression Video Assessment	50 minutes	Subject	Visits 2-6
TOVA	109 minutes	Subject	Visits 2-6
Game and equipment introduction (if applicable)	40-60 minutes	Parent	Visit 1
BioStream Training	6.67-33.33 hours	Subject	Over a period of 28 days following Visit 2
Weekly Parental/Teacher/Therapist Survey	20 minutes	Parent	1 survey/per week
Subject Interview/Survey (if applicable)	40 minutes	Subject	Visits 3 - 6
Parent/Teacher/Therapist Interview/Survey (if applicable)	40 minutes	Parent	Visits 3 - 6
Equipment return (if applicable)	5 minutes	Parent	Visit 6
TOTAL	**13.48 hours – 41.15 hours**	**Subject**	
TOTAL	**~4.25 hours***	**Parent**	***Excludes time spent for oversight of subject during BioStream Training**

TABLE 3: PHASE II SCHEDULE OF STUDY PROCEDURES (HOME, SCHOOL, THERAPY CENTER, OR RESEARCH ORGANIZATION)

	Estimated time (total over all visits)	**Parent or subject?**	**Visits applicable (Total 4 Visits)**
SRS (Parent-Report)	40 minutes	Parent	All Visits
C-JARS (Parent-Report)	40 minutes	Parent	All Visits
Dyadic Interaction Task	40 minutes	Subject	All Visits
Eye Tracking Video Assessment	40 minutes	Subject	All Visits
Ekman 60 Faces Test	40 minutes	Subject	All Visits
Emotional Expression Video Assessment	40 minutes	Subject	All Visits
TOVA	87 minutes	Subject	All Visits
BioStream Training	6.67-33.33 hours	Subject	Over a period of 28

			days
Weekly Parental/Teacher/Therapist Survey	20 minutes	Parent	1 survey/per week
Subject Interview/Survey (if applicable)	40 minutes	Subject	All Visits
Parent/Teacher/Therapist Interview/Survey (if applicable)	40 minutes	Parent	All Visits
Equipment return (if applicable).	5 minutes	Parent	Visit 4
TOTAL	**11.45 hours – 38.12 hours**	**Subject**	
TOTAL	**~2.5 hours***	**Parent**	***Excludes time spent for oversight of subject during BioStream Training**

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized in part by difficulty in social interaction and communication [1], which can affect individuals' employability, educational achievement, and quality of interpersonal relationships [2-4]. Existing therapies targeting the deficits of the school-age population with ASD require significant therapist or educator time and are thus very costly. Given this, as well as the fact that ASD affects 1 in 59 children in the United States according to the Center for Disease Control, there is a pressing need for cost-effective, evidence-based interventions to support the ASD community.

BioStream Technologies, LLC (BioStream), in collaboration with the Center for Autism Research (CAR) at the Children's Hospital of Philadelphia (CHOP), has developed one such intervention: the BioStream Computer-Based Social Skills Training (BioStream Training). The intervention to be used in this protocol has been piloted at CHOP as part of an existing IRB protocol (eIRB #15-12594). As part of that protocol, we have run 32 children (18 with ASD and 14 TDC) through procedures that to some degree are overlapping with the present protocol (up to 5 study sessions of gameplay per subject).

The primary objective of this study is to evaluate changes in subject performance on social skills assessments after engaging in BioStream Training, including in comparison to an alternate game. The secondary objective of this study is to evaluate changes in subject gaze patterns during social skills assessments after engaging in BioStream Training, including in comparison to an alternate game and to evaluate possible correlations in changes in social skills assessments with changes in subject gameplay performance.

Subject computer gameplay data (including game performance, game usage, and eye tracking data), certain subject assessment data, and certain subject survey data, may be

collected and stored using a laptop computer with integrated use of a consumer grade eye tracker connected to it via a USB wired connection (Subject Computer). **All data collected and stored on the Subject Computer will be de-identified data.**

Certain subject assessment and survey data may also be collected manually using a handwritten paper-based data collection method and stored digitally through use of digital scanning.

Additionally, subject eye contact video recording data may also be collected using a video camera and/or video camera embedded glasses worn by any member of the study team during real world dyadic interaction task assessment. **This data will not be stored on or collected using the Subject Computer. This data will be stored on a separate computer and/or computer data storage device maintained by any member of the study team. This data may also be sent to HIPAA compliant third parties including therapist practitioners or research organizations. These HIPAA compliant third parties, blinded to the study group of each subject, will be used to annotate the video recordings of the dyadic interaction task assessments including for subject eye contact during the task.**

Certain Parent assessment and survey data may be collected using a computer **(excluding the Subject Computer except in the case of the Weekly Parental/Teacher/Therapist Survey)** and/or manually using handwritten paper-based data collection methods. The parent or teacher or therapist (as applicable) may complete a Weekly Parental/Teacher/Therapist Survey using a form on the Subject Computer. **This survey is configured so that only de-identified data may be included in the survey or associated with the survey.**

1.2 Name and Description of Investigational Product or Intervention

The study intervention is the BioStream Computer-Based Social Skills Training (BioStream Training). BioStream Training is computer-based social skills training that includes interactive game-like applications involving images (e.g. photos and/or videos of people making different emotional expressions), animation (e.g. animated characters), video (e.g. tutorials), and audio (e.g. sound effects). Subjects can interact with portions of BioStream Training using an eye tracker (i.e. by using their gaze as a real-time input to the game-like applications).

Computer tasks will involve presenting various stimulus types to the participant. These will include action sequences, designed to be very engaging, and social sequences, designed to task users with social skills exercises.

Audiovisual stimuli: The social stimuli will include photographs and videos as well as animated characters. Animated social stimuli will include various characters that may interact with the user, may present the user with tasks, and may guide the user in tasks. Characters may socially interact with the user by eye contact, facial expression, body language, and speech.

The non-social stimuli will include computer graphics of various environments in which the user will navigate and interact in different ways including collection of items that the user may use in the game-like application segment. Parents/guardians will be given the

opportunity to see examples of the pictures and graphics that will be shown to their child as part of the consent process.

