UNIVERSITY OF CALIFORNIA,
IRVINE

Salivary Inflammatory Biomarkers in the Context of Children's Cancer-Related Pain, Psychosocial Well-Being, and Caregiver Perceived Stress

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Psychological Science

by

Crystle-Joie Guerrero Agbayani

Dissertation Committee:
Associate Professor Michelle A. Fortier, Chair
Chancellor's Professor Douglas Granger
Professor Candice Odgers

2024

© 2024 Crystle-Joie Guerrero Agbayani

DEDICATION

In memory of Estrella Agbayani:

My grandma, my number 1 cheerleader, my golden lady!

You lifted me up every step of the way.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

If I learned anything in grad school, it's that science is a team effort – and I am *so* blessed to have had the most supportive team of individuals both inside and outside of UCI supporting me throughout the years. *We* did it!

To my advisor, Dr. Michelle Fortier: thank you for taking a chance on me and welcoming me into the Center on Stress & Health after our phone conversation during interview week in 2017 (pre-Zoom days!). Receiving my offer letter from UCI changed the trajectory of my life, and again I apologize for holding out so long after the official decision deadline to tell you all that I was going to accept (haha!). You've been a true mentor to me and have helped me navigate so many of my "firsts" in academia – designing/conducting research in a clinical setting, publishing, grant writing – even amid a global pandemic! I'm so thankful for your guidance and encouragement, and for the safe space you've cultivated for me to learn.

To the rest of the UCI Center on Stress and Health, my grad school experience would not have been the same without you all! To my director Dr. Zeev Kain, thank you for the opportunity to collaborate with you, and for providing your invaluable insight as a member of my advancement committee. This research center is truly special, and I have you to thank for that. To Haydee Cortes, who literally does it all, thank you for everything – and I mean EVERYTHING! There aren't enough pages to list everything you've done to help me throughout the years. To Lessley Torres, thank you for all your hard work consenting each Pain Buddy family, and learning about the saliva collection protocol to get this project running. This dissertation simply would not have happened without you. And to soon-to-be-Dr. Beverly Mendoza, thank you for the support and laughs along with Lessley throughout the years. You both have made being part of the Center a highlight of my Ph.D. journey! To my last research assistants as a graduate student, Manasi Patel and Cassidy Doan, thank you both so much for helping me with data entry, finding literature, and sifting through medical records for this dissertation! Thank you to our post-baccs Emily Lopez and Andrea Barrientos (now a full-time member of the Center!) as well for your help with data entry. To all my previous research assistants who I have worked with throughout the years – thank you!

To my dissertation committee, Dr. Douglas Granger and Dr. Candice Odgers, I am so grateful for your time, expertise, and kindness. Doug, your passion for salivary bioscience has been infectious since our first conversation during my interview week – thank you for bringing me into IISBR and this entire world of scientific possibilities! Candice, thank you for not only providing mentorship to me as a student in your classes, but as one of my committee members! I also must acknowledge my advancement committee, Dr. Jenna Riis and Dr. Michael Hoyt. Jenna, thank you for taking time to meet with me as I conceptualized this project for my proposal, serving on my advancement committee, and for all your

mentorship during the dozens of times I TAed for your Health Psych class. Michael, thank you for truly going above and beyond to help see this project to the end. A special shoutout here to Hillary Piccerillo as well for all your patience with answering my questions about biomarkers, assuring me as I fretted over my assays, and helping me with every aspect of the lab science (you're the best!!).

To remain on brand, I need to quote Sora here and say that truly, "My friends are my power!" Fams: You all keep me sane, thank you for the silly goose time amidst my trek to the finish line of this degree. Jossy and Stefie: Our groupchat sustains me until we can see each other again (it has been TOO long since Beyoncé), and I know that you both are on-call when I need you the most, like for an entire weekend of all-nighters for this dissertation. Azn Gurl Squad: Y'all already know. TAing with each of you, boba runs, taking stats classes together, and game night at Jin's apartment are just a few treasured memories I have of us all. Dr. Nicole Froidevaux and Dr. Jocelyn Lai: You two have been my rocks as we worked through our dissertation era – thank you for being my sounding board, and for all the social/emotional support. Shoutout to my cohort (Cohort Quarantini Time/Scientific Ninjas) for the stats consulting, the laughs, and the cute pet photos!

To my family, thank you for the support through my very long academic journey. I promise I'm done with school now! Thank you to my parents for allowing me to pursue my passions, even though my education has kept me a whole ocean away for over a decade. To my brothers and the rest, thank you for cheering me on every step of the way!

To Dalton, thank you for literally everything. We've known each other half our lives and you've gone from being my boyfriend to my fiancé to my husband all in the span of me finishing this degree – holy moly! You've been nothing but supportive, especially in the final stretch of me working on this dissertation. From walking and feeding Toph, to getting groceries and ensuring that I'm fed, to patiently listening to me talk in circles, to being my Uber driver, and letting me take over your desk, you've been an S-rank partner. I'm so excited for the next chapter of our life together – a chapter where I'm finally no longer a student!

Finally, a funding acknowledgement: The present study was supported under the Research Supplements to Promote Diversity in Health-Related Research Program by the National Cancer Institute of the National Institutes of Health (Award Number R01CA222012). The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

VITA

Crystle-Joie Guerrero Agbayani

EDUCATION

Ph.D., University of California, Irvine 2017-2024
Psychological Science
Major: Health Psychology
Minor: Quantitative Methods

B.A., Chapman University 2013-2017
Major: Psychology
Minor: Holistic Wellness

RESEARCH OVERVIEW

My program of research examines how sociocultural factors (e.g., race, ethnicity, language) influence biobehavioral stress in the cancer context. I also implement salivary bioscience methods to more precisely measure the physiological factors associated with clinical outcomes such as depression, anxiety, and pain among pediatric cancer patients and their caretakers. I am particularly interested in identifying actionable risk markers in the service of providing tailored care for individuals who may be at-risk for poorer psychosocial or pain outcomes.

RESEARCH EXPERIENCE

Graduate Student Researcher 2017-2024
UCI Center on Stress & Health
Director: Dr. Zeev N. Kain, M.D., M.B.A.
Co-Director & Advisor: Dr. Michelle A. Fortier, Ph.D.
University of California, Irvine

Research Assistant 2014-2017
Culture, Evolution, and Behavior Lab
Advisor: Dr. David Frederick, Ph.D.
Chapman University

PEER-REVIEWED PUBLICATIONS

**Denotes a post-baccalaureate mentee I directly supervised in research.*

Saatchi, B., **Agbayani, C. J. G.**, Clancy, S., & Fortier, M. A. (2023). Measuring irritability in young adults: An integrative review of measures and their psychometric properties. *Journal of Psychiatric and Mental Health Nursing*, *30*(1), 35-53. doi.org/10.1111/jpm.12851.

Agbayani, C. J. G., Tucker, J. A., Nelson, E. L., *Martinez, F., Cortes, H., Khoury, D., Kain, Z. N., Lin, C., Torno, L., & Fortier, M. A. (2022). Immunological and psychosocial functioning in parents of children with cancer. *Supportive Care in Cancer*, *30*(4), 3379-3388. doi.org/10.1007/s00520-021-06770-0.

*Acosta, E., **Agbayani, C. J. G.**, Jenkins, B. N., Cortes, H. G., Kain, Z. N., & Fortier, M. A. (2022). The impact of primary language spoken on the pain experience of children with cancer. *Journal of Pediatric Hematology/Oncology, 44*(4), 135-141. doi.org/10.1097/mph.0000000000002440.

Rosales, P., Evangelista, L., Guo, Y., **Agbayani, C. J. G.**, Kain, Z. N., & Fortier, M. A. (2021). Exploring differences in perceived satisfaction, resilience, and achievement between Hispanic and Non-Hispanic White childhood cancer survivors. *Journal of Pediatric Health Care, 35*(2), 196-204. doi.org/10.1016/j.pedhc.2020.10.003.

Agbayani, C. J. G., Fortier, M. A., & Kain, Z. N. (2020). Non-pharmacological methods of reducing perioperative anxiety in children. *BJA Education, 20*(12), 424-430. doi.org/10.1016/j.bjae.2020.08.003.

Chung, W. W., **Agbayani, C. J. G.**, Martinez, A., Le, V., Cortes, H., Har, K., Kain, Z. N., & Fortier, M. A. (2018). Improving children's cancer pain management in the home setting: Development and formative evaluation of a web-based program for parents. *Computers in Biology and Medicine, 101*, 146-152. doi.org/10.1016/j.compbiomed.2018.08.014.

POSTER PRESENTATIONS AND INVITED TALKS

**Denotes a post-baccalaureate mentee I directly supervised in research.*
***Denotes an undergraduate mentee.*

Agbayani, C. J. G. & Fortier, M. A. (2023, February) *Inflammatory biomarkers and cancer – investigating links to children's psychosocial symptoms and pain.* Talk presented to the UCI Chao Family Comprehensive Cancer Center as part of the UCI Institute for Interdisciplinary Salivary Bioscience Research Seminar Series.

Patel, M., **Agbayani, C. J. G., Hoyt., M, Kain, Z. N., Torno, L., Lin, C., & Fortier, M. A. (2022, November). *Goal-focused emotion-regulation therapy for adolescent cancer survivors.* Poster presented at CHOC Research Day Virtual Symposium.

Agbayani, C. J. G., Tucker, J. A., Nelson, E. L., *Martinez, F., Cortes, H., Khoury, D., Kain, Z. N., Lin, C., Torno, L., & Fortier, M. A. (2022, September). *Examining immune, psychological, and quality of life outcomes among parents of children with cancer.* Poster presented at the UCI Chao Family Comprehensive Cancer Center Annual Scientific Retreat in Lake Arrowhead, CA.

Fortier, M. A., **Agbayani, C. J. G.**, Hoyt., M, Kain, Z. N., Torno, L., & Lin, C. (2022, September). *A goal-directed intervention for adolescent cancer survivors*. Poster presented at the 54th Congress of the International Society of Paediatric Oncology in Barcelona, Spain.

Rosales, P., **Agbayani, C. J. G.**, Lin, C., Torno, L., Cortes, H., & Fortier, M. A. (2022, April). *Culture, language, and health related quality of life in Latinx survivors of childhood cancer.* Poster session cancelled for the Society of Pediatric Nurses 32nd Annual Conference in Anaheim, CA.

*Amaya, C., **Agbayani, C. J. G.**, Torres, L., Cortes H., Torno, L., Lin, C., Rosales, P., Kain, Z. N., & Fortier, M. A. (2022, March). *Language differences in quality of life among childhood and adolescent cancer survivors*. Poster presented at the American Psychosomatic Society 80th Annual Meeting in Long Beach, CA.

Agbayani, C. J. G., *Amaya, C., Torres, L., Cortes H., Torno, L., Lin, C., Moran, D., Yun, C., Kain, Z. N., & Fortier, M. A. (2021, November). *Cultural differences in self reported quality of life among children and adolescents with cancer*. Poster presented at CHOC Research Day Virtual Symposium.

Agbayani, C. J. G., *Zavala, S., Torres, L., Cortes, H., Torno, L., Lin, C., & Fortier, M. A. (2021, May). *Cultural differences in self-reported quality of life among children and adolescents with cancer.* Poster presented at the Association for Psychological Science Annual Virtual Convention.

Agbayani, C. J. G., Torres, L., Torno, L., Lin, C., **Martinez, F. Cortes, H., & Fortier, M. A. (2020, November). *Differences in quality of life reports between pediatric cancer patients and survivors of childhood cancer.* Poster presented at CHOC Children's Virtual Research Day.

Agbayani, C. J. G., Torres, L., Cortes, H., & Fortier, M. A. (2020, August). *Depression, anxiety, and quality of life in a culturally diverse sample of pediatric cancer patients.* Poster presented at the American Psychological Association Annual Virtual Convention.

Agbayani, C. J. G., *Schmid, L., Torres, L., Nelson E., Tucker, J. A., & Fortier, M. A. (2020, December). *Immunological and psychosocial functioning in parents of children with cancer.* Poster presented at the American Psychosomatic Society Annual Virtual Meeting.

*Schmid, L., **Agbayani, C. J. G.**, Torres, L., Jenkins, B. N., Kain, Z. N., & Fortier, M. A. (2020, December). *Cultural associations with pediatric oncology pain*. Poster presented at the American Psychosomatic Society Annual Virtual Meeting.

Torres, L., **Agbayani, C. J. G.**, *Schmid, L., *Zavala, S., Mendoza B., Cortes H., Kain, Z. N., Campos, B., & Fortier, M. A. (2020, December). *Evaluation of a community based participatory research collaboration with Latino caregivers of children with cancer.* Poster presented at the American Psychosomatic Society Annual Virtual Meeting.

Esslinger, J., **Agbayani, C. J. G.**, Stauffer, T., Schoebi, D., & Riis, J. (2019, March). *Time stamped sample collection in salivary bioscience field research: Problems and pitfalls.* Poster presented at the UCI Institute for Interdisciplinary Salivary Bioscience Research inaugural Virtual Salivary Bioscience Conference.

Fortier, M. A., **Ornelas, E., **Agbayani, C. J. G.**, & Kain, Z. (2019, February). *C-TIPS: A web-based program to improve parental management of children's cancer pain*. Poster presented at the International Society for Research on Internet Interventions 10th Scientific Meeting in Auckland, New Zealand.

Agbayani, C. J. G., **Mendoza, B., Cortes, H., & Fortier, M. A. (2018, October). *Development and parent formative evaluation of C-TIPS: A pain management program for children in the home setting*. Poster presented at CHOC Children's Research Week in Orange, CA.

Agbayani, C. J. G., Cortes, H., Gago-Masague, S., & Fortier, M. A. (2018, April). *Preliminary efficacy testing of Pain Buddy: A web-based pain management intervention for children with cancer*. Poster presented at the California Psychological Association Annual Convention in La Jolla, CA.

Agbayani, C. J. G., **Mendoza, B., Cortes, H., & Fortier, M. A. (2018, March). *C-TIPS: A web based program for improving children's cancer pain management in the home setting.* Poster presented at the American Pain Society 37th Annual Scientific Summit in Anaheim, CA.

Agbayani, C. J. G. & Frederick, D. A. (2017, January)*. Men's and women's perceptions of sexual consent and assault: Effects of verbal cues, physical cues, voice, and alcohol.* Poster presented at the Society for Personality and Social Psychology 18th Annual Convention in San Antonio, TX.

Herring, M., **Agbayani, C. J. G.**, & Frederick, D. A. (2016, January). *What constitutes sexual consent and assault? The effects of verbal, physical, and linguistic cues*. Poster presented at the Society for Personality and Social Psychology 17th Annual Convention in San Diego, CA.

AWARDS, FELLOWSHIPS, AND GRANTS

UCI Post-Baccalaureate Mentoring Award [$200; $600] 2022, 2020
Awarded to graduate students for excellent mentorship of post-baccalaureate students.

California State University, Long Beach Pre-Professor Program Fellowship [$500] 2022
Applied to and was selected for the PREPP fellowship, a competitive semester-long training program designed by CSULB to support doctoral students' transition to a faculty position, particularly at an R2 institution. I honed skills in teaching, curriculum design, and mentoring under the guidance of my faculty mentors Dr. Niloofar Bavarian (CSULB Department of Health Science) and Dr. Karissa Miller (CSULB Department of Psychology). I also had the opportunity to attend CSULB trainings that promoted diversity, equity, and inclusion such as:

- Become a Bob Murphy Access Center (BMAC) Ally Workshop
- Gender, Race, and Immigration Status Biases in the Classroom-Working with Different Intersecting Identities

National Cancer Institute Research Supplement to Promote Diversity in Health-Related Research 2021
[PI: Fortier, Award No. 3R01CA222012-04S1; $67,161]
Applied for and received a Diversity Supplement from NCI of the National Institutes of Health to pursue novel research aims under a funded parent R01. The funded project will address the following aims: 1) Examine associations between depression, anxiety, pain, and inflammatory biomarkers among children with a first-time cancer diagnosis throughout the first six months of treatment and 2) Explore how Pain Buddy, a novel mHealth intervention designed to improve psychosocial symptoms and pain among these same children, may impact inflammatory biomarker levels throughout a two-month intervention period.

UCI Association for Graduate Students Spring Virtual Conference Grant [$250] 2021
Applied and received to present the poster, "*Cultural differences in self-reported quality of life among children and adolescents with cancer*" at the Association for Psychological Science Annual Virtual Convention.

American Psychological Association Travel Award [$500] 2020
Awarded by the Children, Adolescents and Families (CAF) Caucus of the American Psychological Association to defray travel expenses associated with presenting at the annual APA meeting. Applied and received for the poster, "*Depression, anxiety, and quality of life in a culturally diverse sample of pediatric cancer patients*."

Diversity Recruitment Fellowship [$5,000] 2017
Merit-based award from UCI Graduate Division granted upon acceptance to Psychological Science (formerly Psychology & Social Behavior) Ph.D. program.

TEACHING EXPERIENCE

Instructional Assistant Professor Expected start: August 2023
Chapman University
Department of Psychology

Fall 2023 courses planned:

Physiological Psychology

Child Development

Lecturer 2021-2023
Chapman University
Department of Psychology

Course: Physiological Psychology; see table for student evaluations.

Term	Class Size	Response Rate	Median Rating	Mean Rating
Fall 2022	26	84% (22/26)	5.0/5.0	4.81/5.00
Fall 2022	24	75% (18/24)	5.0/5.0	4.85/5.00

Course: Health Psychology; see table for student evaluations.

Term	Class Size	Response Rate	Median Rating	Mean Rating
Spring 2022	20	75% (15/20)	5.0/5.0	4.69/5.00
Fall 2021	25	68% (17/25)	5.0/5.0	4.88/5.00

Teaching Associate 2022
University of California, Irvine
Department of Psychological Science

Course: Psychology Fundamentals C; see table for student evaluations.

Term	Class Size	Response Rate	Median Rating	Mean Rating
Summer II 2022	38	39% (15/38)	9.0/9.0	8.52/9.00

Teaching Assistant 2017-present
University of California, Irvine
Department of Psychological Science
**Denotes a course where instructional duties included planning and leading weekly one-hour discussion section to supplement lecture.*

Health Psychology: Fall 2017, 2020; Winter 2019, 2020, 2023; Spring 2018, 2020

*Clinical Health Psychology: Winter 2018

Psychology Fundamentals C: Summer II 2018

Clinical Sports Psychology: Fall 2018

Clinical Psychology: Spring 2019

*Child Development: Summer I 2019, Fall 2019

Infant Development: Summer I 2020, Spring 2021, Fall 2022

*Naturalistic Field Research: Winter 2021

Guest Lectures:

Agbayani, C. J. G. (2023, April). *Health & development.* Presented to Dominican University Lifespan Development course.

Agbayani, C. J. G. (2022, March). *Community-based participatory research.* Presented to CSULB Graduate Research Methods course as part of PREPP Fellowship training.

Agbayani, C. J. G. (2021, June). *Research in pediatric psychosocial oncology*. Presented to UCI Research Design course.

Agbayani, C. J. G. (2020, November). *Health psychology research in pediatric oncology*. Presented to UCI Health Psychology course.

Agbayani, C. J. G. (2019, November). *Chronic illness in adolescents*. Presented to UCI Human Growth and Development through the Lifespan course in the department of Nursing Science.

Agbayani, C. J. G. (2018, May). *Health psychology.* Presented to UCI Psychology Fundamentals C course.

Agbayani, C. J. G. (2018, October). *Research methods*. Presented to UCI Health Psychology course.

PROFESSIONAL SERVICE

UCI Psychological Science 2022-present; 2018–2020
Graduate Student Peer Mentor
Mentored first-year graduate students in Health and Clinical Psychology.

UCI Psychological Science 2021
Coordinator, Graduate Student Peer Mentor Program
Matched graduate student mentors to first-year graduate mentees and facilitated check-ins throughout the academic year to support mentees' transition into a Ph.D. program.

UCI Psychological Science 2017
Graduate Recruitment Committee
Worked closely with recruitment coordinator Dr. Jessica Borelli to handle logistics of recruitment weekend including scheduling graduate student panels and campus tours.

CERTIFICATES

Course Design Certificate 2020
UCI Division of Teaching Excellence and Innovation. Four-week program for graduate students and university staff on student-centered course design.

Communication Certificate 2018
Activate to Captivate. Eight-week public speaking program designed for UCI graduate students and post-doctoral scholars.

ABSTRACT OF THE DISSERTATION

Salivary Inflammatory Biomarkers in the Context of Children's Cancer-Related Pain, Psychosocial Well-Being, and Caregiver Perceived Stress

By

Crystle-Joie Guerrero Agbayani

Doctor of Philosophy in Psychological Science

University of California, Irvine, 2024

Associate Professor Michelle A. Fortier, Chair

Background. Children diagnosed with cancer experience pain and myriad psychosocial symptoms throughout their disease progression and treatment which negatively impact their quality of life. Caregivers of children with cancer are also at risk for poorer psychosocial outcomes, including increased stress. Oral and systemic inflammation may be associated with psychosocial outcomes and pain in children and stress in caregivers, however this has not been studied extensively. The present study aims to 1) measure salivary inflammatory biomarker concentrations in children undergoing treatment for cancer and their primary caregivers; 2) determine how salivary inflammatory biomarkers are related to patient- and caregiver-reported pain and psychosocial outcomes; 3) investigate the impact of Pain Buddy, a mobile health (mHealth) ambulatory symptom management intervention, on salivary biomarkers of inflammation in both children and caregivers; and 4) explore factors that may be associated with salivary biomarkers of inflammation.