Computer interaction sessions will take place in the home and/or school environment. Subjects will sit facing a computer, tablet, or smartphone screen and be positioned so that the eye tracking device can obtain measurements. Subjects may be provided headphones to wear during the computer interaction to enable audio listening without disturbing others in the home, school, therapy center, or research organization environment. Subjects may be provided with a computer mouse, computer keyboard, touchscreen, and/or game controller device. Subjects may be presented with multiple action and social sequences. Perspectives in the computer application may include first-person and third-person views. An eye tracker will record participants' gaze patterns. Participants' use of the controls in the game-like application—computer mouse, computer keyboard, touchscreen, and/or game controller device—will be recorded. All measurements will be taken in real time and may affect the stimuli presented.

Action Sequences: Subjects will view and navigate animated environments on the screen. Action sequences may include items that can be obtained and used for special abilities in the game environment. Subjects may be instructed to use the controls for abilities including turn, run, jump, obtain item, and use item. Any or all behavioral measures will be used to determine engagement, including game performance data and attention from eye gaze data.

Social Sequences: Subjects will be presented with social tasks in the context of gameplay which may include normative eye contact, facial emotion recognition, joint attention exercises, perspective taking exercises, vocal emotion recognition, and normative internal and external emotional response in social interactions. Social sequences may include simulated interaction with social stimuli presenting different personality traits such as extraversion, caution, and aggression. Social sequences may vary and change based on real-time behavioral measurements (including eye tracking measurements/data) and may include the following examples. Subjects may be encouraged to use appropriate eye contact with social stimuli; eye contact will be measured with the eye tracker. Subjects may be prompted to identify the emotion presented by social stimuli based on facial expression. Subjects may be prompted to identify the emotion presented by social stimuli based on vocal expression. Subjects may be instructed to use the controls including the game controller to choose responses in recognition tasks. General emotions to recognize may include pleasantness, unpleasantness, and neutrality. Specific emotions to recognize may include happiness, surprise, sadness, anger, fear, and disgust.

Data Transfer and Storage: Subject computer gameplay data (including game performance, game usage, and eye tracking data), certain subject assessment data, and certain subject survey data, may be collected and stored using a laptop computer with integrated use of a consumer grade eye tracker connected to it via a USB wired connection (Subject Computer). Additionally, the parent, teacher, or therapist (as applicable) may complete a Weekly Parental/Teacher/Therapist Survey using a form on the Subject Computer. This survey is configured so that only de-identified data may be included in the survey or associated with the survey. **All data collected and stored on the Subject Computer will be de-identified data.**

De-identified study data collected and stored on the Subject Computer may at any time be copied (including for backup purposes or remote data analysis purposes) to a computer data storage device (including a USB flash drive or external computer hard drive) or to a separate computer, by any member of the study team by establishing a wired connection between the computer data storage device or separate computer and the Subject Computer (or direct connection in the case of a USB Flash Drive) to manually copy the de-identified study data from the Subject Computer to such device or computer. This data may at any time be transferred by any member of the study team to the Sponsor including for backup and analysis purposes to ensure data integrity.

Additionally, the Subject Computer may be configured to manually enable transmission and/or automatically and regularly provide for transmission, of de-identified study data to the Sponsor's computer (including for backup and analysis purposes) using a wireless communication link (which may include use the parent/guardian home's, the school's, the therapy center's or the research organizations, as applicable, Wi-Fi internet connection) between the Subject Computer and the Sponsor's computer. N**o subject's internet protocol address (IP Address) will be collected or stored by the Sponsor at any time including as a result of this communication link.** This de-identified study data may be retransmitted to any member of the study team electronically or via access to a password protected web interface, for backup purposes, analysis purposes, study monitoring purposes (including in the case of the Weekly Parental/Teacher/Therapist Survey), or for use in determining the necessity of teacher, therapist, parent and/or subject follow up to prompt subject compliance with the study protocol.

Sponsor may provide a web interface for password protected access by any or select members of the study team that shows and/or permits the download of, subject de-identified study data using secure channels which may include secure FTP or HTTPS. The Sponsor may electronically send to any member of the study team reports containing subject de-identified study data. The Sponsor may otherwise electronically communicate subject de-identified study data to any member of the study team. The communication of this de-identified study data to any member of the study team will facilitate monitoring for compliance and data integrity in a real-time fashion.

Sponsor may notify any member of the study team about any subject ID numbers that failed to play the intervention or the alternate game over any 24-hour period. Any member of the study team may reach out to subjects and/or parents and teachers or therapists (as applicable) to remind them to play (by phone, email, and/or text message). Subjects will also have the opportunity to contact any or select members of the study team through phone or email if any questions arise.

The Sponsor may, from time to time, transmit updates to the software installed on the Subject Computer using the communication link described above.

Alternate Comparison Game:

The BioStream Training is being compared to a modified version of the BioStream Training, which will not include the same therapeutic exercises. The game will be played by subjects in the non-intervention group for the same length of playing time and at the same frequency,

as the BioStream Training intervention group. It will require a similar game controller device as that which is used by the intervention group and will be computer based. The Sponsor may also use as the alternate comparison game any video game that is rated E (Everyone) by the Entertainment Software Rating Board.

1.3 Gameplay Frequency

The study is comprised of two phases.

For **Phase I**, each subject's participation in BioStream Training or play of the alternate game will last for up to 6.67 to 33.33 hours over a 28 day period comprised of up to 20 game play sessions, up to 5 days per week for between 20 minutes and 100 minutes per game play session. The subject will be permitted to complete each daily session through use of the BioStream Training or use of the alternate game at different times throughout each day at the subject's discretion.

For **Phase II**, each subject's participation in BioStream Training or play of the alternate game will last for up to 6.67 to 33.33 hours over a 28 day period comprised of up to 20 game play sessions, up to 5 days per week for between 20 minutes and 100 minutes per game play session. The subject will be permitted to complete each daily session through use of the BioStream Training or use of the alternate game at different times throughout each day at the subject's discretion.

1.4 Relevant Literature and Data

BioStream Training builds on prior literature and shows promise because (1) it incorporates computer games, a medium shown to be particularly promising for helping children with ASD; (2) it leverages educational and behavioral concepts shown to be effective in training social skills; and (3) the social skills currently targeted by BioStream Training, eye contact and emotion recognition, have been shown to be trainable in ways that lend themselves to eye tracking technology.

(1) Video game format

Computers have been shown to be promising teaching tools for children with ASD, and virtual social interactions are thought to have significant potential for improving their real-world social skills [8]. Survey data of subjects that have participated in the BioStream Training has yielded encouraging data with respect to engagement.

(2) Educational and behavioral concepts

BioStream Training leverages the fact that many of the principles shown by research to be effective in teaching and shaping behavior, both generally and in the context of specific social skills, can be directly incorporated into computer-based training. Such principles, including discrete trial training, differential reinforcement, and shaping, are central to BioStream Training, and have an established evidence base, maximizing the likelihood that it will prove efficacious [8, 9].

(3) Social skills targeted, and description of BioStream Training exercises

BioStream Training applies these principles to target social skills including (a) eye contact, and (b) emotion recognition. These have been selected because: they are critical social skills in which children with ASD underperform their typically developing peers; they have been shown to be trainable using methods that can be mimicked in computer-based training [8]; and their training can be augmented through the incorporation of gaze control.

(a) Eye contact is the act of looking at someone's eyes during a social interaction. It is important for conveying attention and for communicating social and emotional information [10, 11]. Deficits in eye contact are one of the most notable indicators of ASD [12]. Nevertheless, discrete trial-based training of eye contact is effective, with such interventions having demonstrated increases in eye contact [12, 13]. BioStream Training includes exercises uniquely enabled by gaze control technology that are focused on leveraging these established approaches to help children with ASD improve at making eye contact. For example, during some discrete trials, a character begins speaking to the player, and the player must then look at part of the character's face (e.g. whole face, upper part of the face, eyes) within a certain time increment, for a few seconds, in order to receive a reward.

(b) Emotion recognition is the ability to identify human emotions, most typically from facial expressions, and is a crucial component of social development [14]. A meta-analysis found that children with autism underperform their typically developing peers on emotion recognition tasks [15]. There is a pressing need to adapt traditional, manual therapeutic approaches targeting emotion recognition skills to more standardized, computerized delivery platforms. Interventions to date have been effective at driving improvements, some even using video game-based training approaches [8, 14, 16]. BioStream Training leverages one of the more advanced psychophysical paradigms to come out of the experimental psychology literature on face processing: the use of gaze-contingent blurring of facial images, often called the "bubble paradigm." [17, 18] For example, during some exercises, the participant interacts with an animated character whose face is fully or partially blurred. The player must use their gaze to unblur key facial features in order identify the character's emotion. Then, the player must select the matching emotion from a set of real children's faces.

Assessments

Social Responsiveness Scale-Parent (SRS [19]). The SRS is a parent-report questionnaire that asks parents to rate the frequency of reciprocal social behaviors, communication, and repetitive and stereotypic behaviors in their child. The questionnaire yields a single summary score that can be used as an index of the child's severity of social deficits. This will be completed by a parent during each in-person visit or by telephone with a member of the study team.

Childhood Joint Attention Rating Scale (C-JARS [20]): This is a parent-report measure of joint attention related behaviors in verbal children and adolescents with ASD. This will be completed by a parent during each in-person visit or by telephone with a member of the study team.

Dyadic Interaction Task: There is a lack of standardized validated reference standards for assessing communication-based autism symptoms [21, 22]. Because of this, an exceptionally

large array of assessments is used in the literature. In fact, there are only three standardized tools that have been used in more than 2% of recent trials of autism interventions (none of which are used in more than 7% of studies) [21]. Therefore, in order to determine how we would assess communication skills we worked closely with our team of certified Applied Behavioral Analysis (ABA) therapists, who have years of experience working with children with ASD, and consulted the literature. The task we have constructed follows the Contextual Assessment of Social Skill [23] and procedures developed by Reilly et al [24]. It involves a structured play scenario, which is standard in other similar studies [21, 25-28].

This is an interaction between the subject and a member of the study team where the study team member poses questions to the subject while the interaction is video recorded. Questions may include but are not limited to the following:

What is your birthday? How old are you?
What do you like to do at school? What do you like about ____?
What is your least favorite thing at school?
What do you like to do at home?
Do you like any sports such as…"?
Do you have any favorite movies? What's [that movie] about?
What's your favorite program on TV? What's [that show] about?

In addition to answering questions, subjects may be asked to respond to certain prompts in the context of the dyadic interaction task, sometimes repeatedly. For example, a subject may be prompted to request an object from the study team member conducting the interaction, return the object, and then request that object again.

Further, a subject may be asked by the study team member questions about an object presented. An example of this is a task in which an item is presented to the subject, after presentation, the subject is asked if they would like it placed in a group of items later to be provided to the subject to create something (such as a structure, in the case of building blocks, or 3D art, by laying out different types of objects on a platform such as white board). As the task continues different additional questions are posed to the subject such as the following (in the case of building blocks):

1) "What's your favorite color?"
2) "What do you like to build with blocks?"
3) "What are you going to build with your blocks?
4) "Which shape block do you like the best?"
5) "What do you think you're going to build today with these blocks?"

After completion of the task, the subject is then permitted to complete the activity, which in the above example, is using the building blocks to create something. Each dyadic interaction will last up to 10 minutes.

During the Dyadic Interaction Task the study team member sits directly across from the subject while wearing a pair of video recording glasses (with the lenses in place or removed) that has a video camera embedded in the bridge of the glasses (such as the Pivothead Kudo Glasses or the Pivothead Durango Glasses) [26, 27]. The study team member activates the

video recording function of the glasses prior to starting the task so that the entire task is recorded. Alternatively or in conjunction with the video recording glasses, single or multiple video cameras may be used to record the task.

Following each Dyadic Interaction Task the video recording file is transferred from the glasses to a data storage device and/or computer maintained by the study team member for subsequent analysis. This analysis may include use of video annotating software such as the BORIS or ELAN video annotating software to annotate different conditions within each frame or group of frames of the video recording which conditions may include: the orientation of the subject's face is oriented towards the camera, the orientation of the subject's face is not oriented towards the camera, the orientation of the subject's eyes are oriented directly toward the camera, the orientation of the subject's eyes are not oriented directly toward the camera, and the subject matter of the video is not applicable. Any member of the study team or third parties with HIPPA compliant policies and procedures, blinded to the study group of each subject, may engage in the above-described annotation including therapist practitioners or research organizations.