Method. Children ages 8 to 18 with a first-time cancer diagnosis were recruited along with one primary caregiver (N = 22 dyads) to take part in a randomized controlled trial (RCT) over 8 weeks. Children reported their daily pain and cancer-related

symptoms via a mobile application while receiving usual care. The intervention group (n = 14) received remote symptom monitoring and skills training for pain management and the attention-control group (n = 8) only reported on their pain. Child-caregiver dyads completed questionnaires at baseline, at the end of the intervention period, and at six months post-intervention. Dyads also collected saliva samples for three consecutive days at questionnaire timepoints along with reports of oral health, diet, and current medications.

Results. Intercorrelations of salivary biomarkers and relationships between salivary biomarkers, pain, and psychosocial outcomes were examined among children and caregivers. In exploratory analyses, correlations were found between cancer diagnosis category and children's T3 salivary TNF-α (r_{pb}= -.358), as well as between cancer diagnosis category and caregivers' baseline salivary IL-6 (r_{pb}= .52).

Conclusion. Overall, findings from the present study provide insight and novel methodology for studying potential immune processes (i.e., inflammation) associated with cancer among children within the first six months of a first-time diagnosis and their caregivers. The present study highlights the need for additional research with larger participant samples among children with cancer and their caregivers to determine how factors such as cancer diagnosis, medications, and self-reports of well-being may be related to salivary inflammatory biomarkers.

Introduction

The Centers for Disease Control and Prevention report that over 15,000 children and adolescents younger than 20 years are diagnosed with cancer each year in the United States alone (Centers for Disease Control and Prevention, 2022). Throughout the course of their illness and treatment, most children will experience moderate to severe pain and disruptive psychosocial issues including anxiety and depression (An et al., 2013; Baggott et al., 2009; Collins et al., 2000; Sung et al., 2011; Van Cleve et al., 2004; Varni et al., 2007). Such disruptions have been reported by children themselves and their caregivers to be related to aggregate chronic symptoms and decreased function during illness and treatment progression as early as the first six months following diagnosis (Tsai et al., 2013). The severity of these undesirable outcomes can be effectively reduced in many cases, and although these experiences are acknowledged by health care providers, pain and psychosocial symptoms are currently under-treated in children with cancer.

Limitations in fully understanding how the cancer experience impacts children's quality of life and shortfalls in the systematic assessment of symptoms contribute to inadequate supportive care (Basch, 2016; McGuire, 2004). Issues with pain and psychosocial symptoms can continue as children transition into cancer survivorship, indicating that there is no simple or straightforward solution to improving these late effects (Alberts et al., 2018; Bitsko et al., 2016; Mory et al., 2010).

Primary caregivers, including parents, are also impacted by a child's cancer diagnosis and treatment. Caregivers are responsible for much of the management of children's ever-evolving physical and psychosocial needs throughout the cancer

experience (Kuster & Merkle, 2004). Modern outpatient treatment protocols for cancer and overall healthcare system structure have contributed to higher patient survival rates as well as a shift in the management of patient care (e.g., administering medication, monitoring symptoms) from the hospital to the home (Hendershot et al., 2005; Kim et al., 2007). This substantial demand can lead to disproportionate stress related to caregiving duties for caregivers of children with cancer compared to caregivers of healthy children (G. E. Miller et al., 2002). In cases where this stress becomes unmanageable and functionally impairing for caregivers, there are negative consequences for children's coping and adjustment (Kearney et al., 2015).

For both children with cancer and their caregivers, there are implications for immunological health when considering children's unresolved pain and psychosocial symptoms and parent's unaddressed stress. It is important to examine these sequelae in the context of immune health rather than studying the prevalence of pain or psychosocial symptoms alone in either children or their caregivers. To this end, the present study will apply a chronic stress framework to investigate the relationships between children's cancer-related pain, psychosocial well-being, caregiver stress, and salivary biomarkers of inflammation.

Cancer Diagnosis and Treatment as a Chronic Stressor for Child-Caregiver Dyads

Acute stressors initiate both behavioral and physiological processes, commonly known as our "fight-or-flight" response. These processes are referred to as allostasis and include activation of the sympathetic nervous system (SNS) and hypothalamic–pituitary–adrenal (HPA) axis. This activation results in the release of primary mediators of our stress response including glucocorticoids, catecholamines, and

proteins which all interact as part of a complex network (Juster et al., 2010; Tottenham & Sheridan, 2010). As one primary mediator increases or decreases in response to acute stress, others will adjust accordingly to maintain homeostasis (Juster et al., 2010; McEwen & Wingfield, 2003). In addition to the SNS and HPA axis, the parasympathetic nervous system also plays a regulatory role in reducing inflammation and cardiovascular response, thus contributing to the negative feedback loop intended to regain physiological stability (Irwin & Cole, 2011; Juster et al., 2010).

Allostasis is evolutionarily adaptive as a short-term response – after an acute stressor is resolved or otherwise absent, physiological systems should return to baseline after activation. However, repeated or chronic activation of the stress response can lead to allostatic load in which prolonged release of glucocorticoids, catecholamines, and proteins is predictive of several detrimental health outcomes including diseases related to gastrointestinal, cardiorespiratory, metabolic, and immune systems (Chrousos, 2009; S. Cohen et al., 2007; McEwen, 2008; McEwen & Wingfield, 2003). These physiological disruptions can then negatively affect psychosocial and physical health across the life span (Juster et al., 2010; Siegel et al., 2012). This process is neither a straightforward nor linear pathway, but rather a complex cascade which may also be influenced by individual differences and behavior, social-ecological environment, and additional physiological factors (McEwen, 2008).

For the purposes of this dissertation, chronic stress is defined as the process by which any stressor leads to a prolonged allostatic response (i.e., allostatic load) that places children and their caregivers at risk for poorer psychosocial and immune health. In childhood and adolescence, chronic stressors examined frequently in

established literature have included extreme experiences such as abuse, neglect, or institutionalization, as well as prevalent stressors including exposure to poverty, food insecurity, interpersonal and community violence, parental mental illness, racism, and discrimination (Shonkoff et al., 2012).

Although cancer diagnosis in childhood is not a classic example of chronic stress used in health and developmental literature, the chronic stress framework is useful in examining how children's illness and treatment experience is related to both short- and long-term well-being. The accumulation of psychological, emotional, and social factors related to a child's cancer diagnosis and treatment culminate in what can be conceptualized as chronic stress. Analogous to individuals who are unable to effectively cope with repeated or prolonged exposure to a stressor in classic chronic stress literature, many children with cancer will have unmet psychosocial needs throughout their illness and treatment progression.

Exposure to chronic stress can lead to brain alterations and physiological disruptions among children with cancer that impact health and developmental outcomes across the lifespan (Johnson et al., 2013; Shonkoff et al., 2012). This exposure can be particularly harmful for children because vulnerability to the effects of chronic stress is clearly heightened during sensitive and critical periods in early childhood and adolescent stages of development (Fox et al., 2010). Understanding the physiological pathways which are related to psychosocial well-being in children is a crucial step toward promoting health and reducing health inequities among young cancer patients under chronic stress (Hertzman & Boyce, 2010; McEwen, 2012).

Chronic stress has been implicated for decades in caregivers' long-term well-being, including impaired immune functioning (Esterling et al., 1996; Kiecolt-Glaser et al., 1996). These trends persist when studying caregivers of children with cancer specifically: parents of children with cancer who reported depression and posttraumatic stress symptoms exhibited altered cellular profiles of blood leukocytes with a lower CD4 to CD8 ratio and increased natural killer (NK) cells compared to parents who were not experiencing the same psychosocial symptoms (Benaroya-Milshtein et al., 2014; Glover et al., 2005). While these trends are well-established, they are not often studied longitudinally, warranting a study design with more frequent observations of parents' psychosocial and immune markers for a more nuanced understanding of their health.

In conceptualizing the cancer experience as a chronic stressor, it is worthwhile to analyze psychosocial and immune outcomes related to the cancer experience in the child-caregiver dyad in tandem. In addition to stress-related shifts in immune system functioning, caregivers' quality of life can also be impacted by a child's cancer diagnosis and illness for many years (Kim & Carver, 2019). Children rely significantly on their caregivers for support in all domains during their illness progression, and a recent meta-analysis supports that distress is significantly associated between children with cancer and their parents (Bakula et al., 2019). The current literature does not, however, draw connections between physiological or immune health between children with cancer and their caregivers. Analyzing dyadic trends in psychosocial and immune functioning may further inform how to best intervene and support well-being at the family level.

The Utility of Salivary Biomarkers

Integration of biological markers (biomarkers) into research among child-caregiver dyads is one approach that may help to further investigate the pathways that link chronic stress exposure and lifelong health for families. Examples of prior research implementing biomarkers among caregivers mentioned in the previous section (Benaroya-Milshtein et al., 2014; Esterling et al., 1996; Glover et al., 2005; Kiecolt-Glaser et al., 1996) indicate that there is great potential for the inclusion of biomarkers in studies of child-caregiver dyadic functioning.

Biomarkers are objective and measurable indicators of biological processes (Condon, 2018; Granger et al., 2007, 2012). Researchers use biomarkers to evaluate normal biological processes and biological responses to interventions, or as surrogates for clinical end-points to assist with diagnosis and monitoring of disease (FDA-NIH Biomarker Working Group, 2016).

In pediatric research, salivary biomarkers are especially promising as a minimally-invasive measurement of underlying pathology and processes associated with the chronic stress of cancer in childhood and adolescence (Condon, 2018; Granger et al., 2012; Riis et al., 2015). Another benefit of salivary sampling is that it can be conducted with no specialized expertise, such that caregivers may collect samples from themselves and assist children in sample collections. Saliva sampling can be completed in ecologically valid settings such as a family's home or in the hospital during an inpatient stay.

While many biomarkers measured in serum can also be measured in saliva, there is a dearth of research which implements salivary sampling methods among

children with cancer. Many examples of research which include salivary biomarkers are studies of adults, including adult survivors of cancer. Based on the current literature, salivary biomarkers of inflammation (henceforth, salivary biomarkers) are most relevant in investigating child and caregiver immune health and immune responses.

Oral and Systemic Inflammatory Biomarkers

For children diagnosed with cancer, the disease itself poses a risk for dysregulated immune response, specifically chronic systemic inflammation (Powell et al., 2013; Zhang et al., 2020). For caregivers of individuals with cancer, chronic psychological stress is associated with altered (i.e., increased) inflammatory biomarkers measured in serum (G. E. Miller et al., 2014; Park et al., 2018). The inflammatory response is a key component of our innate immunity triggered to destroy invasive bodies and to repair damaged tissue. Although this defense mechanism is beneficial and adaptive in general, a poorly regulated inflammatory response can result in an array of negative health outcomes, especially in cancer patients as inflammation can contribute directly to disease progression (Morgenstern & Anderson, 2012).

A number of factors increase the likelihood that cancer patients will experience heightened activation of the innate immune response, including surgery, chemotherapy, and radiation, which are all associated with significant damage and destruction of tissue (A. H. Miller et al., 2008). Evidence also suggests that children with acute lymphoblastic leukemia (ALL), the most common type of childhood cancer,

exhibit an immune profile that predisposes them to a dysregulated inflammatory response from birth (Chang et al., 2011).

Pro-Inflammatory Cytokines as Salivary Biomarkers of Oral Inflammation. Cytokines are a diverse group of molecules that serve as the primary messengers of the immune system (McEwen, 2003). Cytokines are produced locally by immune cells as well as by other organs including the brain and liver (McEwen, 2003). These molecules have multiple mechanisms of action and are influenced by stress-related activity in the SNS and HPA axis. Cytokines also act as part of a negative feedback loop between the immune and central nervous systems: Release of norepinephrine in response to acute stress results in an increase in inflammatory cytokines, which in turn stimulates the HPA axis to release cortisol and inhibit inflammatory cytokine production. However, dysregulation of this feedback loop due to chronic stress over time can result in chronic inflammation and insufficient immune functioning (Riis et al., 2015).

Pro-inflammatory cytokines initiate inflammation and are activated in response to stressors or pathogens. They include interleukins, tumor necrosis factors, fibroblast growth factors, and interferons. Inflammatory cytokines can be detected in serum or saliva, and the development of multiplex immunoassays has allowed for rapid detection of multiple cytokines in a single sample (Vignali, 2000). To date, most research in children and caregivers alike have focused on analysis of single cytokines in either serum or saliva, primarily pro-inflammatory cytokines such as interleukin 6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor alpha (TNF-α). Serum levels of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α are highly correlated with

each other in samples of both healthy children and healthy adults due to the shared mechanisms by which they initiate and regulate inflammation (Irwin & Cole, 2011).

Salivary cytokine levels represent a combination of serum cytokines which enter saliva from general circulation, cytokines from lymphoid cells, and cytokines from the local oral immune environment (Riis, Byrne, et al., 2020). Associations between cytokine levels in serum and saliva can thus differ between biomarkers. As salivary cytokine levels have been associated with indicators of oral inflammation such as loose teeth, bleeding gums, or untreated cavities, it is important to note that salivary cytokine levels largely reflect oral immune activity rather than systemic immune activity (Riis et al., 2015).

Acute Phase Protein CRP as a Salivary Biomarker of Systemic Inflammation. C-reactive protein (CRP) is an acute phase protein widely considered to be an important nonspecific marker of inflammation when measured in serum (Pepys & Hirschfield, 2003; Sproston & Ashworth, 2018). CRP synthesis, which primarily occurs in the liver, is stimulated by inflammatory cytokines (primarily IL-6) and can be altered by glucocorticoid levels (Pepys & Hirschfield, 2003; Sproston & Ashworth, 2018).

Salivary CRP has been found to have medium-to-strong correlations with serum CRP and is commonly utilized in research as a salivary biomarker of systemic inflammation (Ouellet-Morin et al., 2011; Out et al., 2013; Riis, Byrne, et al., 2020).

Inflammation and Psychosocial Outcomes Among Children with Cancer

There are studies which provide evidence of a link between inflammatory biomarkers and psychosocial symptoms among adults with cancer, however this association is under-studied among children with cancer.

Adult female survivors of childhood ALL with higher levels of serum inflammatory biomarkers IL-6 and IL-1β have been observed to demonstrate poorer executive function and behavioral symptoms including inattention and aggression as compared to survivors with typical levels of these biomarkers (Cheung et al., 2017). There is also evidence of high serum inflammatory biomarker levels serving as a risk factor for depressive symptomology in adult colorectal cancer patients – the interaction of proinflammatory cytokines with central nervous system pathways which regulate behavior may explain this connection (Archer et al., 2012; A. H. Miller et al., 2008).

Research to date has not determined if similar associations between inflammatory biomarkers and psychosocial outcomes exist among children and adolescents in general, let alone among children and adolescents with cancer. Measuring these biomarkers in saliva provides a minimally invasive option for studying trends.

Inflammation and Pain Among Children with Cancer

High levels of inflammation, particularly chronic systemic inflammation which lasts for months or even years, has been linked to fatigue, pain, and poorer prognosis in adult cancer patients (Aggarwal & Gehlot, 2009; Archer et al., 2012; Bower, 2007; Bower et al., 2009; Munn, 2017; Pierce et al., 2009). Furthermore, evidence

highlights a relationship between chronic systemic inflammation and poorer physical functioning for adult cancer survivors, which includes the endorsement of acute or chronic pain (Bower, 2007; Collado-Hidalgo et al., 2006; Crosswell et al., 2014; Orre et al., 2011). These links have been drawn mainly using serum and plasma levels of biomarkers such as CRP, rather than salivary biomarkers. There is an overall dearth of literature examining pain in relation to biomarkers of inflammation among populations diagnosed with cancer, let alone children and adolescents specifically.

Utilizing salivary inflammatory biomarkers rather than serum in pain research among children with cancer provides the advantage of a minimally invasive and, in most cases, painless method of biospecimen collection.

Potential Impacts of Psychosocial Interventions for Children on Child-Caregiver Dyad Outcomes

It is important to recognize that children with cancer do not experience pain, psychosocial symptoms, and inflammation as independent events. Given that more frequent and severe endorsement of pain and psychosocial symptoms among adults has been associated with elevated serum and salivary inflammatory biomarkers, it may be worth investigating whether this trend is similar for children.

Recent studies have examined the efficacy of stress-reduction interventions on salivary markers of inflammation among adults. One study of young women with depressive symptomatology found that participation in a 4-week mindfulness-based stress reduction intervention was associated with reduced levels of salivary IL-6 and salivary TNF-α, with changes in salivary IL-6 sustained at 3-months post-intervention (Walsh et al., 2016). Another study of adult breast cancer survivors similarly found

that participation in a 6-week mindfulness-based stress reduction intervention was associated with a significant reduction in salivary IL-6 (Lengacher et al., 2019). However, whether similar salivary biomarker trends would be observed in children with cancer who participate in a stress-reduction intervention are so far unclear, as this methodology has not been applied to the pediatric population.

It is also not entirely known how changes in children's outcomes – pain, psychosocial, or salivary inflammatory biomarkers – as the result of a psychosocial intervention may be related to caregiver's well-being, including stress and salivary biomarkers of inflammation. A positive relationship has been observed between levels of child and caregiver distress in pediatric cancer studies, and preliminary evidence suggests that successful supportive care interventions for children can ameliorate distress among both children and their caregivers (i.e., through the development and practice of problem-solving or coping skills) (Robb & Hanson-Abromeit, 2014). One study has explored reciprocal effects of child-caregiver experiences of adversity (including traumatic events) and salivary inflammation in the dyad, but this study did not investigate the impact of a psychosocial intervention on these outcomes (Huffhines et al., 2021).

The present study: Innovation and Specific Aims

Research among adult cancer survivors indicates that psychosocial interventions have the potential to affect long-term positive change in immune functioning (Antoni, 2013), however no research to our knowledge has specifically measured salivary biomarkers of inflammation among children with cancer enrolled in a psychosocial intervention. Furthermore, no research has been conducted in either adults or children with cancer to assess the association between use of a mobile

health (mHealth) symptom management intervention and salivary biomarkers of inflammation.

The following gaps exist regarding salivary inflammatory biomarkers in the pediatric oncology literature: 1) Measuring levels of salivary inflammatory biomarkers among children with cancer participating in an mHealth intervention and their caregivers and 2) Characterizing the relationship between salivary biomarkers and both cancer pain and negative psychosocial symptoms during treatment among children and their caregivers.

Thus, the present study implements the measurement of salivary inflammatory biomarkers into an ongoing randomized control trial (RCT) of a web-based pain and symptom management intervention for children with cancer: Pain Buddy. Pain Buddy is an interactive application designed to aid in pain management for children undergoing cancer treatment (Fortier et al., 2016; Hunter et al., 2020). Key aspects of Pain Buddy include daily pain and symptom diaries completed by children, remote monitoring of symptoms by uploading self-reported patient data via Internet to a cloud server, cognitive and behavioral skills training, interactive three-dimensional avatars that guide children through the program, and an incentive system to motivate engagement. In the comfort of their own home, children can learn evidence-based pain management and psychosocial coping skills by utilizing the application.

The ongoing Pain Buddy RCT provides a timely opportunity to collect and analyze salivary inflammatory biomarkers with respect to intervention components designed to reduce children's pain and psychosocial symptoms including depression

and anxiety. The present study also offers a unique perspective by observing caregivers' stress and inflammatory biomarkers in tandem with children.

In addition to the novel research direction, the measurement of inflammatory biomarkers via saliva sampling in the present study is a novel methodological approach for this sample. The present study aims to accomplish the following:

Aim 1: Measure salivary inflammatory biomarker concentrations in children undergoing treatment for cancer and their primary caregivers.

Hypothesis 1: Salivary inflammatory biomarkers will be positively intercorrelated at baseline among children undergoing treatment for cancer.

Hypothesis 2: Salivary inflammatory biomarkers will also be positively intercorrelated among primary caregivers at baseline.

First, it is important to assess the presence and concentrations of salivary inflammatory biomarkers in children with cancer and their caregivers. Children with cancer undergo painful medical procedures throughout their treatment including chemotherapy, radiation therapy, blood draws, port implants, lumbar punctures, and surgery. Utilizing salivary biomarkers for research among this population of children provides a minimally invasive and minimally painful option for examining immune processes. For caregivers of these children, collecting and storing saliva samples for research requires minimal time and effort – sample collection can be done in the home, for example, rather than coordinating a visit to a clinic or hospital for a blood draw.

Furthermore, highly intercorrelated concentrations of salivary biomarkers of oral inflammation (e.g., IL-1β, IL-6, TNF-α) among children and their caregivers might suggest that even fewer biomarkers may be sufficient in indexing oral inflammation for this group. In follow-up studies, smaller sample volumes can be collected to reduce participant burden.

There is neither an established normal range nor expected baseline concentration for salivary inflammatory biomarkers in chronically ill children or children with cancer more specifically. In healthy children, inflammatory biomarkers measured in saliva are positively intercorrelated, reflective of shared regulatory mechanisms (Riis et al., 2014, 2015).