Eye Tracking Video Assessment: This task involves the subject viewing social scenes and other types of scenes on a computer monitor while eye gaze patterns and fixations on social (i.e. face) and non-social portions of the scene are measured using an eye tracker. This is similar to eye tracking assessments used in ASD diagnosis research in which measurements of the percent of time looking at faces and eyes while watching video clips are obtained. [29]

Ekman 60 Faces Test: (Ekman & Freisen 1976 [30]), using a selection of 60 photographs from Ekman's Pictures of Facial Affect [30]. These images have been used in several studies of emotion recognition [31, 32]. Subjects will be presented with 60 photographs of different individuals expressing different emotions and asked to label each of the photographs in terms of the emotion expressed with one of the six standard emotional labels: happiness, sadness, anger, surprise, disgust, and fear. This assessment may be administered by computer and the study team member administering the assessment may assist in entry of the subject's selection.

Emotional Expression Video Assessment: This task involves the subject viewing videos of scenes where actors exhibit a specific emotion and following each video the subject is asked to label the emotion presented from a group of choices (ie. happiness, sadness, anger, surprise, disgust, and fear) during the assessment eye gaze patterns and fixations are measured using an eye tracker. This is similar to the Ekman 60 Faces Test but adds a level of realism as the emotions expressed are in video form. This is also similar to eye tracking assessments used in ASD diagnosis research in which measurements of the percent of time looking at faces and eyes while watching video clips are obtained [29].

Test of Variables of Attention (TOVA [33]): The TOVA is a highly reliable, computerized neuropsychological measure of sustained attention and response inhibition. This continuous performance measure presents a target and non-target stimulus, and children are instructed to only press the button when they see the target presented. Study members will ask and take

note of the subject's caffeine consumption the day of administration of each TOVA and may note the subject's amount of sleep the night before administration of each TOVA and incidental use of antihistamines or anxiolytic medications, as caffeine consumption, amount of sleep, and use of use of antihistamines or anxiolytic medications, can impact the outcomes of the test.

Peabody Picture Vocabulary Test, 4th Edition (PPVT [34]): The PPVT-4 scale is a norm-referenced, wide-range instrument for measuring the receptive (hearing) vocabulary of children and adults. Enlarged and colorized, this PPVT edition is available in two parallel forms (form A and form B) that are administered individually. Each form contains training items and 228 test items, each consisting of four full-color pictures as response options on a page. For each item, the examiner says a word, and the examinee responds by selecting the picture that best illustrates that word's meaning.

Video Recording of Study Visits: All study team member visits to the home, school, therapy center, or research organization may be video recorded using a single or multiple video cameras and tripods.

Data Collection – Behavioral Data

Eye Tracking: Study participants will be positioned so measurements can be obtained using an eye tracking device available for purchase by consumers e.g. the Tobii Eye Tracker 4C. This device uses a camera to measure eye positions and gaze direction to trace where study participants are focused (point of gaze) as they look at stimuli presented on a screen. The device may also measure pupil dilation.

Behavior: Recorded behavioral measures may include computer mouse click, computer keyboard/touchscreen keystroke, touchscreen contact, and game controller game play data.

1.5 Compliance Statement

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

2.1 Primary Objective (or Aim)

The primary objective of this study is to determine whether 6.67 to 33.33 hours of BioStream Training improves the performance of children with ASD on social skills assessments including in comparison to an alternate game. Specifically with respect to the following:

The mean change from baseline score on the following assessments based on post-final game play session assessment compared to the alternate game group:

a. Subject gaze patterns as measured by video eye tracking assessment.
b. Subject gaze patterns as measured by the dyadic interaction task.
c. Mean change in baseline score on the Ekman 60 Faces Test.
d. Mean change in baseline score on the TOVA.
e. Mean change in baseline score on the SRS.

2.2 Secondary Objectives (or Aim)

The secondary objectives are to observe changes in subject gaze patterns during additional social skills assessments after engaging in BioStream Training, including in comparison to an alternate game.

Specifically with respect to the following:

The mean change from baseline score on the following assessments based on post-final game play session assessment compared to the alternate game group:

a. Subject gaze patterns as measured on the videos of emotional expression assessment.
b. Subject responses to the CJARS survey.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This protocol is a comparison group controlled trial of a computer-based social skills training intervention. The BioStream Training is being compared to a modified version of the BioStream Training, which will not include the same therapeutic exercises or any video game that is rated E (Everyone) by the Entertainment Software Rating Board. The game will be played by subjects in the non-intervention group for the same length of playing time and at the same frequency, as the BioStream Training intervention group. It will require a similar game controller device as that which is used by the intervention group and will be computer based.

3.1.1 Initial Screening

Potential subjects will be first screened by any study team member in an in-person or telephone interview with the parent/guardian. After written informed consent is obtained to the initial screening (in the case of an in-person interview) or verbal informed consent is obtained to the initial screening (in the case of a telephone interview), protocol inclusion and exclusion criteria will be reviewed with the parent/guardian. The parent/guardian will also be asked for a list of the medications the subject is currently taking and that information will be recorded. A subset of questions from the SRS will be asked as part of the screening. The parent/guardian will also be asked if the subject has a diagnosis of ADHD and if so, that will be noted.

Prospective Study Participant Initial Screening Form. This short questionnaire will be administered to the parent/guardian in-person or by telephone prior to the study visit to

query their children's video game playing habits. This questionnaire should take less than 15 minutes to complete. Questions related to the inclusion/exclusion criteria will also be asked to determine eligibility.

3.1.2 Phase I Visit 1

Parental/guardian permission (informed consent) and, if applicable, child assent, will be obtained. Subjects will participate in social skills assessments. The subject will participate in the Peabody Picture Vocabulary Test, 4th Edition [34]. If the study team member determines that the subject's intelligence standard score is below 40, then the subject will not be able to continue to be included in the study per the exclusion criteria (see page 4). The subject will participate in the Ekman 60 Faces Test. If the subject score is greater than 75% correct, then the subject will not be able to continue to be included in the study per the exclusion criteria.