Aim 2: Determine how salivary inflammatory biomarker concentrations are related to pain and patient- and caregiver-reported psychosocial outcomes.

Hypothesis 3: Higher concentrations of salivary inflammatory biomarkers will be positively associated with higher reported pain among children with cancer.

Hypothesis 4: Higher concentrations of salivary inflammatory biomarkers will be positively associated with poorer psychosocial outcomes among children with cancer, specifically more depression and anxiety symptoms.

Hypothesis 5: Caregivers who report higher perceived stress will have higher levels of salivary biomarkers of inflammation.

Associations between salivary inflammatory biomarkers and pain and psychosocial outcomes among children with cancer have not been investigated to date. Research in adult cancer survivors suggests a positive association between

systemic inflammation and cancer-related symptoms including fatigue – this is consistent with literature demonstrating an increase in sickness behaviors when the feedback loop between the immune system and central nervous system is dysregulated. Similar patterns, if found in children with cancer, can inform strategies for improving immune, physical, and psychosocial health.

For caregivers, there is a strong basis for the stress-inflammation link, specifically among caregivers of children with cancer, though studies to date have often used serum inflammatory biomarkers rather than saliva (Agbayani et al., 2022).

Aim 3: Investigate the impact of the Pain Buddy intervention on salivary biomarkers of inflammation.

Hypothesis 6: Children with cancer who learn and practice cognitive and behavioral coping skills through the Pain Buddy application (i.e., the intervention group) will have lower average salivary inflammatory biomarker concentrations at T2 and T3 than children who do not receive the same coping skills training (i.e., the attention-control group).

Hypothesis 7: Caregivers whose children are in the intervention group will also have lower average salivary inflammatory biomarker concentrations at T2 and T3 as compared to caregivers whose children are in the attention-control group.

As mentioned in the literature review, interventions designed to improve psychosocial outcomes in adults have shown promise in reducing levels of salivary inflammatory biomarkers, suggesting a potential non-invasive and non-pharmacological avenue for improving immune functioning. However, no research has been conducted to measure the levels of salivary inflammatory biomarkers

among children with cancer participating in an mHealth intervention; nor have there been studies to investigate how such an intervention designed for children might impact caregiver outcomes. The present study will utilize an established mHealth intervention to begin bridging these gaps in the literature.

Aim 4: Explore participant factors that may be associated with salivary biomarkers of inflammation.

Several factors which may also be associated with salivary biomarkers of inflammation will be explored. Factors to be explored include type of cancer diagnosis and primary language spoken at home.

Method

Participants

The present study was implemented as part an ongoing large-scale RCT to determine if Pain Buddy, a multicomponent mHealth intervention for cancer-related pain and symptom management, is more effective than an attention-control in reducing pain severity among children ages 8 to 18 years old undergoing outpatient cancer treatment (see Hunter et al., 2020 for a report of the preliminary efficacy of Pain Buddy). All participants consisted of child-caregiver dyads recruited from Children's Hospital of Orange County (CHOC) in Orange, California.

A total of 22 child-caregiver dyads who were recruited to the ongoing Pain Buddy RCT between June 2021 and January 2023 participated in the present study. Using the Pain Buddy RCT inclusion criteria, children recruited for the present study were between the ages of 8 and 18 years and were within 16 weeks of a first-time cancer diagnosis. As such, children were currently undergoing outpatient treatment

for cancer at the time of enrollment. Children who can speak, read, and write in English and whose primary caregiver can speak, read, and write in either English or Spanish were eligible for enrollment. Child-caregiver dyads needed internet access at home to use the Pain Buddy intervention on their mobile devices. Children were excluded if they had a cognitive or developmental delay that would prevent them from using Pain Buddy program. Children diagnosed with acute promyelocytic leukemia (APL) were also excluded as the treatment protocols for APL are largely inpatient, precluding use of the Pain Buddy intervention.

Procedures

The CHOC Institutional Review Board approved the present study protocol. Caregivers provided electronic informed consent and HIPAA authorization for both the ongoing Pain Buddy RCT and for the present study via Research Electronic Data Capture (REDCap) – this gave families the option to participate in the Pain Buddy RCT without participating in the present study, which required at-home saliva sample collections. Participant dyads were randomized to the Pain Buddy intervention or attention-control group using a blocked randomization scheme stratified by age group. Participants were randomized at a 1:1 ratio with equal numbers randomized to the intervention and attention-control groups.

Children in the Pain Buddy intervention group continued to receive standard of care for cancer- and treatment-related pain and symptoms, which may include medications, medical visits, and physical interventions. Participants in this condition were taught cognitive and behavioral coping skills (i.e., deep breathing, imagery, relaxation) to deal with pain and symptoms. The skills were taught remotely through the Pain Buddy application. Pain and symptom information, reported daily by children

in the Pain Buddy application, was sent to a designated health care provider on the oncology treatment team. This health care provider contacted participants when certain symptom thresholds were triggered and instructed participants on best ways to control pain and symptoms.

Children in the Pain Buddy attention-control group also continued to receive standard of care for cancer- and treatment-related pain and symptoms. Participants in this condition completed the same data collection through Pain Buddy as intervention group participants but did not receive skills training through the application or engage in the same live symptom monitoring with tailored response from a designated health care provider. However, participants who were part of the attention-control group were still able to contact the CHOC Hyundai Cancer Institute or physician on call to discuss any matters of pain or illness in a timely fashion, as is standard of care.

Throughout the study period, pain and symptom assessments were administered through the Pain Buddy application twice daily for all participants. Child-caregiver dyads also received instructions and materials to collect saliva at home for 3 consecutive days at key study timepoints: baseline, 8 weeks (T2; the end of the intervention period), and at a 6-month follow-up (T3). Saliva collection materials included a soft-sided cooler bag containing labelled cryogenic vials for both child and caregiver samples, optional saliva collection aids (sterile plastic straws), and a reusable ice pack.

Participant dyads completed study questionnaires assessing child and caregiver psychosocial outcomes at baseline, T2, and T3 via REDCap. A survey

collecting demographic information was completed by caregivers via REDCap at baseline only, and any relevant information for children that could be obtained from medical records (e.g., body mass index, medication information) was abstracted by the research team throughout the study.

Measures

The measurement strategy for the present study is detailed in Table 1. All study measures except for the oral health and medication use questionnaire and saliva samples/salivary biomarkers of inflammation are part of the ongoing Pain Buddy RCT.

Table 1. *Study measurement strategy.*

Outcome/Construct:	**Measure/Parameter:**	**Assessment Points**
Child		
Oral health	Oral health questionnaire	Baseline, T2 and T3
Inflammation	Salivary CRP, IL-1β, IL-6, and TNF-α	Baseline, T2 and T3
Anxiety and depression	Revised Child Anxiety and Depression Scale (RCADS)	Baseline, T2 and T3
Pain	Pediatric Quality of Life-Cancer Module (PedsQL Cancer)	Baseline, T2 and T3
Caregiver		
Demographic data	Demographic questionnaire	Baseline
Oral health	Oral health questionnaire	Baseline, T2 and T3
Inflammation	Salivary CRP, IL-1β, IL-6, and TNF-α	Baseline, T2 and T3
Child's pain	MSAS	Baseline, T2
Child's anxiety and depression	RCADS	Baseline, T2 and T3
Child's pain	PedsQL	Baseline, T2 and T3
Perceived stress	Perceived Stress Scale (PSS)	Baseline, T2 and T3
Research Team		

Demographic data, child body mass index	Medical record abstraction	Baseline, T2 and T3

Demographic data (caregiver report and research team medical abstraction). Baseline demographic questionnaires completed by caregivers collected information about sex, race/ethnicity, highest level of education, age, and income for participants. Children's body mass index (BMI) percentile was abstracted from electronic medical records of an appointment within ten days of each saliva collection date unless children did not have an appointment, or caregivers did not report the exact sampling date.

Memorial Symptom Assessment Scale (MSAS) 7-12 (caregiver proxy report). This 8-symptom instrument evaluates whether the child experienced a particular symptom since the prior assessment (Collins et al., 2000, 2002). Caregivers completed a proxy version of the MSAS at baseline and T2 using REDCap. If the caregiver said "yes" to their child having experienced any of the symptoms, then the caregiver was prompted to describe the frequency (i.e., a very short time, a medium amount, or a lot), severity (i.e., a little, a medium amount, or a lot), and distress (i.e., not at all, a little, a medium amount, or very much) experienced due to the symptom. For the purposes of this dissertation, only reports from the "pain" item from the caregiver proxy version will be used in analyses to quantify caregivers' report of children's pain.

Memorial Symptom Assessment Scale (MSAS) 10-18 (caregiver proxy report). This 30-symptom instrument evaluates whether the child experienced a particular symptom (Collins et al., 2000, 2002). Caregivers completed a proxy version of the MSAS 10-18 at baseline and T2 using REDCap. If the caregiver said

"yes" to their child having experienced any of the symptoms, then the caregiver was prompted to describe the frequency, severity, and distress experienced due to the symptom. The MSAS 10-18 is comprised of 3 subscales: psychological (i.e., "difficulty concentrating or paying attention"), physical (i.e., "pain") and general distress index (GDI; i.e., "how much did [the symptom] bother your child?"). For the purposes of this dissertation, only reports from the "pain" item from the caregiver proxy version will be used in analyses to quantify caregivers' report of children's pain.

The Revised Child Anxiety and Depression Scale (RCADS) (child self-report and caregiver proxy report). RCADS contains a 10-item major depressive disorder subscale and a 5-item generalized anxiety disorder subscale (Chorpita et al., 2000). There are both child self-report and caregiver-report versions. *t*-scores are calculated based on child's gender and grade in school. Completed at baseline, T2, and T3 using REDCap.

Pediatric Quality of Life-Cancer Module (PedsQL Cancer) (child self-report and caregiver proxy report). The PedsQL Cancer Module is administered along with the generic core scale in order to get a better understanding of a child's health related quality of life (HRQOL) due to cancer (Varni et al., 2002). This 27-item multidimensional scale encompasses eight categories: pain & hurt, nausea, procedural anxiety, treatment anxiety, cognitive problems, perceived physical appearance and communication. This measure is scored on a 5-point Likert scale with 0 being "never" to 4 being "almost always" on items such as "I worry that my cancer will come back or relapse." and "It is hard for me to tell the doctors and nurses how I feel." Higher scores on the scale suggest better HRQOL related to cancer. For the present study, the two-item pain composite score will be utilized. There are both child

self-report and caregiver-report versions completed at baseline, T2, and T3 using REDCap.

Perceived Stress Scale (PSS) (caregiver self-report). The PSS is a widely-used 14-item self-report measure of perceived stress (S. Cohen et al., 1983). Caregivers were asked to rate statements such as "In the past month, how often have you been upset because of something that happened unexpectedly?" and "In the past month how often have you felt that things were going your way?" Caregivers rated the items on a 5-point Likert-type scale with higher scores reflecting greater perceived stress. Seven items are reverse-scored and items are summed to obtain the final score. Completed at baseline, T2, and T3 using REDCap.

Collection, Storage, and Assay of Saliva

Salivary CRP, IL-1β, IL-6, and TNF-α can be reliably measured using highly sensitive enzyme immunoassays utilizing "whole saliva" collected with an unstimulated passive drool method (Riis, Ahmadi, et al., 2020; Slavish et al., 2015). As salivary cytokines are strongly influenced by the oral immune environment, it is important to statistically control for poor oral health in all analyses (Riis et al., 2014, 2015). Information regarding oral health was collected from child-caregiver dyads, including the presence of any open cuts or sores in the mouth, untreated cavities, or sensitive/bleeding gums. Saliva samples were also assayed for blood leakage into saliva (transferrin) due to the potential of blood contamination influencing accurate detection of salivary inflammatory biomarker levels (Riis, Ahmadi, et al., 2020; Riis, Byrne, et al., 2020).

Another consideration for sample quality is the standardization of sample collection times; participants were instructed to collect samples at the same time of day for each sampling event, ideally in the afternoon, to control for independent diurnal rhythms observed in salivary cytokines (Chiappelli et al., 2006). While sampling times varied between participant dyads to accommodate individual or family schedules, the research team encouraged dyads to keep sampling times consistent as possible across baseline, T2, and T3 samples (e.g., kept at 12:00PM for all three timepoints, or kept at 5:00PM for all three timepoints).

Children with cancer are already predisposed to factors which increase the likelihood that they will experience heightened activation of the innate immune response, and thus possible elevations in inflammatory biomarkers (i.e., immune profile, procedures including surgery, chemotherapy, radiation; Miller et al., 2008, Chang et al., 2011). In order to minimize the influence of cancer treatment-related factors on the measurement of basal salivary biomarker levels for children recruited, saliva samples were scheduled (when possible) on days when children were not having labs completed, receiving chemotherapy, or otherwise having their port device accessed as this may contribute to heightened inflammation (Liaw et al., 2008). To this end, analyses for the present study take into consideration patient medical records and comprehensive notes regarding medication use including asthma medication, corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDS), and other medications which may influence detectable biomarker levels.

Participants were instructed to avoid eating or brushing their teeth for one hour prior to each sample collection. Participants were to rinse their mouths thoroughly with water before a sample was collected, and they washed their hands

for 20 seconds immediately prior to collecting the sample. Anyone assisting with the collection (e.g., other family members) also practiced the same hand hygiene. Participants were instructed to passively drool into a collection vial either with or without a collection aid for up to two minutes or until 2.0 milliliters (mL) of fluid were collected. Samples were always immediately capped and sealed before being placed in a secondary container such as a sealed plastic bag. Participants and anyone assisting with sample collection once again washed hands for at least 20 seconds after collecting the saliva sample. Participants immediately placed samples into their freezer at home until bringing them to their next appointment at CHOC, where the research team coordinated pick-up and storage in the on-site -20°C freezer. Saliva samples were transported from CHOC to the UCI Institute for Interdisciplinary Salivary Bioscience Research (IISBR) for assay.

Salivary CRP and salivary cytokines (IL-1β, IL-6, and TNF-α) were assayed in duplicate using a commercially available, V-plex pro-inflammatory cytokine panel using the Meso Scale Discovery (MSD)® Multi-Spot Assay system (MSD® www.mesocale.com). Assays were run following the manufacturer's recommended protocol without modification. Detection antibodies are coupled to SULFOTAG™ labels that emit light when electrochemically stimulated via carbon-coated electrodes in the bottom each microwell. Salivary CRP and salivary cytokine concentrations (pg/mL) were determined with MSD Discovery Workbench Software (v. 4.0) using curve fit models (4-PL with a weighting function option of $1/y^2$).

For salivary CRP, samples were diluted 5-fold in MSD® Assay Diluent 101 prior to adding to the coated plate with a test volume of 5 µL. The mean intra- and inter-assay coefficients of variation (CVs) for salivary CRP were within the acceptable range

at 3.6% and 5.1%, respectively. The assay sensitivity range for salivary CRP was 9.40 pg/mL – 38600 pg/mL.

For salivary cytokines, samples were diluted 2-fold in MSD® Assay Diluent 2 prior to adding to the coated plate with a test volume of 25 µL. Intra- and inter-assay CVs and assay sensitivity ranges were as follows: salivary IL-1β (2%, 6%, 0.31 pg/mL – 1288 pg/mL), salivary IL-6 (6.1%, 6.7%, 0.35 pg/mL – 1440 pg/mL), and salivary TNF-α (13.1%, 8.1%, 0.18 pg/mL – 724 pg/mL). All CVs were within the acceptable ranges except for the intra-assay CV for salivary TNF-α.

Compensation

All participant dyads received a $50 gift card at each time point for completing study questionnaires (baseline, T2, T3). Dyads were compensated with an additional $5 gift card for each completed saliva sample (three samples assigned at each study timepoint).

In addition to baseline, T2, and T3 compensation, children in both the control and intervention groups were compensated $5 per week in the form of a gift card for completing at least 10 out of 14 daily diaries per week in the Pain Buddy app. Participants earned up to $40 in the form of a gift card for completing at least 10 diaries per week for the 8-week diary portion of the study. Participants in the intervention condition earned an additional $25 gift card for completing at least 40 CBT exercises throughout the 8-week study period (5 exercises per week).

Dyads in the attention-control group could be compensated up to $280 in the form of a gift card for completion of all the parts of the study: Diary, baseline, T2 and T3 measures, and 18 saliva samples between child and caregiver. Participants in the

intervention group could be compensated up to $305 in the form of a gift card for completion of all parts of the study: Diary, CBT exercises (intervention), baseline, T2 and T3 measures, and 18 saliva samples between child and caregiver.

Data Analytic Plan

Preliminary data processing and sensitivity analyses

Prior to main analyses, raw concentrations of salivary CRP and salivary cytokines were examined. The present study considered 259 individual saliva samples donated by participant dyads: 127 from children and 132 from caregivers. Saliva samples with a transferrin concentration of 1 mg/dL or higher were excluded from final analyses ($n_{transferrin}$ = 65, 25% of the total samples assayed, 38 of which were samples from children). Salivary CRP and salivary cytokine concentrations were also examined for values which fell above or below the MSD assay calibration curve range or fell outside of the limits of quantification for the assay. The values of biomarker concentrations which were detectable but fell below the assay calibration curve range were replaced by half the lowest level of sensitivity for the relevant biomarker (pg/mL). For salivary CRP, one sample's value was replaced with 4.7 pg/mL; for salivary IL-6, two samples' values were replaced with 0.175 pg/mL; and for salivary TNF-α, six samples' values were replaced with 0.09 pg/mL. Additional samples which fell outside of the assay limits of quantification were excluded from final analyses (n_{CRP} = 8; $n_{IL\text{-}1\beta}$ = 10; $n_{IL\text{-}6}$ = 11; $n_{TNF\text{-}\alpha}$ = 6).

At each of the three study timepoints, participant dyads were asked to collect saliva samples for three consecutive days, thus a summary metric (composite score) for salivary CRP and salivary cytokines at each timepoint was created for use in

statistical models with pain and psychosocial variables. Salivary CRP composite scores were derived for children and caregivers at each timepoint by averaging raw salivary CRP values from participants' three subsequent days of sampling. Salivary CRP composite scores showed generally moderate to high levels of internal consistency (Cronbach's *α* range 0.49 – 0.94) except for caregivers' T2 salivary CRP composite score with a Cronbach's *α* of 0.10 indicating low reliability. Salivary cytokine composite scores were derived by averaging the *z*-scores of salivary IL-1β, IL-6, and TNF-α levels from participants' three subsequent days of sampling. Each salivary cytokine composite score showed moderate to high levels of internal consistency (Cronbach's *α* range 0.62 – 0.92).

Preliminary analyses were conducted to examine study group differences in participant demographic characteristics. Independent samples *t*-tests were used to compare differences in normally distributed continuous variables (age, income) and Mann-Whitney *U* tests were used to compare differences in BMI, a skewed continuous variable. Chi-squared analyses were used to compare differences in categorical variables (sex, race/ethnicity, primary language spoken at home, caregiver education level, and child's diagnosis).

Main analyses

Statistical analyses were conducted in IBM SPSS Statistics Version 29.0.0.

Aim 1: Measure salivary inflammatory biomarker concentrations in children undergoing treatment for cancer and their primary caregivers.

Hypothesis 1: Salivary inflammatory biomarkers will be positively intercorrelated at baseline among children undergoing treatment for cancer;

Hypothesis 2: Salivary inflammatory biomarkers will also be positively intercorrelated among primary caregivers at baseline. Intercorrelations among individual salivary biomarkers at baseline were examined using Spearman's correlations.

Aim 2: Determine how salivary inflammatory biomarker concentrations are related to pain and patient- and caregiver-reported psychosocial outcomes.

Hypothesis 3: Higher concentrations of salivary inflammatory biomarkers will be positively associated with higher reported pain among children with cancer.; Hypothesis 4: Higher concentrations of salivary inflammatory biomarkers will be positively associated with poorer psychosocial outcomes among children with cancer, specifically more depression and anxiety symptoms. Separate linear regression models were run with each salivary biomarker (CRP, cytokine) at T2 or T3 as an outcome predicted by each pain and psychosocial symptom at the previous timepoint. For example, analyte concentrations at T2 were predicted by pain and psychosocial symptoms reported at baseline. Pain was measured via caregiver proxy on the MSAS, with higher scores denoting higher pain experienced by the child for whom the report was for. Pain was also measured via a two-item measure comprising the "pain and hurt" domain within the PedsQL Cancer Module, which had a self-report version for children and caregiver proxy. Depression and anxiety were measured using the RCADS, which had a self-report version for children and a caregiver proxy.

Hypothesis 5: Caregivers who report higher perceived stress will have higher levels of salivary biomarkers of inflammation. Linear regression models utilized each analyte at T2 and T3 as an outcome predicted by caregiver perceived stress at the previous timepoint as measured on the PSS. A manual backward stepwise deletion approach was applied to the selection of covariates to assess whether any conceptually relevant covariates (e.g., BMI, age, annual family income) significantly altered findings.

Aim 3: Investigate the impact of the Pain Buddy intervention on salivary biomarkers of inflammation.