Additionally, to confirm certain inclusion criteria, the parent/guardian will be required to present the study team member with a note from a licensed medical professional indicating the subject has been diagnosed with ASD and also if the subject has been diagnosed with ADHD. The study team member will inspect the note, but will not take possession of it. The study team member will provide training to the parent/guardian (in the case of in-home use) or the teacher (in the case of in school use) or the therapist (in the case of therapy center use) regarding use of the Subject Computer, and review the protocol for the subject's training or use of the alternate game (as applicable).

3.1.3 BioStream Training Group

The BioStream Training group will undergo an estimated 40-60 minute training session on how to use the BioStream Training game. Further details on further assessments conducted during the visits can be found in section 4.2.

3.1.4 Alternate Game Group

The alternate game group will undergo an estimated 40-60 minute training session on how to use the alternate game on the first visit. Otherwise, their visits will be exactly the same as the BioStream Training Group. Further details on further assessments conducted during the visits can be found in section 4.2

3.2 Allocation to Treatment Groups and Blinding

Subjects will be randomly assigned to one of the two groups (BioStream Training group, or alternate game group) as an ongoing process throughout enrollment.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

Enrollment for this study will last 18 months (starting on August 7, 2018 and ending on February 7, 2020). When a subject is enrolled, their first home visit will be within four weeks of their enrollment. Each phase lasts four weeks, but it is possible there will be delays of up to four weeks between phases for some participants. Therefore, for each subject, we expect to have all of our data for both phases 16 weeks from the date of enrollment at the latest. We expect the study to be complete by the beginning of February of 2020 at the latest.

Expected time commitment for parents and subjects for each phase can be found in tables 1 and 2 on pages 4-6.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

There will be 10 sites for this study. Each site is an operating public school, private school, therapy center, therapist practitioner, or research organization where a therapist practitioner provides regular treatment for children with autism or regularly conducts research involving children with autism. Therefore, we expect to potentially enroll up to 150 subjects.

3.3.3 Offer of Free BioStream Training and Follow-up Study to Control Group

If funding permits, at the end of Phase II of the study, the study Sponsor would like to invite all participants in the control group (alternate game group) to participate in the BioStream Training. The study team will collect performance data on these participants over the course of 2 months. The study PI will submit a detailed protocol amendment to the IRB for approval prior to enrolling any of the control group participants in this study. Participant permission to be contacted regarding this additional part of the project is included in the consent form attached to this IRB package.

3.4 Study Population

3.4.1 Inclusion Criteria

1) Parental/guardian permission (informed consent) and if appropriate, child assent.

2) The parent who participates in the screening, completes the questionnaires, and provides informed consent must be proficient in English.

3) Age 4 years 0 months up to 14 years, 11 months, and 29 days.

4) English as the child's primary/first language (the intervention does not currently have alternate language versions).

5) Capable of using a handheld video game controller (as reported by parent).

6) Has successfully played a video game using a Microsoft Xbox game controller, Sony PlayStation game controller, or other comparable game controller.

7) Parent reports AT LEAST ONE of the following:

    a) Score of ≤ 2 on a modified version of Question 15 of the Social Responsiveness Scale (SRS) ("Is able to understand the meaning of other people's facial expressions.")

    b) Score of ≥ 2 on a modified version of Question 16 of the Social Responsiveness Scale (SRS) ("Avoids eye contact.")

8) Confirmed diagnosis of ASD from a licensed medical professional.

9) Estimated intelligence standard score of at least 40 (can be substituted for a receptive language score such as PPVT or use of the Differential Ability Scales, 2nd Edition (DAS-II)).

10) Attains a score equal to or less than 75% correct on the Ekman 60 Faces Test.

3.4.2 Exclusion Criteria

1) History of seizures.

2) History of traumatic brain injury or other significant medical or neurological abnormality affecting motor or higher cortical functioning. This includes gestational age below 32 weeks or perinatal injury, where the risk for brain injury is greatly increased. Rationale: a primary aim of the study is to identify sequences in our game-like applications that are appealing to youth with ASD; it is not clear that individuals with any type of acquired brain injury would provide useful data on what appeals to an individual with ASD.

3) A visual disorder that cannot be corrected through the use of corrective lenses to a level of 20-40 in both eyes.

4) Use of corrective visual lenses that would significantly impede the valid collection of visual attention and gaze pattern data during dyadic interaction tasks.

5) Auditory impairment (that cannot be corrected by a hearing aid) that would significantly impede the valid collection of test measures.

6) Profound intellectual disability or sensory-motor difficulties that would preclude valid use of diagnostic instruments and/or use of a computer or mobile computing device.

7) A DSM 5 disorder or other psychiatric symptoms that would interfere with the participant's ability to participate in the study (e.g., active psychosis), per parent report.

8) History of one or more psychiatric hospitalizations.

9) Presence of significant symptoms of a conduct disorder.

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

4 STUDY PROCEDURES

4.1 Initial Screening

See section 3.1.1 for a full description of the initial screening.

4.2 In-Person Study Visits

During the in-person study visits, parents and subjects will complete the following assessments, described in section 1.4 unless otherwise noted. Tables 1 and 2 also show details on the schedule of all in-person assessments for both phases.

Social Responsiveness Scale – Parent (SRS) – 10 minutes, parent only (may be administered by phone).

Childhood Joint Attention Rating Scale (C-JARS) – 10 minutes, parent only (may be administered by phone).

Dyadic Interaction Task – 10 minutes, subject only.

Eye Tracking Video Assessment – 10 minutes, subject only.

Ekman 60 Faces Test – 10 minutes, subject only.

Emotional Expression Video Assessment – 10 minutes, subject only.

Test of Variables of Attention (TOVA) – 22 minutes, subject only.

An introduction to game and equipment (Only Phase I Visit 1) – 40 – 60 minutes, with the parent/guardian, teacher, or therapist.

Subject Interview/Survey (described in section 5.4.1) – 10 minutes, subject only.

Parent/Teacher/Therapist Interview/Survey (described in section 5.4.2) – 10 minutes, parent, teacher, or therapist only.