Hypothesis 6: Children with cancer who learn and practice cognitive and behavioral coping skills through the Pain Buddy application (i.e., the intervention group) will have lower average salivary inflammatory biomarker concentrations at T2 and T3 than children who do not receive the same coping skills training (i.e., the attention-control group).; Hypothesis 7: Caregivers whose children are in the intervention group will also have lower average salivary inflammatory biomarker concentrations at T2 and T3 as compared to caregivers whose children are in the attention-control group. Independent-samples *t*-tests were used to compare differences between study groups on salivary CRP and salivary cytokine composite scores at T2 and T3 for children and caregivers.

Aim 4: Explore participant factors that may be associated with salivary biomarkers of inflammation.

As the present study is one of the first in its inclusion of caregiver-child dyads (versus individual participants) and measurement of salivary inflammatory biomarkers, the expected relationships among salivary biomarkers and participant outcomes is unclear. Several factors which may be associated with salivary biomarkers of inflammation beyond the above hypotheses will be explored using correlations. Factors to be explored include type of cancer diagnosis and primary language spoken at home.

Results

Descriptives and preliminary analyses

Children recruited for the present study were 8 to 17 years old (M = 11.59, SD = 2.92) and most children were male (59%). Caregivers recruited for the present study were 30 to 57 years old (M = 42.43, SD = 7.92) and most caregivers in the study were mothers (59%). In addition to thirteen mothers and thirteen fathers, the present sample included one dyad with an aunt as primary caregiver and one dyad with a grandmother as primary caregiver.

Average household income for families recruited to the present study was $75,682 ($SD$ = 49032.75). Most dyads recruited for the present study identified as Hispanic, Latino/a/x, or Chicano/a (over 72% of the study sample, or sixteen dyads). For the eight dyads who indicated that Spanish was their primary language spoken at home, all recruitment and study communication was done in Spanish. All study materials (questionnaires, saliva sampling instructions) were also available in Spanish.

There were no statistically significant differences observed in demographic characteristics between individuals assigned to the Pain Buddy intervention group and individuals assigned to the attention-control group, as seen in Table 2 below.

Table 2. Study sample (N=22 dyads) characteristics of Pain Buddy intervention group participants and attention-control group participants.

Demographic Characteristic	Pain Buddy Intervention Group, *n*=14 dyads	Attention-Control Group, *n*=8 dyads	$T/\chi^2/u$	*p*	Missing *n* (%)
Child sex[a]			.51	.66	0 (0)
Male	9 (40.91)	4 (18.18)			
Female	5 (22.73)	4 (18.18)			
Caregiver sex[a]			.14	.19	0 (0)
Male	6 (27.27)	1 (4.55)			
Female	8 (36.36)	7 (31.82)			
Dyad race/ethnicity[a]			4.04	.26	1 (4.55)
Hispanic, Latino/a/x, Chicano/a	8 (36.36)	8 (36.36)			
Non-Hispanic White	2 (9.09)	0 (0)			
Asian	2 (9.09)	0 (0)			
Pacific Islander	1 (4.55)	0 (0)			
Caregiver education[a]			6.16	.41	1 (4.55)
Less than high school degree	2 (9.09)	4 (18.18)			
High school degree	3 (13.64)	1 (4.55)			
Some college	4 (18.18)	2 (9.09)			
College degree	3 (13.64)	1 (4.55)			
Professional degree	1 (4.55)	0 (0)			

Dyad primary language spoken at home[a]			3.71	.05	0 (0)
English	11 (50)	3 (13.64)			
Spanish	3 (13.64)	5 (22.73)			
Child diagnosis[a]			3.27	.35	0 (0)
Leukemias	9 (40.91)	3 (13.64)			
Lymphomas	1 (4.55)	2 (17)			
Sarcomas	3 (13.64)	1 (4.55)			
CNS tumors	1 (4.55)	2 (9.09)			
Child age[b]	14.64 (8.91)	10.63 (2.56)	-1.24	.23	0 (0)
Caregiver age[b]	42 (7.46)	43.13 (9.09)	.31	.76	1 (4)
Annual family income (USD)[b]	88,017 (51,756)	46,080 (26,431)	-1.70	.11	5 (22.73)
Child baseline BMI percentile[c]	71.14 (10.28 – 94.64)	69.34 (53.12 – 94.15)	40	.90	3 (13.64)

[a]*n* (%); [b]*M* (*SD*); [c]Median (interquartile range)
Note: Some demographic data were missing and reports above reflect the proportion of available data (i.e., valid percent). Any race/ethnicity categories that received zero participant responses are not included in the above table. For caregiver education, the "Less than high school degree" category consists of three separate choices from the demographic questionnaire, collapsed for ease of presentation in this table: Ten to eleven years of school (part high school); Seven to nine years of school; Less than seven years of school.
Abbreviations: CNS = central nervous system; SD = standard deviation; BMI = body mass index.

Raw salivary biomarker concentrations were examined at each study timepoint for highly influential values which were not already excluded in preliminary processing steps. For example, any individual saliva samples with a concentration greater than four standard deviations above the sample mean would be flagged for exclusion – no such sample concentrations were observed in the present study. Table 3 summarizes salivary biomarker concentrations among the 22 child-caregiver dyads recruited.

Table 3. Summary of raw salivary biomarker concentrations (pg/mL) at each study timepoint among participant dyads (N=22 dyads) assigned to the Pain Buddy intervention group and attention-control group.

Baseline salivary biomarkers	Pain Buddy Intervention Group, *n*=14 dyads	Attention-Control Group, *n*=8 dyads	Missing *n* (%)
Child salivary CRP	2215.25 (125.29-6646.44)	62.25 (43.34-360.18)	6 (27.27)
Child salivary IL-1β	29.03 (12.07-50.37)	40.74 (22.42-93.13)	6 (27.27)
Child salivary IL-6	2.65 (0.76-9.26)	3.70 (2.23-4.43)	6 (27.27)
Child salivary TNF-α	0.50 (0.18-2.75)	1.06 (0.23-1.77)	6 (27.27)
Caregiver salivary CRP	290.16 (103.13-857.52)	342.76 (49.41-11215.75)	7 (31.82)
Caregiver salivary IL-1β	68.34 (34.77-422.35)	103.26 (34.79-536.90)	7 (31.82)
Caregiver salivary IL-6	1.56 (1.18-2.16)	3.43 (0.93-10.26)	7 (31.82)
Caregiver salivary TNF-α	0.79 (0.44-2.28)	0.79 (0.30-1.86)	7 (31.82)
T2 salivary biomarkers	**Pain Buddy Intervention Group, *n*=14 dyads**	**Attention-Control Group, *n*=8 dyads**	**Missing *n* (%)**
Child salivary CRP	493.33 (68.18-1829.07)	471.84 (373.98-2004.20)	8 (36.36)
Child salivary IL-1β	42.87 (16.94-81.32)	91.48 (54.79-153.18)	8 (36.36)
Child salivary IL-6	3.68 (1.39-5.34)	6.02 (1.65-8.18)	8 (36.36)
Child salivary TNF-α	0.85 (0.53-1.07)	1.69 (0.83-2.89)	8 (36.36)
Caregiver salivary CRP	1048.72 (313.37-2098.55)[a]	257.87 (118.17-1605.54)	4 (18.18)
Caregiver salivary IL-1β	115.95 (16.49-569.66)	62.83 (29.34-422.10)	3 (13.67)

Caregiver salivary IL-6	2.75 (1.12-4.53)	2.06 (0.89-5.01)	4 (18.18)
Caregiver salivary TNF-α	2.11 (0.71-4.18)	0.60 (0.36-4.30)	4 (18.18)
T3 salivary biomarkers	**Pain Buddy Intervention Group, *n*=14 dyads**	**Attention-Control Group, *n*=8 dyads**	**Missing *n* (%)**
Child salivary CRP	217.50 (8.75-217.50)[a]	938.76 (298.41-2054.68)	15 (68.18)
Child salivary IL-1β	71.40 (42.70-71.40)[a]	62.70 (27.04-128.89)	15 (68.18)
Child salivary IL-6	4.24 (1.99-4.24)[a]	4.53 (1.33-5.89)	15 (68.18)
Child salivary TNF-α	0.50 (0.41-0.50)[a]	0.66 (0.17-0.99)	15 (68.18)
Caregiver salivary CRP	471.63 (69.62-2319.46)	304.70 (81.08-304.70)[a]	15 (68.18)
Caregiver salivary IL-1β	167.80 (126.72-539.92)[a]	90.23 (50.14-90.23)[a]	15 (68.18)
Caregiver salivary IL-6	2.44 (0.80-5.25)	2.07 (0.22-2.07)[a]	15 (68.18)
Caregiver salivary TNF-α	1.25 (1.06-13.31)	0.38 (0.10-0.38)	15 (68.18)

[a]Quartile 2 presented instead of quartile 3 due to inadequate sample size (three or fewer observations).

Note: Descriptive statistics for salivary biomarkers at each timepoint were calculated using raw data from each timepoint. Median and interquartile range statistics are provided. Distributions are not accurately reflected by the sample mean for salivary biomarker concentrations due to the high skewness and/or kurtosis of all salivary biomarkers.

Main analyses

Aim 1: Measure salivary inflammatory biomarker concentrations in children undergoing treatment for cancer and their primary caregivers.

Hypothesis 1: Salivary inflammatory biomarkers will be positively intercorrelated at baseline among children undergoing treatment for cancer. Spearman's correlations revealed that overall, salivary CRP and all salivary cytokines were weakly to strongly, and significantly, positively intercorrelated at baseline for children in the current study (see Table 3). Intercorrelations of salivary analytes at baseline for children were also examined separately by study group (Pain Buddy intervention group and attention-control group) (see Table 4).

Table 4. Spearman's correlations of children's salivary biomarkers of inflammation (pg/mL) at baseline for all study participants.

	1	2	3	4
1. Salivary C-reactive protein (CRP)	1	.33*	.38*	.34*
2. Salivary interleukin 1-beta (IL-1β)	-	1	.36*	.53**
3. Salivary interleukin 6 (IL-6)	-	-	1	.66*
4. Salivary tumor necrosis factor alpha (TNF-α)	-	-	-	1

Note: *p<.05; **p<.01

Table 5. Spearman's correlations of children's salivary biomarkers of inflammation (pg/mL) at baseline by study group: Pain Buddy intervention group and attention-control group.

	1	2	3	4
1. Salivary C-reactive protein (CRP)	1	.47	.14	.24
2. Salivary interleukin 1-beta (IL-1β)	.66**	1	**-.03**	.51*
3. Salivary interleukin 6 (IL-6)	.74**	**.03**	1	.43
4. Salivary tumor necrosis factor alpha (TNF-α)	.78**	.48*	.77**	1

Note: Attention-control group sample correlations are to the right of the diagonal. Pain Buddy intervention group sample correlations are to the left of the diagonal. Bolded correlations differ in direction (i.e., positive, negative) between the Pain Buddy intervention and attention-control groups.
*p<.01; **p<.01

Interestingly, among children in the attention-control group, statistically significant correlations were not observed for salivary CRP and salivary cytokines, though correlations were positive. Among the Pain Buddy intervention group, salivary CRP was strongly, and statistically significantly, positively correlated at baseline with

all salivary cytokines. A moderate, positive, statistically significant correlation was observed between salivary TNF-α and salivary IL-1β among both the attention-control group and the Pain Buddy intervention group. The direction of the correlation between salivary IL-6 and salivary IL-1β was negative in the attention-control group and positive in the Pain Buddy intervention group, and did not achieve statistical significance, though the strength of the correlation was the same between groups (absolute value .03).

Hypothesis 2: Salivary inflammatory biomarkers will also be positively intercorrelated among primary caregivers at baseline. Spearman correlations revealed that overall, salivary CRP and all salivary analytes were weakly to strongly, and significantly, positively intercorrelated at baseline for caregivers in the current study *except* for salivary IL-1β and salivary IL-6 (see Table 5). While not statistically significant, the correlation between salivary IL-1β and salivary IL-6 for caregivers at baseline was positive. Intercorrelations of salivary analytes at baseline for caregivers were also examined separately by intervention group (i.e., whether their child was assigned to the control group or Pain Buddy intervention group) (see Table 6).

Table 6. Spearman's correlations of caregivers' salivary biomarkers of inflammation (pg/mL) at baseline for all study participants.

	1	2	3	4
1. Salivary C-reactive protein (CRP)	1	.48**	.47**	.51**
2. Salivary interleukin 1-beta (IL-1β)	-	1	0.27	.73**
3. Salivary interleukin 6 (IL-6)	-	-	1	.36*
4. Salivary tumor necrosis factor alpha (TNF-α)	-	-	-	1

Note: *p<.05; **p<.01

Table 7. Spearman's correlations of caregivers' salivary biomarkers of inflammation (pg/mL) at baseline by child's study group: Pain Buddy intervention group and attention-control group.

	1	2	3	4
1. Salivary C-reactive protein (CRP)	1	0.25	.47**	.39*
2. Salivary interleukin 1-beta (IL-1β)	.80**	1	0.16	.58**
3. Salivary interleukin 6 (IL-6)	.57*	.53*	1	0.32
4. Salivary tumor necrosis factor alpha (TNF-α)	.71*	.87**	.58*	1

Note: Attention-control group sample correlations are to the right of the diagonal. Pain Buddy intervention group sample correlations are to the left of the diagonal.
*p<.05; **p<.01

Among caregivers whose children were in the attention-control group, statistically significant correlations were not observed for salivary CRP and salivary IL-1β at baseline, though the correlation observed was positive. Correlations between salivary CRP and salivary IL-6 and salivary TNF-α were moderate and weak (respectively), positive, and statistically significant among caregivers whose children were in the attention-control group. Among caregivers whose children were in the Pain Buddy intervention group, salivary CRP was moderately-to-strongly, and statistically significantly, positively correlated at baseline with all salivary cytokines. Salivary TNF-α was also moderately-to-strongly, and statistically significantly, positively correlated at baseline with salivary IL-1β and salivary IL-6 among caregivers whose children were in the intervention group.

Aim 2: Determine how salivary inflammatory biomarker concentrations are related to pain and patient- and caregiver-reported psychosocial outcomes.

Hypothesis 3: Higher concentrations of salivary inflammatory biomarkers will be positively associated with higher reported pain among children with cancer. Linear regression revealed that children's pain at baseline as reported on the MSAS did not explain a significant proportion of variation in their T2 salivary CRP composite score (Adj. R^2 = -.05, $F(1,5)$ = .73, p = .43) or T2 salivary cytokine concentrations (Adj. R^2 = -.13, $F(1,6)$ = .19, p = .68). Similarly, linear regression models utilizing children's pain as reported on the MSAS at T2 could not predict salivary CRP composite score (Adj. R^2 = -.05, $F(1,5)$ = .73, p = .43) or salivary cytokine composite score (Adj. R^2 = -.05, $F(1,5)$ = .73, p = .43) at T3. Linear regression models utilizing pain scores from the PedsQL Cancer Module to predict salivary CRP and salivary cytokine composite scores did not have an improved model fit.

Hypothesis 4: Higher concentrations of salivary inflammatory biomarkers will be positively associated with poorer psychosocial outcomes among children with cancer, specifically more depression and anxiety symptoms and lower. Separate linear regressions assessing the relationships between child depression, anxiety, and inflammatory biomarker composite scores did not reveal statistically significant associations:

Major depression symptoms reported by children on the RCADS at baseline did not explain a significant proportion of variation in their T2 salivary CRP composite

scores (Adj. R^2 = -.08, F(1,12) = .01, p = .91) or T2 salivary cytokine composite scores (Adj. R^2 = -.02, F(1,12) = .71, p = .42). Similarly, major depression symptoms reported by children on the RCADS at T2 did not statistically predict salivary CRP (Adj. R^2 = .12, F(1,3) = 1.54, p = .30) or salivary cytokine (Adj. R^2 = .138, F(1,3) = 1.64, p = .29) composite scores at T3. Model fits did not improve when major depression symptom scores from the RCADS caregiver proxy were utilized instead.

General anxiety symptoms reported by children on the RCADS at baseline did not explain a significant proportion of variation in their T2 salivary CRP composite scores (Adj. R^2 = -.05, F(1,12) = .36, p = .56) or T2 salivary cytokine composite scores (Adj. R^2 = .02, F(1,12) = 1.22, p = .29). General anxiety symptoms reported by children on the RCADS at T2 also did not statistically predict salivary CRP (Adj. R^2 = .10, F(1,3) = 1.43, p = .32) or salivary cytokine (Adj. R^2 = -.33, F(1,3) = 8.27, p = .995) composite scores at T3. Model fits did not improve when general anxiety symptom scores from the RCADS caregiver proxy were utilized.

Hypothesis 5: Caregivers who report higher perceived stress will have higher levels of salivary biomarkers of inflammation. Linear regression revealed that caregivers' perceived stress reported at baseline on the PSS did not explain a significant proportion of variation in their T2 salivary CRP composite scores (Adj. R^2 = .139, F(1,15) = 3.58, p = .08) or salivary cytokine composite scores (Adj. R^2 = -.004, F(1,16) = .93, p = .35). Similarly, linear regression models utilizing caregivers' perceived stress reported on the PSS at T2 were unable to statistically predict salivary CRP (Adj. R^2 = -.25, F(1,4) = .001, p = .97) or salivary cytokine (Adj. R^2 = -.14, F(1,4) = .37, p = .57) composite scores at T3.

Aim 3: Investigate the impact of the Pain Buddy intervention on salivary biomarkers of inflammation.

Hypothesis 6: Children with cancer who learn and practice cognitive and behavioral coping skills through the Pain Buddy application (i.e., the intervention group) will have lower average salivary inflammatory biomarker concentrations at T2 and T3 than children who do not receive the same coping skills training (i.e., the attention-control group). Mann-Whitney *U* tests revealed that the distribution of all individual salivary inflammatory biomarkers (CRP, IL-1β, IL-6, and TNF-α), as well as the distribution of the salivary biomarker composite scores, was similar between the attention-control and intervention groups for children at each of the three study timepoints (p range = 0.11-1.00). Mean rank comparisons of salivary inflammatory biomarker composite scores for children are visualized in Figures 1 and 2.

Hypothesis 7: Caregivers whose children are in the intervention group will also have lower salivary inflammatory biomarker concentrations at T2 and T3 as compared to caregivers whose children are in the attention-control group. Mann-Whitney *U* tests revealed that the distribution of all individual salivary inflammatory biomarkers, as well as the distribution of the salivary biomarker composite scores, was similar between caregivers whose children were in the attention-control group and intervention group at each of the three study timepoints (p range = 0.06-1.00). Mean rank comparisons of salivary inflammatory biomarker composite scores for caregivers are visualized in Figures 3 and 4.

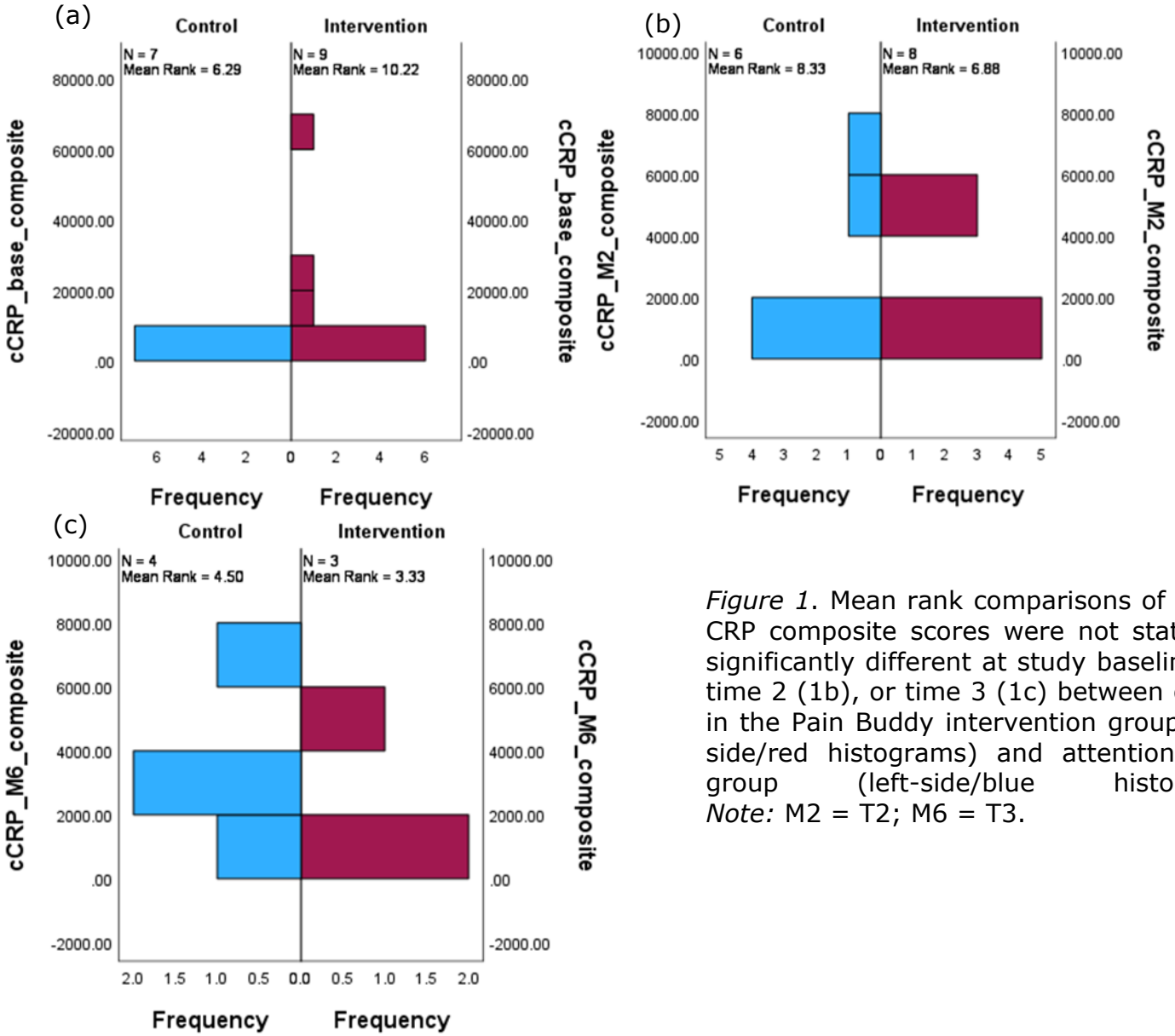

Figure 1. Mean rank comparisons of salivary CRP composite scores were not statistically significantly different at study baseline (1a), time 2 (1b), or time 3 (1c) between children in the Pain Buddy intervention group (right-side/red histograms) and attention-control group (left-side/blue histograms). *Note:* M2 = T2; M6 = T3.

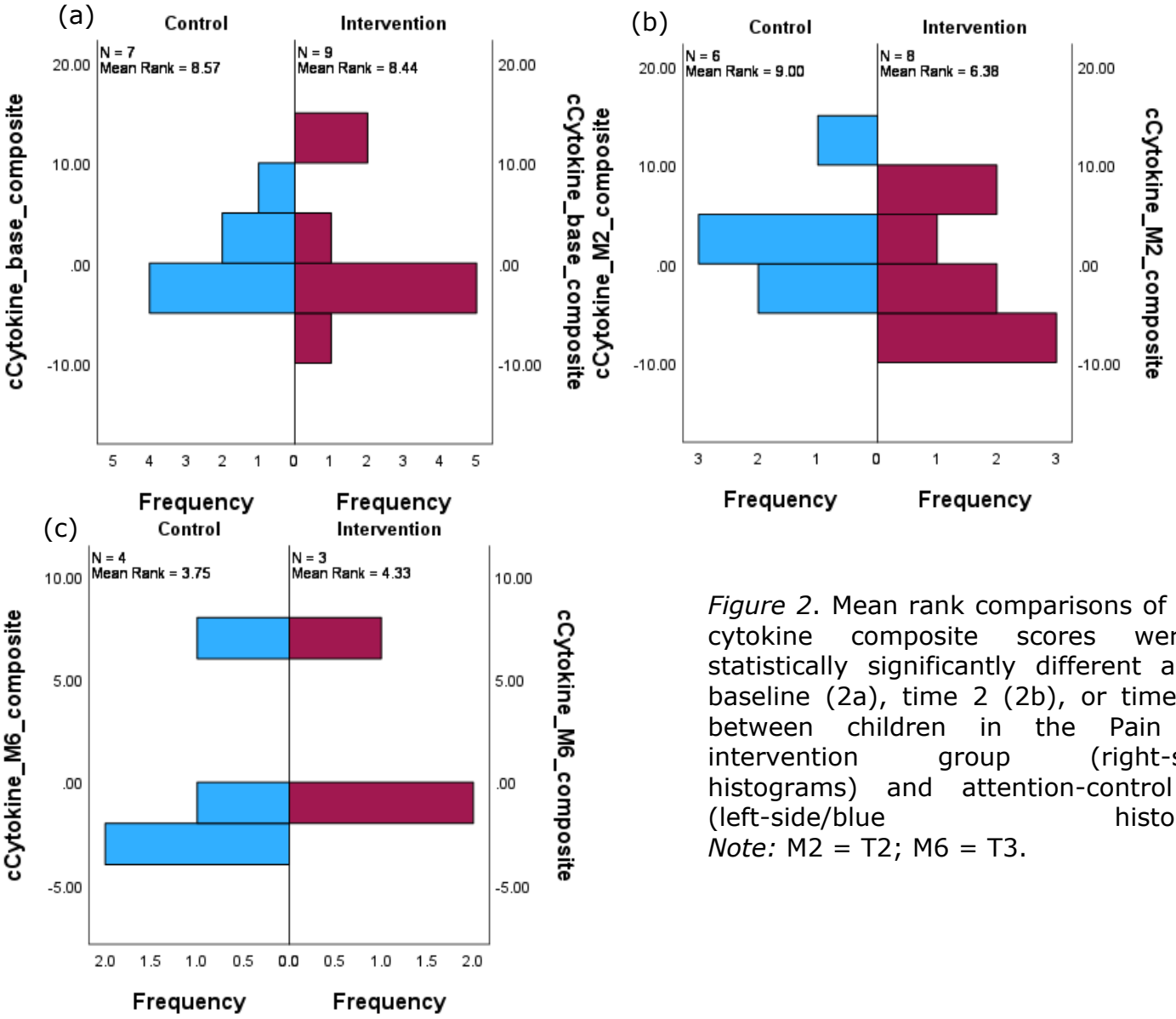

Figure 2. Mean rank comparisons of salivary cytokine composite scores were not statistically significantly different at study baseline (2a), time 2 (2b), or time 3 (2c) between children in the Pain Buddy intervention group (right-side/red histograms) and attention-control group (left-side/blue histograms). *Note:* M2 = T2; M6 = T3.

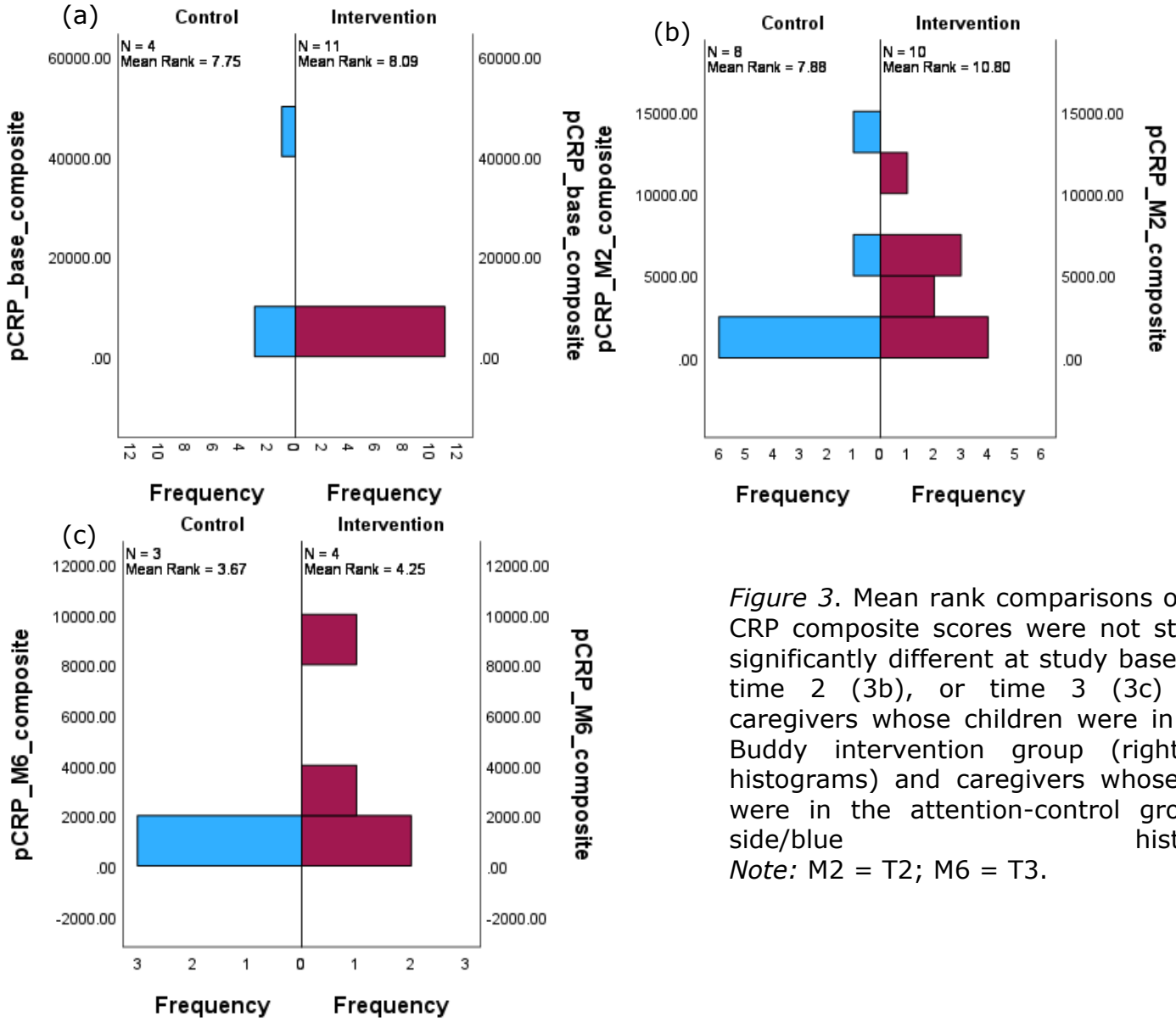

Figure 3. Mean rank comparisons of salivary CRP composite scores were not statistically significantly different at study baseline (3a), time 2 (3b), or time 3 (3c) between caregivers whose children were in the Pain Buddy intervention group (right-side/red histograms) and caregivers whose children were in the attention-control group (left-side/blue histograms).
Note: M2 = T2; M6 = T3.

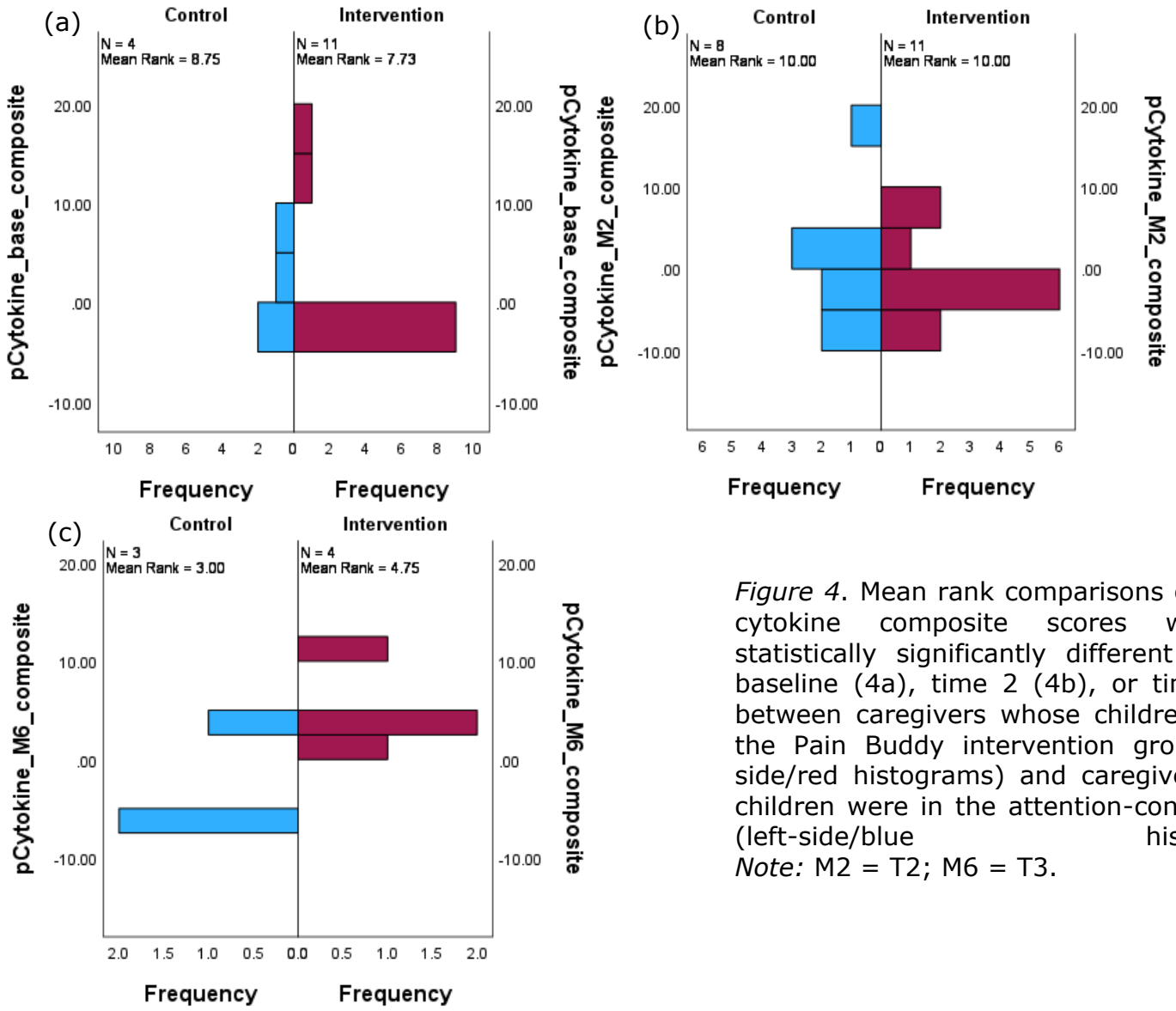

Figure 4. Mean rank comparisons of salivary cytokine composite scores were not statistically significantly different at study baseline (4a), time 2 (4b), or time 3 (4c) between caregivers whose children were in the Pain Buddy intervention group (right-side/red histograms) and caregivers whose children were in the attention-control group (left-side/blue histograms). *Note:* M2 = T2; M6 = T3.

Aim 4: Explore participant factors that may be associated with salivary biomarkers of inflammation.

As the present study is one of very few in its inclusion of caregiver-child dyads (versus individuals), the expected relationships among salivary inflammatory biomarkers and participant outcomes is unclear. Two participant demographic characteristics were chosen to be explored further: type of cancer diagnosis and primary language spoken at home. Furthermore, as BMI is associated with salivary inflammatory biomarkers (particularly CRP), correlations between children's BMI percentile and salivary inflammatory biomarkers were explored.

Point-biserial correlations were utilized to assess the association between individual salivary inflammatory biomarkers and cancer diagnosis category (i.e., leukemias, lymphomas, sarcomas, central nervous system tumors) among both children and caregivers at each timepoint. As the treatment protocols and prognosis for each diagnosis can vary widely, even within each diagnosis category, this exploratory analysis was conducted to determine whether those differences may be reflected in salivary inflammatory biomarker levels in either children or caregivers. There was a statistically significant weak negative correlation between diagnosis category and children's T3 salivary TNF-α concentrations (pg/mL): $r_{pb}(5)= -.358$, $p = .03$. There was also a statistically significant moderate positive correlation between diagnosis category and caregiver's baseline salivary IL-6 concentrations (pg/mL): $r_{pb}(13)= .52$, $p = .048$.

Since over 36 percent of participants recruited for the present study spoke Spanish as their primary language, point-biserial correlations were also utilized to

assess associations between primary language (English or Spanish) and inflammatory biomarker concentrations (pg/mL) to begin exploring differences based on culture (e.g., cultural values, acculturation). Across all study timepoints and all individual inflammatory biomarkers, no statistically significant correlations were observed between primary language and biomarkers.

Spearman's correlations were run to determine the relationship between children's BMI percentile and individual salivary inflammatory biomarker concentrations (pg/mL) at each study timepoint. There was a statistically significant, moderate positive relationship between BMI percentile and salivary CRP concentrations at T2: $r_s(8) = .67$, $p = .03$. No other statistically significant correlations between BMI and salivary inflammatory biomarkers were observed.

Discussion

Measuring salivary inflammatory biomarkers among children with cancer and their caregivers is a promising first step toward better understanding how oral and systemic immune processes for the dyad may be related to psychosocial and pain outcomes during the course of a child's cancer treatment. Prior research suggests that these salivary inflammatory biomarkers have implications for cancer-related outcomes in adulthood after children have transitioned into survivorship and can predict long-term functioning for caregivers as well. Children diagnosed with cancer experience myriad symptoms throughout their disease progression and treatment, including moderate to severe pain, fatigue, insomnia, and worry (Baggott et al., 2009; Collins et al., 2000). Substantial research has accordingly been devoted to understanding, managing, and improving children's psychosocial outcomes and

cancer-related pain. There is, however, a gap regarding how inflammatory biomarkers are related to pain and psychosocial sequelae commonly experienced by children with cancer, providing an opportunity to extend current knowledge and inform the development of more comprehensive care.

For caregivers, research in psychoneuroimmunology has established links between stress and heightened immune response (i.e., increased inflammation), though these links are often observed using serum biomarker levels. For example, higher perceived stress among clinically normal adults is linked to disproportionate elevations in serum monocyte concentrations as compared to adults who do not report high perceived stress (Casaletto et al., 2018). Measures of immune system functioning utilizing saliva samples rather than serum samples may more feasibly provide information regarding caregivers' physiological health which may not be reflected in their self-reports (i.e., reports of health or reports of perceived stress). Research to date has also not analyzed the potential associations between children's functioning (or changes in symptom severity) during cancer treatment and caregivers' salivary inflammatory biomarkers.

The present study is the first, to our knowledge, which aims to investigate differences in salivary inflammatory biomarkers at multiple timepoints among both children with cancer and their primary caregivers, alongside reports of children's pain, anxiety, and depression, and reports of caregivers' perceived stress. The present study was designed to address current gaps and questions in the literature concerning relationships between salivary biomarkers of inflammation, reports of pain, and psychosocial outcomes among children with cancer. Rather than providing definitive answers to these questions, the present study is a stepping stone toward

understanding how best to approach research in this field and will inform methodology for follow-up studies.

Unique aspects of the present study include recruitment of a sample of children utilizing an mHealth intervention to reduce pain and cancer-related symptoms, as well as the measurement of caregivers' salivary inflammatory biomarker concentrations alongside their children during the study period. We did not find statistical support for all hypothesized relationships between study variables; however, conducting the present study provided us with valuable insight regarding how best to approach and engage this population of participants moving forward. The present study also highlights the potential importance of participant factors, such as medication use and oral health, which were considered during the initial study design but should be further investigated in future research. Below is a discussion of study findings in relation to the proposed aims of the current dissertation, as well as limitations, lessons learned, and directions for follow-up research.

Aim 1: Measure salivary inflammatory biomarker concentrations in children undergoing treatment for cancer and their primary caregivers.

Consistent with established literature, salivary inflammatory biomarkers were generally intercorrelated at baseline among both children and caregivers. When children were separated by study intervention group (Pain Buddy intervention and attention-control) and correlations were reassessed, the correlation between salivary IL-6 and salivary IL-1β among attention-control group children was found to be negative, though this correlation did not reach statistical significance. Among caregivers separated by study intervention group (Pain Buddy intervention and

attention-control), correlations between salivary biomarkers of inflammation remained positive. The general trend of these associations indicate that salivary inflammatory biomarkers used in the current study may be positively intercorrelated within children diagnosed with cancer, as well as within their adult caregivers. More research, specifically the inclusion of more participants, is needed to be confident that salivary inflammatory biomarker concentrations are consistently intercorrelated among children with cancer as they are in healthy children and adults (Riis et al., 2014, 2015).

Positive associations found in the present study, if corroborated by future research, mean that we may be able to utilize fewer salivary inflammatory biomarkers to index oral inflammation for children with cancer. The current study protocol included three salivary cytokines – IL-6, IL-1β, and TNF-α – which all are highly influenced by the oral immune environment and thus typically regarded as indices of oral inflammation rather than systemic inflammation. Utilizing fewer salivary inflammatory biomarkers to assess oral inflammation could be a benefit to children with cancer and their caregivers as they would not have to devote as much of their vital time or effort to collecting saliva samples for research purposes.

The current study protocol asked participants to passively drool into a collection vial for up to two minutes or until 2.0 mL of fluid were collected, to account for at least 0.5 mL per salivary biomarker included in the protocol (CRP, IL-6, IL-1β, TNF-α) for assay purposes. At least four caregivers recruited for the current project communicated with the research team that they were unable to collect at least 2.0 mL of saliva from their child at points throughout the study due to their child

experiencing pain, dry mouth, saliva being more viscous than typical, or simply being uncomfortable with passively drooling for that amount of time.

Even with a minimally invasive and minimally painful biospecimen collection such as saliva sampling, there are ways we can improve participant comfort and compliance with study procedures, especially for children undergoing treatment for a first-time cancer diagnosis. If follow-up studies find that salivary biomarkers of oral inflammation (i.e., cytokines IL-6, IL-1β, and TNF-α) are indeed consistently positively associated, future research designs may be able to include just one of these biomarkers, such as salivary IL-6, as representative of oral inflammation. Along with salivary CRP as an index of systemic inflammation, just one additional salivary inflammatory biomarker would mean that participants only need to collect 1.0 mL of saliva (0.5 mL for each salivary biomarker) to provide similar insights into inflammatory processes. This smaller sample volume could be quicker and more manageable for child-participant dyads to collect, especially on days when children have scheduled medical procedures or planned family events. A smaller required sample volume may increase the amount of viable saliva samples from children who would not have otherwise been able to produce double the amount (2.0 mL) of saliva at multiple study assessment points and thus allow us to capture more participant data.