Video Recording of Study Visits: All study team member visits to the home, school, or therapy center may be video recorded using a single or multiple video cameras and tripods.

Please note that for subjects who are using the BioStream Training in their school, therapy center or research organization, their parent/guardian only needs to be present for the first visit. After that visit, surveys involving the parent including the Social Responsiveness Scale and Childhood Joint Attention Rating Scale can be conducted over the phone.

4.3 Subject Completion/Withdrawal

Study participants may withdraw from the study at any time. They may also be discontinued from the study:

1) For failing to meet all inclusion criteria.

2) For meeting an exclusion criterion.

3) For not adhering to scheduled visits (at the discretion of the PI).

4) For failing to comply with study requirements (at the discretion of the PI).

5) For reasons of safety or for administrative reasons (at the discretion of the PI).

6) If the participant is otherwise not well-suited for further participation in the study (at the discretion of the PI).

4.3.1 Early Termination Study Visit

Subject Exit Interview/Survey

In the case that a subject decides to leave the study early, the subject will be asked to complete a final interview. This interview will discuss the reasons for leaving the study, will be completely voluntary, and may be discontinued at any time during the interview by the subject.

Parent Exit Interview/Survey

In the case that a subject decides to leave the study early, the parent/guardian will also be asked to complete a final interview. This interview will discuss the reasons for the subject leaving the study, will be completely voluntary, and may be discontinued at any time during the interview by parent/guardian.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Initial Screening Interview

See section 3.1.1 for a full description of the initial screening.

5.1.2 ASD/ADHD Diagnosis Verification

To confirm certain inclusion criteria, during the first visit the parent/guardian will be required to present the study team member with a note from a licensed medical professional indicating the subject has been diagnosed with ASD and also if the subject has been diagnosed with ADHD. The study team member will inspect the note, but will not take possession of it.

5.1.3 Medication List

During the initial screening, the parent/guardian will be asked for a list of the subject's medications in the initial screening in order to determine the medication's possible impact on the therapeutic outcomes and research outcomes.

5.1.4 Video Game Interview Form (Parent-Report)

Parents or teachers or therapists will complete a weekly survey on the laptop provided by the Sponsor. The survey will ask questions concerning the subject's enjoyment and progress with the training as well as administration in the home or school environment. Survey is fully described in section 5.4.2.

5.2 Efficacy Evaluations

5.2.1 Primary Endpoints

Please see the primary endpoints listed on page 3-4.

5.2.2 Secondary Endpoints

Please see the secondary endpoints listed on page 3-4.

5.3 Biosensor Data Collection

- *Eye Tracking*: Study participants will be positioned so measurements can be obtained using an eye tracking device available for purchase by consumers Tobii Eye Tracker 4C, or another device of a similar nature. This device uses a camera to measure eye positions and gaze direction to trace where study participants are focused (point of gaze) as they look at stimuli presented on a screen. The device may also measure pupil dilation.

- *Behavior*: Recorded behavioral measures may include computer mouse click, computer keyboard/touchscreen keystroke, touchscreen contact, and game controller game play data.

5.4 Subject and Parent Interviews

5.4.1 Subject Interviews/Surveys

At each study visit and at other times during the course of the study, the subject will participate in brief interviews and/or surveys (which may be conducted in-person or by phone by any member of the study team) including questions about their enjoyment of BioStream Training or alternate game. These interviews will provide an opportunity for subjects to share feedback about BioStream Training with the study team and compare that feedback to feedback regarding the alternate game. All interviews and surveys will be configured (if computer based) so as to include only de-identified subject data. If the surveys are in paper form, the study team member conducting the surveys will only record data in such a manner so that it is de-identified. All such paper based surveys may be converted to digital format (including by use of a scanner) and transmitted to the Sponsor. Additionally, the subject may be asked to complete a brief survey about BioStream Training and/or the alternate game on the Subject Computer which may be transmitted to the Sponsor as described in Section 1.2 Data Transfer and Storage.

5.4.2 Parent/Teacher/Therapist Interviews/Surveys and Equipment Handoff/Return

At each study visit and at other times during the course of the study, the parent/guardian (in the case of the at-home subjects) or teacher or therapist (in the case of in-school subjects or in-center subjects) will participate in brief interviews and/or surveys (which may be conducted in-person or by phone) including questions about the subject's enjoyment of BioStream Training or alternate game. These interviews will provide an opportunity for parents/guardians or teachers or therapists (as applicable) to share feedback about BioStream Training and/or alternate game with the study team. All interviews and surveys will be configured (if computer based) so as to include only de-identified subject data. If the surveys are in paper form, study team member conducting the surveys will only record data in such a manner so that it is de-identified. All such paper based surveys may be converted to digital format (including by use of a scanner) and transmitted to the Sponsor. Additionally, the parent/guardian or teacher or therapist (as applicable) may be asked to complete a brief survey about BioStream Training, the alternate game, and/or provide observational

information regarding the subject (including in the form of a Weekly Parental/Teacher/Therapist survey), on the Subject Computer which may be transmitted to the Sponsor as described in Section 1.2 Data Transfer and Storage. The interviews and surveys will provide an opportunity for parents/guardians or teachers or therapists (as applicable) to share feedback about the at-home, in-school, or in-center administration process (as applicable) with the study team.

Additionally, the parent/guardian or teacher or therapist (as applicable) will be provided with at-home or in-school or in-center equipment (such as a laptop, an eye tracker, such as the Tobii Eye Tracker 4C, and a game controller, such as an Xbox 360 Controller), as well as instructions regarding at-home or in-school or in-center administration of BioStream Training or alternate game. The parent/guardian or teacher or therapist (as applicable) will return the equipment to the study team at the appropriate in-person visit.

STATISTICAL CONSIDERATIONS

5.5 Primary Endpoint

Paired-sample t-tests will be used to measure changes from baseline within each group, and independent sample t-tests will be used to measure changes from baseline relative to the alternate game group for all endpoints.

For all endpoints, we will determine whether there were statistically significant changes in scores from baseline or relative to the alternate game group. Significance will be set at $p<0.05$. Multiplicity of primary endpoints will be managed via statistical corrections.