For associations between salivary inflammatory biomarkers which were not statistically significant or positive in the current study, there are additional factors to consider. These samples were taken at baseline, prior to engagement in the Pain Buddy intervention, which suggests that participant characteristics which naturally varied in the present sample, such as individual differences in sample collection and

handling at home, may contribute to differences. Child-caregiver dyads were allowed to select a time of day that worked best for their saliva collections at the beginning of the study, and there was some variation in timing. For example, some dyads were able to collect samples consistently in the mornings while some dyads could only consistently collect samples in the late afternoon after a child's scheduled medical appointments. Yet more participant dyads reported to the current research team that there was inconsistency in their sample collection times throughout the study due to personal, work, or medical schedules (e.g., attending a family event where immediate sample collection and storage was not possible, or being admitted to the emergency department during a typical sample collection time). These variations in sample collection timing should be accounted for in future analyses as many serum cytokines have a circadian rhythm, though we do not know precisely how salivary CRP and salivary cytokines may vary throughout the day (Szabo & Slavish, 2021).

Aim 2: Determine how salivary inflammatory biomarker concentrations are related to pain and patient- and caregiver-reported psychosocial outcomes.

Children's Pain

The measures of pain utilized for the present study, the MSAS and the PedsQL Cancer Module, were not predictive of CRP and cytokine composite scores among children in the present study. Based on analyses conducted for the present study, it is still unclear to what extent acute or chronic pain experienced by children with cancer is associated with local oral immune activity (i.e., salivary cytokines) or systemic immune activity (i.e., salivary CRP). Children's self-reports of pain at baseline and T2 were not predictive of composite salivary cytokine scores at the next

study timepoints (i.e., T2, T3) when using linear regression (p range = .43-.68). We are also unable to make definite conclusions regarding the impact of pain on salivary CRP composite scores as there was no predictive association at T2 or T3 (p = .43 for both timepoints) for this group of children. Of note, regression analyses for the current dissertation only allowed for the inclusion of participant dyads who provided at least one saliva sample at each of the three study timepoints, resulting in 15 participant dyads who had missing samples being excluded from the current analyses. An analytic approach such as latent growth modeling, which can consider levels of salivary biomarkers at each separate day within study timepoints (i.e., three days of baseline sampling, three days of T2 sampling, and three days of T3 sampling) rather than creating one composite value, may be able to more accurately detect the relationship between pain and salivary biomarkers over time.

Self-reports of pain among cancer patients has been associated with serum biomarkers including CRP, however this link is not as well-established using salivary biomarkers of inflammation (Oliveira et al., 2014). Relationships between salivary biomarkers of inflammation and pain are under-studied among children in general: across samples of all ages, salivary alpha-amylase and salivary cortisol are the most widely studied as biomarkers of pain (Payne & Fortier, 2020). In future research, the inclusion of salivary CRP and salivary cytokines such as in the present study can extend knowledge of how self-reported pain is related to salivary biomarkers of inflammation among children with cancer throughout their treatment course. Future research investigating this relationship between pain and salivary inflammatory biomarker concentrations may help with determining if assessment of pain is possible

through the measurement of salivary biomarkers, such as in cases where a child is not able to self-report pain levels due to age or other factors.

Children's and Caregivers' Psychosocial Outcomes

Psychosocial well-being (anxiety, depression) as self-reported by children and reported by caregivers via proxy were also not predictive of CRP and cytokine composite scores among children in the present study. This null finding is particularly interesting since there were detectible levels of proinflammatory cytokines for both children and caregivers in the present study, and these cytokines in serum have been found to be related to anxiety and depression in humans (Anisman et al., 1999). The circulating levels of salivary cytokines or reports of psychosocial symptoms observed in the present sample of children may not be illustrative of this typical relationship, perhaps due to having a small sample and inadequate power to detect this relationship.

In a study of adults caring for a spouse with dementia, serum CRP was linked to pain among spousal caregivers, but not the adults with dementia they cared for, suggesting that pain may be specifically associated with chronic caregiving stress (Graham et al., 2006). It is possible that this relationship may exist for caregivers of children with cancer as well, though the current project did not investigate this association. Similarly, among caregivers in the current study, perceived stress was not predictive of either salivary CRP or cytokine composite scores. It was proposed that perceived stress would be associated with higher salivary biomarker composite scores, and while that was not the observed pattern, results from the present study may still be in line with prior research. The Th1 (proinflammatory) cytokine response

in serum, including IL-1β and TNF-α activity, may be decreased or blunted within individuals under chronic psychological stress such as caregivers of children with cancer (Ambrée et al., 2019; Miyasaka et al., 2018; Nelson et al., 2008; Wenzel et al., 2015). If future research corroborates the trends observed in the current study regarding perceived stress and lower biomarker composite scores, there may evidence for a pattern that is reflected in salivary concentrations of Th1 cytokines as it is for serum concentrations.

Aim 3: Investigate the impact of the Pain Buddy intervention on salivary biomarkers of inflammation.

While results did not indicate statistically significant differences in inflammatory biomarkers between children or caregivers in the Pain Buddy intervention and attention-control groups, these observations are still informative. Rather than inflammatory biomarkers being associated with just study group membership, perhaps changes in inflammatory biomarkers can be studied in follow-up studies as a function of Pain Buddy intervention engagement (i.e., a dose-response relationship). There is already a basis for engagement in mindfulness-based interventions reducing serum CRP and IL-6 (Dunn & Dimolareva, 2022), and there is an opportunity to extend this body of work with findings from interventions tailored for children undergoing cancer treatment. Follow-up studies will benefit from considering children's engagement in different components of the Pain Buddy intervention, such as length of time spent learning cognitive-behavioral skills via the mobile application.

The sampling procedure of three timepoints across a 90-day study period from recruitment to completion of follow-up questionnaires may not have been sufficient to capture nuanced changes in inflammatory biomarker concentrations for child-caregiver dyads, or it is possible that the components of the Pain Buddy intervention, including mindfulness training, simply did not result in significant changes to inflammatory biomarkers as found in prior research (Z. P. Cohen et al., 2021; Oswald et al., 2022).

Aim 4: Explore participant factors that may be associated with salivary biomarkers of inflammation.

Exploratory analyses indicated that there may be an association between children's diagnosis and salivary inflammatory biomarker concentrations within children (TNF-α) and caregivers (IL-6). In a study of Latino children aged 5 to 10 years, elevated plasma TNF-α levels were associated with stressful life events, a relationship which was not modified by child sex or family history of type 2 diabetes mellitus (Dixon et al., 2009). Conceptualizing the cancer illness and treatment experience as a chronic stressor, trends from the present study supports a need to further investigate the relationship between significant stressful life events and elevated salivary biomarker levels, such as TNF-α, among children. Elevations in serum IL-6 in response to stress, both acute and chronic, are well-established among otherwise healthy adults (Slavish et al., 2015). However, the mechanisms underlying a possible relationship between a child's specific cancer diagnoses and salivary IL-6 concentrations among their caregivers are unclear and warrant further investigation.

Limitations and Considerations for Future Research

The present study contributes to the current literature by examining salivary biomarkers of inflammation among children with cancer and their caregivers. The present study offers insight into improving methodological design and practice in working with this vulnerable population, which may improve patient comfort and compliance with research procedures. This investigation also extends immune biomarker research by beginning to examine the associations between caregivers' salivary biomarkers of inflammation and children's pain and psychosocial outcomes. When considering these strengths, it is also important to address the limitations of this work and how lessons learned from the present study can contribute to future directions in this field.

The present study was a small, single-site study of children with cancer and their primary caregivers, which limits the generalizability of findings and increases the likelihood of a Type II error. For example, study group (Pain Buddy intervention, attention-control) differences in caregivers' T3 salivary IL-1β concentrations approached statistical significance (p = 0.057) but a significant difference was not ultimately detected, perhaps due in part to the sample size.

In addition to the potential of time-based peak values in salivary CRP and salivary cytokines as mentioned in discussion of Aim 1, other important considerations include how sleep and stress may impact salivary inflammatory biomarkers. A recent study among university students suggests that salivary IL-1β may be related to symptoms of insomnia or poor sleep quality as a function of its role in helping recover physiological function after sleep loss via sympathetic nervous system pathways (i.e., serum IL-1β levels may increase to help induce sleep, and

these levels are partially reflected in salivary IL-1β) (Ballestar-Tarín et al., 2023). During a child's initial diagnosis and treatment for cancer, children and their caregivers often concurrently experience sleep disturbances including difficulties with falling asleep, experiencing fragmented sleep, and reported lower quality of sleep (Rensen et al., 2019; Stavinoha et al., 2021). While sleep disturbances may be related to respiratory issues such as sleep apnea, this is not the case for the majority of reported sleep disturbances among children with cancer or their caregivers in research to date, and these disturbances can persist even as children transition into cancer survivorship (Rensen et al., 2019). It is plausible that poor sleep quality, sleep duration, and symptoms of insomnia are related to salivary IL-1β levels within children with cancer and their caregivers as it is among healthy university students, though the current study did not account for these factors in its design and analysis. Future research is needed to investigate bidirectional links between sleep and salivary biomarkers of inflammation.

In addition to sleep, another health behavior that should be considered in future research is engagement in oral hygiene habits (e.g., tooth brushing, flossing, regular dentist visits). In the present study, we asked participants to report any current dental issues such as bleeding gums or oral caries to help explain transferrin (blood leakage) concentrations in saliva. About a quarter of individual saliva samples donated by participants (both children and caregivers) for the present study were excluded due to blood contamination, which is exceptionally high compared to studies where less than five percent of samples are excluded due to blood contamination (Kamodyová et al., 2015). However, we did not account for aspects of oral hygiene or health in either children or caregivers such as inflamed gums, last dental visit, or

tooth-brushing habits. Given current knowledge regarding the prevalence of chronic stress among caregivers of a family member with cancer, and research which indicates that these caregivers may be less likely to engage in self-care practices to maintain their own health (Dionne-Odom et al., 2017), chronic stress and lack of self-care may manifest in a poorer oral immune environment for caregivers. In humans, salivary TNF-α has been implicated in detecting the onset of periodontal issues including periodontitis, and is a potential clinical diagnostic marker of oral cancer (Kibune et al., 2022; Sahibzada et al., 2017). For children with cancer, there is literature highlighting the prevalence of oral complications related to cancer therapies including mucositis, opportunistic infection, and salivary gland dysfunction which may influence biomarker concentrations (Epstein et al., 2012). Chronic stress itself as experienced by children with cancer and their caregivers may pose an increased risk for higher concentrations of salivary inflammatory biomarkers even aside from impacting oral health or hygiene habits: In animal models, salivary IL-1β and mRNA expression levels of IL-1β in the submandibular glands of mice subjected to daily chronic stress were significantly elevated as compared to mice in a control group (Paudel et al., 2020). While these factors were not specifically measured in the present study, it is important to consider the potential influence of both stress and oral health habits in tandem when investigating salivary inflammatory biomarkers among this population.

Another piece of information which must be investigated further is how the types of medications used throughout children's cancer treatment, particularly corticosteroids, may influence levels of detectable salivary inflammatory biomarkers. Literature investigating the impact of corticosteroids on salivary inflammatory

biomarkers in children is largely limited to studies among children with asthma who use inhaled corticosteroid medications, and while there is a lower level of systemic absorption for these children, there are still concerns that prolonged use may lead to adverse effects including stunted growth or HPA axis suppression (Amato et al., 2015; Ballerini et al., 2023; Smy et al., 2015). For children with cancer who are administered corticosteroid medication as part of their treatment for prolonged periods of time (over the course of weeks to months), careful monitoring for HPA axis recovery following corticosteroid treatment is necessary. During the active administration of corticosteroid medication as well as the weeks following, children with cancer will have lower levels of detectible salivary inflammatory biomarkers than typical (Ballerini et al., 2023) before HPA axis recovery. While we attempted to account for this in the present study by timing children's samples in between cycles of corticosteroid medication, there are some cases where a child's care team may have initiated corticosteroid treatment earlier than anticipated, or delayed treatment. In general, there was a wide variety in treatment courses for children recruited for the present study, as we did not restrict enrollment to children with just one diagnosis. It may be that in cases where there were not detectable levels of salivary inflammatory biomarkers, those children were still experiencing blunted HPA axis functioning as a result of their standard corticosteroid treatment. Appendices A through D are examples of the standard courses of treatment for the children recruited for the present study, separated by diagnosis – however, even within children with the same diagnosis, there were slight variations in the timing of medication administration. These are factors which can, and should, be explored in

a more qualitative fashion in future research due to the variety resulting from tailoring of patient care.

The group of caregivers in the intervention group reported higher levels of education and higher household income than the group of caregivers in the control group. Prior research has linked socioeconomic stress to inflammation and immune function (O'Connor et al., 2009), so it is possible that the present limited sample size and analytical methods masked the detection of interactions between these factors and participant outcomes.

There are also notable differences between the two study groups in race and ethnicity such that all child-caregiver dyads in the attention-control group identified as Hispanic, Latino/a/x, or Chicano/a. The analytical methods used in the present study, while suitable for the data, could not account for demographic variables such as caregiver race and ethnicity. Related to this, lack of adjustment for multiple outcome comparisons in the current analyses necessitates confirmation, such as in a larger follow-up study. Finally, this study did not account for salivary flow rate or caregiver BMI, which can influence salivary biomarker concentrations (Szabo & Slavish, 2021).

Caring for a child with cancer is a significant chronic stressor, and caregivers who may have lower socioeconomic status or who do not speak English as their primary language may not have adequate resources to cope throughout a child's disease progression (Ullrich et al., 2021; Wang et al., 2018). Knowing that immune health is potentially impacted by caregiving stress, early intervention efforts to improve caregiver health should include stress management interventions tailored

specifically for caregivers of children with cancer. An RCT of one such intervention, designed for caregivers of stem cell transplant patients, resulted in lower depression and anxiety, and showed promise in impacting physiological pathways associated with inflammation (Laudenslager et al., 2015). The development of a timely and tailored psychosocial intervention thus has implications for all domains of wellness and functioning, including immune health.

Conclusion

In light of these limitations, this study has important strengths that contribute to the existing literature. Methodologically, this study employs a prospective design that includes salivary inflammatory measurement for children with cancer and their caregivers. This is a key strength of the present study, as many studies to date have studied individuals with cancer independently, without a caregiver (Mundy-Bosse et al., 2011; Thornton et al., 2007). The present study builds upon previous research highlighting the psychological, emotional, and practical difficulties faced by caregivers of cancer patients (Wang et al., 2018), offering new insight to their immune functioning via salivary bioscience, which has not been studied previously. Many studies recruit caregivers of adults with medical conditions, or spousal caregivers, and the present study is one of the few which presents findings on caregivers of children with cancer.

Caregivers of children with cancer had measurable levels of salivary inflammatory biomarkers at baseline in our study, and if these biomarkers remain at elevated levels long-term, it could place them at risk for long-term health deficits and potentially more immediate difficulties in fulfilling their role as caregivers. Higher inflammation among otherwise healthy caregivers is indicative of chronic stress,

which may lead to increased behavioral symptoms and poorer mental health (Wohleb et al., 2015; Wohleb & Delpech, 2017). Observations from the present study provide further support for continued research to assist caregivers in identifying and advocating for their psychological, emotional, and social needs to maintain their well-being.

In addition to meeting the immune needs of caregivers of a child with cancer, addressing any concerns for children's pain, psychosocial, and inflammation is imperative. While there were no statistically significant associations between salivary inflammatory biomarkers and pain or psychosocial symptoms (depression, anxiety), the correlation between diagnosis category and children's T3 TNF-α warrants further investigation with a larger participant sample. Future research will also benefit from further examining how medical characteristics of a child with cancer are related to caregivers' outcomes as there has been prior research indicating that caregiver well-being is related to children's illness prognosis (Ilic et al., 2020; Litzelman et al., 2011). Based on the present study, characteristics to be considered in future research should include specific diagnosis and treatment protocol, and length of time since diagnosis.

Overall, the present study provides further insight into how we can continue to improve upon methodology, including saliva sampling and collecting self-reports and medical information, to study the potential immune processes (i.e., inflammation) associated with cancer among children within the first six months of a first-time diagnosis and their caregivers. The present study highlights the need for further research investigating how a child's diagnosis or treatment protocol may be related to salivary inflammatory biomarkers.

References

Agbayani, C., Tucker, J. A., Nelson, E. L., Martinez, F., Khoury, D., Kain, Z. N., Lin, C., Torno, L., & Fortier, M. A. (2022). Immunological and psychosocial functioning in parents of children with cancer. *Supportive Care in Cancer*, *30*(4), 3379–3388. https://doi.org/10.1007/s00520-021-06770-0

Aggarwal, B. B., & Gehlot, P. (2009). Inflammation and cancer: how friendly is the relationship for cancer patients? *Current Opinion in Pharmacology*, *9*(4), 351–369. https://doi.org/10.1016/j.coph.2009.06.020

Alberts, N. M., Gagnon, M. M., & Stinson, J. N. (2018). Chronic pain in survivors of childhood cancer: A developmental model of pain across the cancer trajectory. In *Pain* (Vol. 159, Issue 10, pp. 1916–1927). Lippincott Williams and Wilkins. https://doi.org/10.1097/j.pain.0000000000001261

Ambrée, O., Ruland, C., Zwanzger, P., Klotz, L., Baune, B. T., Arolt, V., Scheu, S., & Alferink, J. (2019). Social defeat modulates T helper cell percentages in stress susceptible and resilient mice. *International Journal of Molecular Sciences*, *20*(14). https://doi.org/10.3390/ijms20143512

An, K. J., Joung, Y. S., Sung, K. W., & Kim, J. H. (2013). Health-related quality of life and cognitive functioning at on- and off-treatment periods in children aged between 6-13 years old with brain tumors: A prospective longitudinal study. *Yonsei Medical Journal*. https://doi.org/10.3349/ymj.2013.54.2.306

Anisman, H., Ravindran, A. V, Griffiths, J., & Merali, Z. (1999). Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. *Molecular Psychiatry*, *4*(2), 182–188.

Antoni, M. H. (2013). Psychosocial intervention effects on adaptation, disease course and biobehavioral processes in cancer. *Brain, Behavior, and Immunity*, *30*(SUPPL.), S88–S98. https://doi.org/10.1016/j.bbi.2012.05.009

Archer, J. A., Hutchison, I. L., Dorudi, S., Stansfeld, S. A., & Korszun, A. (2012). Interrelationship of depression, stress and inflammation in cancer patients: A preliminary study. *Journal of Affective Disorders*, *143*(1–3), 39–46. https://doi.org/10.1016/j.jad.2012.05.023

Baggott, C., Dodd, M., Kennedy, C., Marina, N., & Miaskowski, C. (2009). Multiple symptoms in pediatric oncology patients: a systematic review. *Journal of Pediatric Oncology Nursing*, *26*(6), 325–339.

Bakula, D. M., Sharkey, C. M., Perez, M. N., Espeleta, H. C., Gamwell, K. L., Baudino, M., Delozier, A. M., Chaney, J. M., Matt Alderson, R., & Mullins, L. L. (2019). The Relationship between Parent and Child Distress in Pediatric Cancer: A Meta-Analysis. *Journal of Pediatric Psychology*, *44*(10), 1121–1136. https://doi.org/10.1093/jpepsy/jsz051

Ballestar-Tarín, M. L., Ibáñez-del Valle, V., Mafla-España, M. A., Cauli, O., & Navarro-Martínez, R. (2023). Increased Salivary IL-1 Beta Level Is Associated with Poor Sleep Quality in University Students. *Diseases*, *11*(4), 1–16. https://doi.org/10.3390/diseases11040136

Basch, E. (2016). Missing patients' symptoms in cancer care delivery - The importance of patient-reported outcomes. In *JAMA Oncology*. https://doi.org/10.1001/jamaoncol.2015.4719

Benaroya-Milshtein, N., Apter, A., Yaniv, I., Yuval, O., Stern, B., Bengal, Y., Kodman, Y., Shemer, E., Pick, C. G., Buchval, I., & Valevski, A. (2014). Neuroimmunological function in parents of children suffering from cancer. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *121*(3), 299–306. https://doi.org/10.1007/s00702-013-1098-6

Bitsko, M. J., Cohen, D., Dillon, R., Harvey, J., Krull, K., & Klosky, J. L. (2016). Psychosocial Late Effects in Pediatric Cancer Survivors: A Report From the Children's Oncology Group. In *Pediatric Blood and Cancer* (Vol. 63, Issue 2, pp. 337–343). John Wiley and Sons Inc. https://doi.org/10.1002/pbc.25773

Bower, J. E. (2007). Cancer-related fatigue: Links with inflammation in cancer patients and survivors. *Brain, Behavior, and Immunity*, *21*(7), 863–871. https://doi.org/10.1016/j.bbi.2007.03.013

Bower, J. E., Ganz, P. A., May, L. T., Hu, W., Belin, T. R., Sepah, S., Cole, S., & Aziz, N. (2009). Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer. *Clinical Cancer Research*, *15*(17), 5534–5540. https://doi.org/10.1158/1078-0432.CCR-08-2584

Casaletto, K. B., Staffaroni, A. M., Elahi, F., Fox, E., Crittenden, P. A., You, M., Neuhaus, J., Glymour, M., Bettcher, B. M., Yaffe, K., & Kramer, J. H. (2018). Perceived Stress is Associated with Accelerated Monocyte/Macrophage Aging Trajectories in Clinically Normal Adults. *American Journal of Geriatric Psychiatry*, *26*(9), 952–963. https://doi.org/10.1016/j.jagp.2018.05.004

Centers for Disease Control and Prevention. (2022). *Tracking Pediatric and Young Adult Cancer Cases*. https://www.cdc.gov/cancer/npcr/pediatric-young-adult-cancer.htm

Chang, J. S., Zhou, M., Buffler, P. A., Chokkalingam, A. P., Metayer, C., & Wiemels, J. L. (2011). Profound deficit of IL10 at birth in children who develop childhood acute lymphoblastic leukemia. *Cancer Epidemiology Biomarkers and Prevention*. https://doi.org/10.1158/1055-9965.EPI-11-0162

Cheung, Y. T., Brinkman, T. M., Mulrooney, D. A., Mzayek, Y., Liu, W., Banerjee, P., Panoskaltsis-Mortari, A., Srivastava, D., Pui, C. H., Robison, L. L., Hudson, M. M., & Krull, K. R. (2017). Impact of sleep, fatigue, and systemic inflammation on neurocognitive and behavioral outcomes in long-term survivors of childhood acute lymphoblastic leukemia. *Cancer*, *123*(17), 3410–3419. https://doi.org/10.1002/cncr.30742

Chiappelli, F., Iribarren, F. J. I., & Prolo, P. (2006). Salivary biomarkers in psychobiological medicine. *Bioinformation*, *1*(8), 331–334. https://doi.org/10.6026/97320630001331

Chorpita, B. F., Yim, L., Moffitt, C., Umemoto, L. A., & Francis, S. E. (2000). Assessment of symptoms of DSM-IV anxiety and depression in children: A

revised child anxiety and depression scale. *Behaviour Research and Therapy*, *38*(8), 835–855. https://doi.org/10.1016/S0005-7967(99)00130-8

Chrousos, G. P. (2009). Stress and disorders of the stress system. In *Nature Reviews Endocrinology* (Vol. 5, Issue 7, pp. 374–381). Nature Publishing Group. https://doi.org/10.1038/nrendo.2009.106

Cohen, S., Janicki-Deverts, D., & Miller, G. E. (2007). Psychological stress and disease. In *Journal of the American Medical Association* (Vol. 298, Issue 14, pp. 1685–1687). American Medical Association. https://doi.org/10.1001/jama.298.14.1685

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.2307/2136404

Cohen, Z. P., Cosgrove, K. T., Akeman, E., Coffey, S., Teague, K., Hays-Grudo, J., Paulus, M. P., Aupperle, R. L., & Kirlic, N. (2021). The effect of a mindfulness-based stress intervention on neurobiological and symptom measures in adolescents with early life stress: a randomized feasibility study. *BMC Complementary Medicine and Therapies*, *21*(1), 123. https://doi.org/10.1186/s12906-021-03295-1

Collado-Hidalgo, A., Bower, J. E., Ganz, P. A., Cole, S. W., & Irwin, M. R. (2006). Inflammatory biomarkers for persistent fatigue in breast cancer survivors. *Clinical Cancer Research*, *12*(9), 2759–2766. https://doi.org/10.1158/1078-0432.CCR-05-2398

Collins, J. J., Byrnes, M. E., Dunkel, I. J., Lapin, J., Nadel, T., Thaler, H. T., Polyak, T., Rapkin, B., & Portenoy, R. K. (2000). The Measurement of Symptoms in Children with Cancer. *Journal of Pain and Symptom Management*, *19*(5), 363–377. http://www.sciencedirect.com/science/article/B6T8R-40JFY9K-B/2/4272e608a07530760583334c446fc96a

Collins, J. J., Devine, T. D., Dick, G. S., Johnson, E. A., Kilham, H. A., Pinkerton, C. R., Stevens, M. M., Thaler, H. T., & Portenoy, R. K. (2002). The measurement of symptoms in young children with cancer: the validation of the Memorial Symptom Assessment Scale in children aged 7-12. *J Pain Symptom Manage*, *23*(1), 10–16. https://doi.org/S088539240100375X [pii]

Condon, E. M. (2018). Chronic Stress in Children and Adolescents: A Review of Biomarkers for Use in Pediatric Research. *Biological Research for Nursing*, *20*(5), 473–496. https://doi.org/10.1177/1099800418779214

Crosswell, A. D., Bower, J. E., & Ganz, P. A. (2014). Childhood adversity and inflammation in breast cancer survivors. *Psychosomatic Medicine*, *76*(3), 208–214. https://doi.org/10.1097/PSY.0000000000000041

Dionne-Odom, J. N., Demark-Wahnefried, W., Taylor, R. A., Rocque, G. B., Azuero, A., Acemgil, A., Martin, M. Y., Astin, M., Ejem, D., Kvale, E., Heaton, K., Pisu, M., Partridge, E. E., & Bakitas, M. A. (2017). The self-care practices of family caregivers of persons with poor prognosis cancer: differences by varying levels

of caregiver well-being and preparedness. *Supportive Care in Cancer*, *25*(8), 2437–2444. https://doi.org/10.1007/s00520-017-3650-7

Dixon, D., Meng, H., Goldberg, R., Schneiderman, N., & Delamater, A. (2009). Stress and Body Mass Index Each Contributes Independently to Tumor Necrosis Factor-α Production in Prepubescent Latino Children. *Journal of Pediatric Nursing*, *24*(5), 378–388. https://doi.org/10.1016/j.pedn.2008.02.034

Dunn, T. J., & Dimolareva, M. (2022). The effect of mindfulness-based interventions on immunity-related biomarkers: a comprehensive meta-analysis of randomised controlled trials. *Clinical Psychology Review*, *92*, 102124. https://doi.org/10.1016/j.cpr.2022.102124

Esterling, B. A., Kiecolt-Glaser, J. K., & Glaser, R. (1996). Psychosocial modulation of cytokine-induced natural killer cell activity in older adults. *Psychosomatic Medicine*, *58*(3), 264–272. https://doi.org/10.1097/00006842-199605000-00010

FDA-NIH Biomarker Working Group. (2016). *BEST (Biomarkers, EndpointS, and other Tools) Resource*. Food and Drug Administration (US). http://www.ncbi.nlm.nih.gov/books/NBK326791/

Fortier, M. A., Chung, W. W., Martinez, A., Gago-Masague, S., & Sender, L. (2016). Pain Buddy: A Novel Use of m-Health in the Management of Children's Cancer Pain. *Computers in Biology and Medicine*, *76*, 202–214. https://doi.org/10.1016/j.compbiomed.2016.07.012

Fox, S. E., Levitt, P., & Nelson, C. A. (2010). How the timing and quality of early experiences influence the development of brain architecture. In *Child Development* (Vol. 81, Issue 1, pp. 28–40). Child Dev. https://doi.org/10.1111/j.1467-8624.2009.01380.x

Glover, D. A., Steele, A., Stuber, M., & Fahey, J. (2005). Preliminary evidence for lymphocyte distribution differences at rest and after acute psychological stress in PTSD-symptomatic women. *Brain, Behavior, and Immunity*, *19*(3), 243–251. https://doi.org/10.1016/j.bbi.2004.08.002

Graham, J. E., Robles, T. F., Kiecolt-Glaser, J. K., Malarkey, W. B., Bissell, M. G., & Glaser, R. (2006). Hostility and pain are related to inflammation in older adults. *Brain, Behavior, and Immunity*, *20*(4), 389–400. https://doi.org/https://doi.org/10.1016/j.bbi.2005.11.002

Granger, D. A., Johnson, S. B., Szanton, S. L., Out, D., & Schumann, L. L. (2012). Incorporating Salivary Biomarkers Into Nursing Research: An Overview and Review of Best Practices. *Biological Research for Nursing*, *14*(4), 347–356. https://doi.org/10.1177/1099800412443892

Granger, D. A., Kivlighan, K. T., Fortunato, C., Harmon, A. G., Hibel, L. C., Schwartz, E. B., & Whembolua, G. L. (2007). Integration of salivary biomarkers into developmental and behaviorally-oriented research: Problems and solutions for collecting specimens. *Physiology and Behavior*, *92*(4), 583–590. https://doi.org/10.1016/j.physbeh.2007.05.004

Hendershot, E., Murphy, C., Doyle, S., Van-Clieaf, J., Lowry, J., & Honeyford, L. (2005). Outpatient chemotherapy administration: Decreasing wait times for patients and families. *Journal of Pediatric Oncology Nursing*, *22*(1), 31–37. https://doi.org/10.1177/1043454204272539

Hertzman, C., & Boyce, T. (2010). How experience gets under the skin to create gradients in developmental health. In *Annual Review of Public Health* (Vol. 31, pp. 329–347). Annu Rev Public Health. https://doi.org/10.1146/annurev.publhealth.012809.103538

Huffhines, L., Jackson, Y., McGuire, A., & Schreier, H. M. C. (2021). The intergenerational interplay of adversity on salivary inflammation in young children and caregivers. *Psychoneuroendocrinology*, *128*(April), 105222. https://doi.org/10.1016/j.psyneuen.2021.105222

Hunter, J. F., Acevedo, A. M., Gago-Masague, S., Kain, A., Yun, C., Torno, L., Jenkins, B. N., & Fortier, M. A. (2020). A pilot study of the preliminary efficacy of Pain Buddy: A novel intervention for the management of children's cancer-related pain. *Pediatric Blood & Cancer*, *67*(10), e28278. https://doi.org/https://doi.org/10.1002/pbc.28278

Ilic, V., Nikitovic, M., Maric, G., Jovanovic, A., Paripovic, L., Bokun, J., Stanic, D., Vukovic, M. P., Tepavcevic, D. K., & Pekmezovic, T. (2020). Assessment of health-related quality of life among parents of children with solid tumors in Serbia. *Supportive Care in Cancer*, *28*(11), 5109–5115. https://doi.org/10.1007/s00520-020-05348-6

Irwin, M. R., & Cole, S. W. (2011). Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol*, *11*(9), 625–632. https://doi.org/10.1038/nri3042

Johnson, S. B., Riley, A. W., Granger, D. A., & Riis, J. (2013). The science of early life toxic stress for pediatric practice and advocacy. *Pediatrics*, *131*(2), 319–327. https://doi.org/10.1542/peds.2012-0469

Juster, R. P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. In *Neuroscience and Biobehavioral Reviews* (Vol. 35, Issue 1, pp. 2–16). Pergamon. https://doi.org/10.1016/j.neubiorev.2009.10.002

Kearney, J. A., Salley, C. G., & Muriel, A. C. (2015). Standards of Psychosocial Care for Parents of Children With Cancer. *Pediatric Blood & Cancer*, *62*(S5), S632–S683. https://doi.org/10.1002/pbc.25761

Kibune, R., Muraoka, K., Morishita, M., Ariyoshi, W., & Awano, S. (2022). Relationship between Dynamics of TNF-α and Its Soluble Receptors in Saliva and Periodontal Health State. *Dentistry Journal*, *10*(2), 1–10. https://doi.org/10.3390/dj10020025

Kiecolt-Glaser, J. K., Glaser, R., Gravenstein, S., Malarkey, W. B., & Sheridan, J. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proceedings of the National Academy of Sciences of the United*

States of America, *93*(7), 3043–3047. https://doi.org/10.1073/pnas.93.7.3043

Kim, Y., & Carver, C. S. (2019). Unmet needs of family cancer caregivers predict quality of life in long-term cancer survivorship. *Journal of Cancer Survivorship*, *13*(5), 749–758. https://doi.org/10.1007/s11764-019-00794-6

Kim, Y., Schulz, R., & Carver, C. S. (2007). Benefit finding in the cancer caregiving experience. *Psychosomatic Medicine*, *69*(3), 283–291. https://doi.org/10.1097/PSY.0b013e3180417cf4

Kuster, P. A., & Merkle, C. J. (2004). Caregiving stress, immune function, and health: Implications for research with parents of medically fragile children. *Issues in Comprehensive Pediatric Nursing*, *27*(4), 257–276. https://doi.org/10.1080/01460860490884165

Laudenslager, M. L., Simoneau, T. L., Kilbourn, K., Natvig, C., Philips, S., Spradley, J., Benitez, P., Mcsweeney, P., & Mikulich-Gilbertson, S. K. (2015). A randomized control trial of a psychosocial intervention for caregivers of allogeneic hematopoietic stem cell transplant patients: Effects on distress. *Bone Marrow Transplantation*, *50*(8), 1110–1118. https://doi.org/10.1038/bmt.2015.104

Lengacher, C. A., Reich, R. R., Paterson, C. L., Shelton, M., Shivers, S., Ramesar, S., Pleasant, M. L., Budhrani-Shani, P., Groer, M., Post-White, J., Johnson-Mallard, V., Kane, B., Cousin, L., Moscoso, M. S., Romershausen, T. A., & Park, J. Y. (2019). A Large Randomized Trial: Effects of Mindfulness-Based Stress Reduction (MBSR) for Breast Cancer (BC) Survivors on Salivary Cortisol and IL-6. *Biological Research for Nursing*, *21*(1), 39–49. https://doi.org/10.1177/1099800418789777

Liaw, C. C., Chen, J. S., Chang, H. K., Huang, J. S., Yang, T. S., & Liau, C. T. (2008). Symptoms and signs of port-related infections in oncology patients related to the offending pathogens. *International Journal of Clinical Practice*, *62*(8), 1193–1198. https://doi.org/10.1111/j.1742-1241.2008.01746.x

Litzelman, K., Catrine, K., Gangnon, R., & Witt, W. P. (2011). Quality of life among parents of children with cancer or brain tumors: The impact of child characteristics and parental psychosocial factors. *Quality of Life Research*, *20*(8), 1261–1269. https://doi.org/10.1007/s11136-011-9854-2

McEwen, B. S. (2003). Interacting mediators of allostasis and allostatic load: Towards an understanding of resilience in aging. *Metabolism: Clinical and Experimental*, *52*(SUPPL. 2), 10–16. https://doi.org/10.1016/S0026-0495(03)00295-6

McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. In *European Journal of Pharmacology* (Vol. 583, Issues 2–3, pp. 174–185). Eur J Pharmacol. https://doi.org/10.1016/j.ejphar.2007.11.071

McEwen, B. S. (2012). Brain on stress: How the social environment gets under the skin. *Proceedings of the National Academy of Sciences of the United States of*

America, *109*(SUPPL.2), 17180–17185. https://doi.org/10.1073/pnas.1121254109

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. In *Hormones and Behavior* (Vol. 43, Issue 1, pp. 2–15). Academic Press Inc. https://doi.org/10.1016/S0018-506X(02)00024-7

McGuire, D. B. (2004). Occurrence of cancer pain. *Journal of the National Cancer Institute*, *32*, 51-6.

Miller, A. H., Ancoli-Israel, S., Bower, J. E., Capuron, L., & Irwin, M. R. (2008). Neuroendocrine-immune mechanisms of behavioral comorbidities in patients with cancer. *Journal of Clinical Oncology*, *26*(6), 971–982. https://doi.org/10.1200/JCO.2007.10.7805

Miller, G. E., Cohen, S., & Ritchey, A. K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. *Health Psychology*, *21*(6), 531–541. https://doi.org/10.1037/0278-6133.21.6.531

Miller, G. E., Murphy, M. L. M., Cashman, R., Ma, R., Ma, J., Arevalo, J. M. G., Kobor, M. S., & Cole, S. W. (2014). Greater inflammatory activity and blunted glucocorticoid signaling in monocytes of chronically stressed caregivers. *Brain, Behavior, and Immunity*, *41*(1), 191–199. https://doi.org/10.1016/j.bbi.2014.05.016

Miyasaka, T., Dobashi-Okuyama, K., Takahashi, T., Takayanagi, M., & Ohno, I. (2018). The interplay between neuroendocrine activity and psychological stress-induced exacerbation of allergic asthma. In *Allergology International* (Vol. 67, Issue 1, pp. 32–42). Japanese Society of Allergology. https://doi.org/10.1016/j.alit.2017.04.013

Morgenstern, D. A., & Anderson, J. (2012). Inflammation: What role in pediatric cancer? In *Pediatric Blood and Cancer*. https://doi.org/10.1002/pbc.24008

Mory, N., Coyle, N., Essandoh, S., & Glare, P. (2010). Chronic pain management in cancer survivors. In *JNCCN Journal of the National Comprehensive Cancer Network* (Vol. 8, Issue 9, pp. 1104–1110). Harborside Press. https://doi.org/10.6004/jnccn.2010.0079

Mundy-Bosse, B. L., Thornton, L. M., Yang, H. C., Andersen, B. L., & Carson, W. E. (2011). Psychological stress is associated with altered levels of myeloid-derived suppressor cells in breast cancer patients. *Cellular Immunology*, *270*(1), 80–87. https://doi.org/10.1016/j.cellimm.2011.04.003

Munn, L. L. (2017). Cancer and inflammation. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *9*(2), 1–13. https://doi.org/10.1002/wsbm.1370

Nelson, E. L., Wenzel, L. B., Osann, K., Dogan-Ates, A., Chantana, N., Reina-Patton, A., Laust, A. K., Nishimoto, K. P., Chicz-DeMet, A., Du Pont, N., & Monk, B. J. (2008). Stress, immunity, and cervical cancer: Biobehavioral

outcomes of a randomized clinical trail. *Clinical Cancer Research*, *14*(7), 2111–2118. https://doi.org/10.1158/1078-0432.CCR-07-1632

O'Connor, M. F., Bower, J. E., Cho, H. J., Creswell, J. D., Dimitrov, S., Hamby, M. E., Hoyt, M. A., Martin, J. L., Robles, T. F., Sloan, E. K., Thomas, K. M. S., & Irwin, M. R. (2009). To assess, to control, to exclude: Effects of biobehavioral factors on circulating inflammatory markers. *Brain, Behavior, and Immunity*, *23*(7), 887–897. https://doi.org/10.1016/j.bbi.2009.04.005

Oliveira, K. G., von Zeidler, S. V., Lamas, A. Z., de Podesta, J. R. V., Sena, A., Souza, E. D., Lenzi, J., Lemos, E. M., Gouvea, S. A., & Bissoli, N. S. (2014). Relationship of inflammatory markers and pain in patients with head and neck cancer prior to anticancer therapy. *Brazilian Journal of Medical and Biological Research*, *47*(7), 600–604. https://doi.org/10.1590/1414-431X20143599

Orre, I. J., Reinertsen, K. V., Aukrust, P., Dahl, A. A., Fosså, S. D., Ueland, T., & Murison, R. (2011). Higher levels of fatigue are associated with higher CRP levels in disease-free breast cancer survivors. *Journal of Psychosomatic Research*, *71*(3), 136–141. https://doi.org/10.1016/j.jpsychores.2011.04.003

Oswald, L. B., Fox, R. S., Murphy, K. M., Salsman, J. M., Sanford, S. D., McDade, T. W., & Victorson, D. E. (2022). Preliminary Effects of Mindfulness Training on Inflammatory Markers and Blood Pressure in Young Adult Survivors of Cancer: Secondary Analysis of a Pilot Randomized Controlled Trial. *International Journal of Behavioral Medicine*, *29*(5), 676–684. https://doi.org/10.1007/s12529-021-10050-w

Ouellet-Morin, I., Danese, A., Williams, B., & Arseneault, L. (2011). Validation of a high-sensitivity assay for C-reactive protein in human saliva. *Brain, Behavior, and Immunity*, *25*(4), 640–646. https://doi.org/10.1016/j.bbi.2010.12.020

Out, D., Granger, D. A., Sephton, S. E., & Segerstrom, S. C. (2013). Disentangling sources of individual differences in diurnal salivary alpha-amylase: reliability, stability and sensitivity to context. *Psychoneuroendocrinology*, *38*(3), 367–375. https://doi.org/10.1016/j.psyneuen.2012.06.013

Park, J., Ross, A., Klagholz, S. D., & Bevans, M. F. (2018). The Role of Biomarkers in Research on Caregivers for Cancer Patients: A Scoping Review. *Biological Research for Nursing*, *20*(3), 300–311. https://doi.org/10.1177/1099800417740970

Paudel, D., Morikawa, T., Yoshida, K., Uehara, O., Giri, S., Neopane, P., Khurelchuluun, A., Hiraki, D., Sato, J., & Abiko, Y. (2020). Chronic stress-induced elevation of IL-1β in the saliva and submandibular glands of mice. *Medical Molecular Morphology*, *53*(4), 238–243. https://doi.org/10.1007/s00795-020-00250-w

Payne, L. A., & Fortier, M. A. (2020). Salivary Bioscience and Pain. In D. A. Granger & M. K. Taylor (Eds.), *Salivary Bioscience: Foundations of Interdisciplinary Saliva Research and Applications* (pp. 321–348). Springer International Publishing. https://doi.org/10.1007/978-3-030-35784-9_14