5.6 Secondary Endpoints

See statistical analysis for primary endpoints listed above.

5.7 Statistical Methods

5.7.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

5.7.2 Efficacy Analysis

The primary analysis will be based on all subjects randomized at the initial visit for who initial visit and final visit data is collected.

Paired-sample t-tests will be used to measure changes from baseline within each group, and independent sample t-tests will be used to measure changes from baseline relative to the alternate game group for all endpoints.

For all endpoints, we will determine whether there were statistically significant changes in scores from baseline or relative to the alternate game group. Significance will be set at $p<0.05$. Multiplicity of primary endpoints will be managed via statistical corrections.

Secondary endpoints will be evaluated in a similar manner. Analysis will include all subjects for who initial visit and final visit data is collected. The treatment and control groups will be compared using t-tests as described above.

5.7.3 Safety Analysis

Clinical AEs will be monitored throughout the study. All SAEs will be reported to the IRB in accordance with IRB policies. AEs that are not serious will be summarized in narrative or other format and submitted to the IRB at the time of continuing review. Since the study procedures are not greater than minimal risk, AEs are not expected. See section 6.2 on page 22 for further details.

5.8 Sample Size

This study will enroll up to 150 participants (see section 3.3.1 for details in enrollment timeline).

5.9 Interim Analysis

No interim efficacy analyses or corresponding stopping rules are proposed. Interim safety analyses will consist of ongoing reviews of AEs (if any) and will result in stopping the study only if life-threatening or serious diseases are found to be caused by the study, which is not anticipated.

6 SAFETY MANAGEMENT & REPORTING

6.1 Clinical Adverse Events

Clinical (AEs) will be monitored throughout the study. Since the study procedures are not greater than minimal risk, AEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) they will be reported to the IRB. As with any computer video game, seizure is a rare risk and possible SAE. However, subjects are required to have played video games in the past and a history of seizures excludes participants from taking part in this study.

6.2 Adverse Event Reporting

If, at any time, during any BioStream Training session or play of the alternate game session, the parent/guardian or teacher or therapist (as applicable) observes the subject displaying seizure symptoms, or becoming agitated, frustrated, disturbed, or in any other manner being negatively impacted (including in a manner that may pose a risk to the subject's safety or well-being or the safety or well-being of others) or in their judgment, believes the subject will or may become negatively impacted in any way (including in a way that may pose a risk to the subject's safety or well-being or the safety or well-being of others), they will immediately discontinue the session, stop the subject from engaging in any further sessions, and contact the Principal Investigator to inform him or her of this occurrence. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

7 STUDY ADMINISTRATION

7.1 Treatment Assignment Methods

7.1.1 Randomization

Subjects will be randomly assigned to one of the two groups (BioStream training group, or alternate game group) as an ongoing process throughout enrollment.

7.2 Data Collection and Management

See page 8 and pages 19-20 for information on data collection and management.

7.3 Confidentiality

All written or electronic forms (except Informed Consent Forms and Assent Forms) will employ a unique alphanumeric identifier; in no case will the participant's name or other identifying information appear on these forms. The user ID for the BioStream Training and alternate game shall use the same unique alphanumeric identifier. The study team will accomplish this on all parent/guardian or teacher or therapist questionnaires (as applicable) which have lines for child and parent name by applying a label with unique alphanumeric identifier and instructing parents/guardians or teacher or therapist to use generic labels (e.g., biological mother) rather than writing their names. The PI will keep a master list containing PHI and subject ID number separate from coded data forms, which will be maintained in a locked file drawer, cabinet or lock box in the PI's office. Only the PI will have access to this location. The master list will be maintained on a separate password-protected computer and be stored in an encrypted password protected file. Video recordings will stored on password-protected and/or encrypted data storage devices including computers, external hard drives, and/or flash drives only accessible by members of the study team including the Sponsor's study team members. Any member of the study team or third party with HIPPA compliant policies and procedures, blinded to the study group of each subject, may engage in in video recording annotation including therapist practitioners or research organizations. The Sponsor may collect and store all de-identified data on password-protected and/or encrypted data storage devices including computers, external hard drives, and/or flash drives.

7.4 Regulatory and Ethical Considerations

7.4.1 Data and Safety Monitoring Plan

The PI will periodically review the data collection and storage practices associated with this study and determine whether changes to enhance confidentiality and privacy are required. The PI will frequently monitor the quality control of the data with staff and research assistants.

7.4.2 Risk Assessment

Use of BioStream Training or Alternate Game: The tasks associated with use of the BioStream Training or alternate game can produce feelings of frustration, anxiety, stress and fatigue including eye fatigue. These risks are no greater than those associated with minimal risk studies. As with any computer video game, seizure is a rare risk and possible SAE.

However, subjects are required to have played video games in the past and a history of seizures excludes participants from taking part in this study.

Psychological Stress and Anxiety: Due to the length of time associated with the assessments, some participants may experience fatigue, anxiety, or stress. Participants will be allowed to take breaks and stop at any time. Additionally, the presentation of unpleasant stimuli (including social interactions that are challenging for some youth with ASD) might be distressing to some participants. To minimize risk associated with the presentation of unpleasant stimuli, several precautions will be taken: (i) only members of the study team that are trained to conduct study visits by a Board Certified Behavior Analyst with experience in working with children with ASD, will conduct study visits, (ii) Parents/guardians will be shown examples of stimuli as part of the consent process and will be asked whether they feel confident that their child will not find these overly distressing, (iii) the parent/guardian or teacher or therapist (as applicable) will be instructed and agree to oversee gameplay to look for signs of distress in the subject and the tasks will be stopped if the subject appears distressed or requests to stop.

Risks Associated with Behavioral Data Collection: These risks are no greater than those associated with minimal risk studies.

Breach of Confidentiality: Confidentiality is carefully guarded to address issues of privacy and insurability. In particular, each subject is assigned a unique alphanumeric identifier immediately upon recruitment. Although a list linking the study number to the subject's name is maintained for future studies and contact, the name does not appear on any other document (other than Informed Consent Forms and Assent Forms). The families are informed of this link and are assured that only the PI has access to that list. The purpose of the list linking study ID number to the name is to record who we have already recruited so that we accidentally do not re-approach any family.

7.4.3 Potential Benefits of Trial Participation

There is no direct benefit to participation in this study, however it is possible that progress made during the training could be generalizable as a therapeutic benefit. Subjects will receive collateral benefits. We anticipate indirect benefits to the community of people with ASD at large. Researchers will be able to increase their understanding of the behavioral foundations of this disorder as well as the propensity of people with ASD towards specific game-like applications, which may be translated into improved future treatments. In addition, it could significantly advance development of an intervention and/or diagnostic tool to help children with ASD**.**

7.4.4 Risk-Benefit Assessment

It is hoped that the knowledge gained will indirectly benefit the participants as well as the larger community of people with ASD. The risks of the study procedures including the effects of behavioral measurements and assessments are all considered minimal risk.

7.5 Recruitment Strategy

In-home Subject Recruitment

With respect to members of the study team that have a therapy practice that includes in-home patients, in-school patients, or in-center patients, they will recruit from within their patient base for in-home study subjects.

Additionally, recruitment will occur through phone and in-person outreach by study team members to organizations that serve the needs of families of children with ASD to help identify families that may have an interest participating in the study. Such organizations will provide a study team member's contact information to those families that they, in their discretion, decide to contact that express an interest in learning more about the study. The study team will not receive the names or telephone numbers of potential study participants without their verbal consent to share that information.

Study team members may also engage in outreach through their personal and professional network of contacts to help identify families that may have an interest participating in the study. Such contacts will provide a study team member's contact information to those families that they, in their discretion, decide to contact that express an interest in learning more about the study. The study team will not receive the names or telephone numbers of potential study participants without their verbal consent to share that information.

In-school, and In-center Subject Recruitment

Schools, therapy centers, and research organizations that serve the needs of children with ASD or conduct research involving children with ASD will be approached by the Sponsor to source potential subjects. These organizations will approach parents of potential subjects and assess interest in the project and ask permission to connect to the study team. No additional subject information, excluding information collected in the ordinary course of operations, will be collected before this permission is given.

7.6 Informed Consent/Assent and HIPAA Authorization

After a thorough discussion of the study and its procedures, the parent/legal guardian will be consenting and giving permission for their child to participate in this study. Children ages 7 to 14 entering the study will give their assent to participate.

Initial Interview Phase. When a parent/legal guardian expresses interest in having their child participate in the study, an in-person meeting or telephone call will be set up by a member of the study team to review the study details, risks, benefits, financial considerations, and HIPAA procedures that are in place to insure privacy and confidentiality. The member of the study team will explain that an initial eligibility interview needs to be conducted as a first step, and they will be told that all information collected will be kept strictly confidential. The nature of the risks and benefits of participating in the screening interview will be explained. The parent/legal guardian will then undergo a short (up to 30 min) in-person or by phone screening interview to determine study eligibility. The specific inclusion or exclusion criteria in question will be described, and the parent/legal guardian will have an opportunity to ask questions. After the results of initial interview phase are received and evaluated, the parent/legal guardian will be contacted again with the results. The parent/legal guardian will be informed of specific inclusion or exclusion criteria that are not met/met based on this more detailed review.

In-Person Study Phase. After the initial interview phase, those parents/legal guardians of children that met the study inclusion and exclusion criteria in the initial interview phase and who are interested in participating in the study will be scheduled for have their first visit in the home, school, therapy center, or research organization (as applicable). Upon the study team member's arrival at the subject's home, school, therapy center, or research organization (as applicable), the first agenda item will be a face-to-face conversation with the parent/legal guardian to review the study protocol. The parent/legal guardian will then be asked to read the consent forms. Prior to signing the forms, the study team member will encourage the parent/legal guardian to ask as many questions as they wish, until they fully understand all aspects of the study, including its risks and benefits, as well as the time demands and inconveniences of study participation. This is critical, as it does not benefit the parent/legal guardian or the researchers to have a misunderstanding at this phase as to what full study participation entails. The study team member will also remind the parent/legal guardian that the subject's participation in the study is voluntary and that the parent/legal guardian or child can terminate participation at any time with no obligations to continue and no penalties whatsoever. Refusal to participate will in no way influence the individual's relationship with the study team member. In addition, if the participants' parent/legal guardian is an employee of the study team member's organization or Sponsor, they will be informed that their participation will not affect their performance evaluations or employment and that the study data will not be shared with their supervisor.

7.6.1 Waiver of Assent

A waiver of assent is requested for the initial screening phase of the study. The short screening interview will be conducted with parent/legal guardian in-person, and the child may not be present to provide assent.

7.7 Payment to Subjects/Families

7.7.1 Compensation

As compensation for time and expenses, child participants will be provided with a gift card upon the completion of each phase of the study. The total value of each gift card provided to each subject over the course of this study will not exceed $50 per completion of each phase of the study. Gift cards may be for retailers such as Amazon and Target. In addition, each study participant will be provided with a gift card not to exceed $5 to retailers such as Amazon and Target for each time he or she completes the TOVA assessment.

8 PUBLICATION

At appropriate scientific junctures, results from these studies will be presented at scientific forums (e.g. national meetings). Similarly, as results are achieved that deserve to be shared with the greater scientific community, they will be submitted for publication in appropriate peer review journals. They may also be presented in book chapters. In all cases, data on specific participants will be stripped of all protected or identifiable health information.

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Document Submission Cover Page

Official Title of Study: Computer-based Social Skills Training for Autism Spectrum Disorder

NCT Number of Study: NCT03672344

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Document Title: Supplement to Statistical Analysis Plan

Document Date: April 25, 2022

Study Sponsor: BioStream Technologies, LLC

Supplement to Statistical Analysis Plan

In addition to the statistical analyses described in the original study protocol, a 2 (Time) × 2 (Condition) mixed ANOVA was conducted to test for between-group differences in Ekman-60 scores from pre- to post-intervention. Mixed ANOVAs also will be used to test for between-group differences in Social Responsiveness Scale (SRS) and Child Joint Attention Rating Scale (C-JARS) scores from pre- to post-intervention. Significance will be set at $p < 0.05$.