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. *Journal of Clinical Investigation*, *111*(12), 1805–1812. https://doi.org/10.1172/jci18921

Pierce, B. L., Ballard-Barbash, R., Bernstein, L., Baumgartner, R. N., Neuhouser, M. L., Wener, M. H., Baumgartner, K. B., Gilliland, F. D., Sorensen, B. E., McTiernan, A., & Ulrich, C. M. (2009). Elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients. *Journal of Clinical Oncology*, *27*(21), 3437–3444. https://doi.org/10.1200/JCO.2008.18.9068

Powell, N. D., Tarr, A. J., & Sheridan, J. F. (2013). Psychosocial stress and inflammation in cancer. In *Brain, Behavior, and Immunity* (Vol. 30, Issue SUPPL., pp. S41–S47). Academic Press. https://doi.org/10.1016/j.bbi.2012.06.015

Rensen, N., Steur, L. M. H., Schepers, S. A., Merks, J. H. M., Moll, A. C., Grootenhuis, M. A., Kaspers, G. J. L., & van Litsenburg, R. R. L. (2019). Concurrence of sleep problems and distress: prevalence and determinants in parents of children with cancer. *European Journal of Psychotraumatology*, *10*(1). https://doi.org/10.1080/20008198.2019.1639312

Riis, J. L., Ahmadi, H., Hamilton, K. R., Hand, T., & Granger, D. A. (2020). Best Practice Recommendations for the Measurement and Interpretation of Salivary Proinflammatory Cytokines in Biobehavioral Research. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2020.09.009

Riis, J. L., Byrne, M. L., Hernández, L. M., & Robles, T. F. (2020). Salivary Bioscience, Immunity, and Inflammation. In D. A. Granger & M. K. Taylor (Eds.), *Salivary Bioscience: Foundations of Interdisciplinary Saliva Research and Applications* (pp. 177–213). Springer International Publishing. https://doi.org/10.1007/978-3-030-35784-9_9

Riis, J. L., Granger, D. A., DiPietro, J. A., Bandeen-Roche, K., & Johnson, S. B. (2015). Salivary cytokines as a minimally-invasive measure of immune functioning in young children: Correlates of individual differences and sensitivity to laboratory stress. *Developmental Psychobiology*, *57*(2), 153–167. https://doi.org/10.1002/dev.21271

Riis, J. L., Out, D., Dorn, L. D., Beal, S. J., Denson, L. A., Pabst, S., Jaedicke, K., & Granger, D. A. (2014). Salivary cytokines in healthy adolescent girls: Intercorrelations, stability, and associations with serum cytokines, age, and pubertal stage. *Developmental Psychobiology*, *56*(4), 797–811. https://doi.org/10.1002/dev.21149

Robb, S. L., & Hanson-Abromeit, D. (2014). A review of supportive care interventions to manage distress in young children with cancer and parents. *Cancer Nursing*, *37*(4), 1–26. https://doi.org/10.1097/NCC.0000000000000095

Sahibzada, H. A., Khurshid, Z., Khan, R. S., Naseem, M., Siddique, K. M., Mali, M., & Zafar, M. S. (2017). Salivary IL-8, IL-6 and TNF-α as potential diagnostic

biomarkers for oral cancer. *Diagnostics*, *7*(2), 1–10. https://doi.org/10.3390/diagnostics7020021

Shonkoff, J. P., Garner, A. S., Health, T. C. on P. A. of C. and F., Childhood, C. on E., Adoption, Care, A. D., Pediatrics, A. S. on D. and B., Siegel, B. S., Dobbins, M. I., Earls, M. F., Garner, A. S., McGuinn, L., Pascoe, J., & Wood, D. L. (2012). The Lifelong Effects of Early Childhood Adversity and Toxic Stress. *Pediatrics*, *129*(1), e232–e246. https://doi.org/10.1542/peds.2011-2663

Siegel, B. S., Dobbins, M. I., Earls, M. F., Garner, A. S., Pascoe, J., Wood, D. L., High, P. C., Donoghue, E., Fussell, J. J., Gleason, M. M., Jaudes, P. K., Jones, V. F., Rubin, D. M., Schulte, E. E., Macias, M. M., Bridgemohan, C., Goldson, E., McGuinn, L. J., Weitzman, C., & Wegner, L. M. (2012). Early childhood adversity, toxic stress, and the role of the pediatrician: Translating developmental science into lifelong health. In *Pediatrics* (Vol. 129, Issue 1, pp. e224–e231). American Academy of Pediatrics. https://doi.org/10.1542/peds.2011-2662

Slavish, D. C., Graham-Engeland, J. E., Smyth, J. M., & Engeland, C. G. (2015). Salivary markers of inflammation in response to acute stress. In *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2014.08.008

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-reactive protein at sites of inflammation and infection. In *Frontiers in Immunology* (Vol. 9, Issue APR, p. 754). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2018.00754

Stavinoha, P. L., Olsthoorn, I. M., Swartz, M. C., Nowakowski, S., Wells, S. J., Hicklen, R. S., Sheikh, I., & Jang, H. J. (2021). Non-pharmacological sleep interventions for pediatric cancer patients and survivors: a systematic review protocol. *Systematic Reviews*, *10*(1), 1–7. https://doi.org/10.1186/s13643-021-01724-3

Sung, L., Yanofsky, R., Klaassen, R. J., Dix, D., Pritchard, S., Winick, N., Alexander, S., & Klassen, A. (2011). Quality of life during active treatment for pediatric acute lymphoblastic leukemia. *International Journal of Cancer*, *128*(5), 1213–1220. https://doi.org/10.1002/ijc.25433

Szabo, Y. Z., & Slavish, D. C. (2021). Measuring salivary markers of inflammation in health research: A review of methodological considerations and best practices. In *Psychoneuroendocrinology* (Vol. 124, Issue 254, pp. 1–28). https://doi.org/10.1016/j.psyneuen.2020.105069

Thornton, L. M., Andersen, B. L., Crespin, T. R., & Carson, W. E. (2007). Individual trajectories in stress covary with immunity during recovery from cancer diagnosis and treatments. *Brain, Behavior, and Immunity*, *21*(2), 185–194. https://doi.org/10.1016/j.bbi.2006.06.007

Tottenham, N., & Sheridan, M. A. (2010). A review of adversity, the amygdala and the hippocampus: A consideration of developmental timing. *Frontiers in Human Neuroscience*, *3*(JAN). https://doi.org/10.3389/neuro.09.068.2009

Tsai, M. H., Hsu, J. F., Chou, W. J., Yang, C. P., Jaing, T. H., Hung, I. J., Liang, H.

F., Huang, H. R., & Huang, Y. S. (2013). Psychosocial and emotional adjustment for children with pediatric cancer and their primary caregivers and the impact on their health-related quality of life during the first 6 months. *Quality of Life Research*, *22*(3), 625–634. https://doi.org/10.1007/s11136-012-0176-9

Ullrich, A., Marx, G., Bergelt, C., Benze, G., Zhang, Y., Wowretzko, F., Heine, J., Dickel, L. M., Nauck, F., Bokemeyer, C., & Oechsle, K. (2021). Supportive care needs and service use during palliative care in family caregivers of patients with advanced cancer: a prospective longitudinal study. *Supportive Care in Cancer*, *29*(3), 1303–1315. https://doi.org/10.1007/s00520-020-05565-z

Van Cleve, L., Bossert, E., Beecroft, P., Adlard, K., Alvarez, O., & Savedra, M. C. (2004). The Pain Experience of Children With Leukemia During the First Year After Diagnosis. *Nursing Research*, *53*(1), 1–10. http://journals.lww.com/nursingresearchonline/Fulltext/2004/01000/The_Pain_Experience_of_Children_With_Leukemia.1.aspx

Varni, J. W., Burwinkle, T. M., Katz, E. R., Meeske, K., & Dickinson, P. (2002). The PedsQL™ in pediatric cancer: Reliability and validity of the Pediatric Quality of Life Inventory™ Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. *Cancer*, *94*(7), 2090–2106. https://doi.org/10.1002/cncr.10428

Varni, J. W., Limbers, C., & Burwinkle, T. M. (2007). Literature review: Health-related quality of life measurement in pediatric oncology: Hearing the voices of the children. In *Journal of Pediatric Psychology* (Vol. 32, Issue 9, pp. 1151–1163). https://doi.org/10.1093/jpepsy/jsm008

Vignali, D. A. A. (2000). Multiplexed particle-based flow cytometric assays. *Journal of Immunological Methods*, *243*(1–2), 243–255. https://doi.org/10.1016/S0022-1759(00)00238-6

Walsh, E., Eisenlohr-Moul, T., & Baer, R. (2016). Brief mindfulness training reduces salivary IL-6 and TNF-α in young women with depressive symptomatology. *Journal of Consulting and Clinical Psychology*, *84*(10), 887–897. https://doi.org/10.1037/ccp0000122

Wang, T., Molassiotis, A., Chung, B. P. M., & Tan, J. Y. (2018). Unmet care needs of advanced cancer patients and their informal caregivers: a systematic review. *BMC Palliative Care*, *17*(1), 1–29. https://doi.org/10.1186/s12904-018-0346-9

Wenzel, L., Osann, K., Hsieh, S., Tucker, J. A., Monk, B. J., & Nelson, E. L. (2015). Psychosocial telephone counseling for survivors of cervical cancer: Results of a randomized biobehavioral trial. *Journal of Clinical Oncology*, *33*(10), 1171–1179. https://doi.org/10.1200/JCO.2014.57.4079

Wohleb, E. S., & Delpech, J. C. (2017). Dynamic cross-talk between microglia and peripheral monocytes underlies stress-induced neuroinflammation and behavioral consequences. In *Progress in Neuro-Psychopharmacology and Biological Psychiatry* (Vol. 79, pp. 40–48). Elsevier Inc. https://doi.org/10.1016/j.pnpbp.2016.04.013

Wohleb, E. S., McKim, D. B., Sheridan, J. F., & Godbout, J. P. (2015). Monocyte trafficking to the brain with stress and inflammation: A novel axis of immune-to-brain communication that influences mood and behavior. In *Frontiers in Neuroscience* (Vol. 9, Issue JAN, p. 447). Frontiers Media S.A. https://doi.org/10.3389/fnins.2014.00447

Zhang, L., Pan, J., Chen, W., Jiang, J., & Huang, J. (2020). Chronic stress-induced immune dysregulation in cancer: implications for initiation, progression, metastasis, and treatment. *American Journal of Cancer Research*, *10*(5), 1294–1307. http://www.ncbi.nlm.nih.gov/pubmed/32509380

Appendix A

B-Cell Acute Lymphoblastic Leukemia (ALL) Treatment Schema at Children's Hospital of Orange County

CHOC Children's

MRN: DOB:
PMD: CC:
NOT ON STUDY

Acute Lymphoblastic Leukemia (ALL), B-Cell

SCHEMA NOT ON STUDY

NCI Standard Risk
Age 1-9.99 years **AND** WBC < 50,000
3 DRUG INDUCTION -DEX for 28 days

NCI High Risk
Age ≥ 10 years **OR** WBC ≥ 50,000
4 DRUG INDUCTION:
< 10 years - DEX for 14 days
≥ 10 years - PDN for 28 days

CNS Status	Cytogenetics	MRD	TPMT	MTHFR
1 2a 2b 2c 3	BCR/ABL___iamp21___MLL____Hypodiploidy____ TEL/AML____DT ____	Day 8 PB ____% Day 29 BM____%		C677T _____ A1298C_____

Post-Induction Risk Stratification

	Average Risk (AR)		High Risk (HR) <13 years with Day 29 BM MRD< 0.01%; AR w/o favorable cytogenetics w/ Day 8 PB MRD ≥ 1% & Day 29 BM MRD <0.01%; AR w/favorable cytogenetics w/ any Day 8 PB MRD & Day 29 BM MRD ≥ 0.01% AR/HR w/ testicular disease (no other VHR factors)			Very High Risk (VHR) ≥13 year; CNS 3 @ dx; iAMP21; MLL rearrangement; Day 29 BM MRD ≥ 0.01%; Hypodiploidy; Ind Failure (M3 BM @ Day 29); AR w/o favorable cytogenetics, with D29 BM MRD ≥ 0.01% Hypodiploidy proceed to transplant after Consolidation.			
NCI Criteria	SR	SR	SR	SR	HR (<13 y/o)	SR	HR	HR (≥13 y/o)	SR or HR
Favorable	Any	No	Yes	No	Any	No	Any	Any	Any
Unfavorable	No	No	No	No	No	No	No	No	Yes
Day 8 PB MRD	≥ 0.01%	<1%	Any	≥1%	Any	Any	Any	Any	Any
Day 29 BM MRD	<0.01%	<0.01%	≥0.01%	<0.01%	<0.01%	≥0.01%	≥0.01%	<0.01%	Any

Average Risk
Based on AALL0932
Arm A

- Consolidation
- Interim Maintenance I (*Capizzi)*
- Delayed Intensification
- Interim Maintenance II *(Capizzi)*
- Maintenance *(Dexamethasone)*

High Risk
MBFM
Based on AALL1131
Arm A

- Consolidation *Testicular XRT if patient has testicular disease*
- Interim Maintenance *(HD Methotrexate)*
- Delayed Intensification
- Maintenance *(Prednisone)*

Very High Risk
MBFM
Based on AALL1131
Control Arm

- Consolidation
- Interim Maintenance I *(HD Methotrexate)*
- Delayed Intensification
- Interim Maintenance II *(Capizzi)*
- Maintenance *(Prednisone)* XRT in Cycle 1 if CNS 3

MBFM: Modified Berlin Frankfurt Munster
Favorable: ETV6-RUNX1 (TEL/AML) fusion OR double trisomy 4 and 10
Unfavorable: CNS3, hypodiploidy (< 44 chromosomes &/or DNA index <0.81), iAMP21, Induction failure (Day 29 M3 marrow),OR MLL rearrangement (not MLL deletion.
Note: BCR/ABL1 positive (Ph + ALL) patients are eligible for different treatment.
(Revised 7-15-2013)

Appendix B

Diffuse Large B-Cell Lymphoma (DLBCL) Treatment Schema at Children's Hospital of Orange County

On-Study: □ Yes □ No

Physician's Outpatient Chemotherapy Orders

Inotuzumab

Name: _____ MRN: _____ Subject ID#: _____

Diagnosis: _____ Protocol: AALL1732

Wt (Kg): _____ Ht (cm) _____ BSA (m^2): _____ InO Block: _____ Day: _____

Initiate Chemotherapy and Supportive Care Orders on: (Date) _____

**** All doses will be based on BSA calculated from the height and weight measured at the beginning of each block****

1. IV fluids: _____
2. Anti-Emetics (for nausea/vomiting):
   (Emetogenic Potential: Likely (>20%) nausea per AALL1732)
   ☐ ondansetron ODT ____ mg PO once. Begin 30 minutes pre-chemo (_____ mg/kg/dose)
   (4-11 yr: 4 - 12 mg/dose; >11 yr: 8 - 24 mg/dose) **- OR-**
   ☐ ondansetron _____ mg IV once over 15 minutes. Start 30 minutes pre-chemo
   (_____ mg/kg/dose) (0.15-0.45 mg/kg/dose; Max=16 mg/dose)
   ☐ _____
3. Premedication (strongly recommended per AALL1732)
   ☐ Diphenhydramine 1 mg/kg = ____ mg (max 50 mg) IV 30-60 min prior to infusion
   ☐ Methylprednisolone 1 mg/kg = ____ mg (max 100 mg) IV 30-60 min prior to infusion
   ☐ Acetaminophen 10 mg/kg = ____ mg (max 650 mg) PO 30-60 min prior to infusion
   ☐ Other: _____
4.

Inv-**Inotuzumab**	**Inotuzumab (Besponsa®)**
□ INVESTIGATIONAL supply Protocol: AALL1732	□ Commercial supply

   0.5 mg/m^2/dose = _____ mg in NS IV over 60 minutes on Days 1, 8, and 15
   - There must be a minimum of 6 days between inotuzumab doses
   - Round inotuzumab to the nearest 0.05 mg in patients with BSA ≥0.64 m^2. See Appendix XIV for BSA based dosing for patients with BSA <0.64 m^2.
   - IV bag (50 mL) conc = 0.01 – 0.1 mg/mL; Syringe conc = 0.025 – 0.1 mg/mL (Min = 2 mL)
   - Attach microbore tubing [Carefusion 30914] if <25 mL; Attach [CH-3147C] tubing if 25-50 mL.
   - Expires 8 hours refrigerated.
   - Protect dose from light. If infusion exceeds 1 hour, also protect IV tubing from light.
   - Monitor vital signs/symptoms of infusion reactions every 15 minutes during inotuzumab ozogamicin infusion, followed by every 30 minutes x 2 thereafter
5. Medications for possible inotuzumab infusion reactions (in Pyxis):
   a. Epinephrine 0.01 mg/kg = _____ mg (Maximum = 0.5 mg) (1:1000) intramuscularly to be repeated q 15-20 minutes prn (anterolateral aspect of the thigh)
   b. Diphenhydramine 1 mg/kg (50 mg MAX) = _____ mg IV once prn
   c. Hydrocortisone 1-2 mg/kg (100 mg MAX) = _____ mg IV once prn
   d. Normal saline 10 ml/kg (1000 ml MAX) = _____ ml IV once prn
   e. Ranitidine/famotidine _____ mg IV once prn

998546 Version Date: 5/5/16

Appendix C

Osteosarcoma Treatment Schema at Children's Hospital of Orange County

CHOC Children's

Osteosarcoma

AOST0331: MAP
Five consecutive weeks (35 days) will constitute one cycle.

LAST, First
MRN: DOB:
PMD: CC:
NOT ON STUDY

Criteria to start each cycle: ANC ≥ 750 and platelet count ≥ 75,000.
MTX at Weeks 4, 5, 9 & 10 can be administered as long as ANC ≥ 250 and platelet count ≥ 50,000

DRUG	ROUTE	DOSAGE	DAYS	NOTES	OBSERVATIONS
DOXOrubicin** (DOXO) w/ Zinecard	IV	37.5 mg/m²/dose	1 & 2 (Weeks 1 and 6)	**Administer w/ Zinecard	a. CBC w/ Diff, Platelets b. CMP c. Magnesium d. Urinalysis e. Echo f. Met Eval Audiogram recommended as baseline in Cycle 1 #Weekly after MTX and twice weekly after DOXO/CDDP
CISplatin (CDDP)	IV	60 mg/m2/dose	1 & 2 (Weeks 1 and 6)		
Methotrexate (MTX)	IV	12 g/m2/dose	1 (Weeks 4, 5, 9, & 10)	**Max Dose 20 g**	
Leucovorin+ (LCV)	PO or IV	According to Institutional Protocol	2 (Weeks 4, 5, 9 & 10)	+Leucovorin rescue to start 24 hours after start of MTX infusion	

Cycle 1 **Ht_____ cm** **Wt_____ kg** **BSA_____ m²**

Date Due	Date Given	Week	Day	DOXO** w/ Zinecard	CDDP	MTX	LCV	Observations	Comments (Include any held dose or dose modifications)
		1	1	mg	mg			a,b,c,d,e	
			2	mg	mg			a#	
		4	1			g		a#	
			2				Q mg hrs		Date of Last LCV Dose:
		5	1			g		a#	
			2				Q mg hrs		Date of Last LCV Dose:
		6	1	*Proceed to Next Cycle*					

Cycle 2 **Ht_____ cm** **Wt_____ kg** **BSA_____ m²**

Date Due	Date Given	Week	Day	DOXO** w/ Zinecard	CDDP	MTX	LCV	Observations	Comments (Include any held dose or dose modifications)
		6	1	mg	mg			a,b,c,d,e	
			2	mg	mg			a#	
		9	1			g		a#	
			2				Q mg hrs		Date of Last LCV Dose:
		10	1			g		a#	
			2				Q mg hrs	f	Date of Last LCV Dose:
			1	*Proceed to Surgery*					

Appendix D

Pilocytic Astrocytoma Treatment Schema at Children's Hospital of Orange County

CHOC Children's

(Treatment modeled from A9952) Maintenance Cycles 1-3

Drug	Route	Dose	Days	Studies
Carboplatin (CB)	IV	175 mg/m²/dose	0, 7, 14 and 21	a. CBC w/diff/plts b. Panel 18 c. Magnesium d. MRI of Brain(Every 3 months) e. Ophthalmology Evaluation (Every 6 months)
VinCristine (VCR)	IV	1.5 mg/m²dose (0.05 mg/kg if < 12 kg) Maximum dose 2.0 mg	0, 7 and 14	

Count Requirements to begin each Cycle: ANC ≥ 1000/µL and Platelets ≥ 100,000/µL for a total of 8 cycles

****Count Requirements for Days 7, 14 & 21: ANC ≥ 500/µL and Platelets ≥ 50,000/µL**

****NOTE**: If ANC < 500/µL and/or Platelets < 50,000/µL, hold Carboplatin and repeat counts every 7 days.
Resume @ 75% of full dose when count requirements have been met.

Cycle #: 1

Date Due	Cycle Day	Date Given	Carboplatin	VinCristine	Studies/Comments
	0				a, b, c, e
	7**				a
	14**				a
	21**				a
	28				
	35				
	41				d

Start next cycle on day 42 or when count criteria have been met.

Cycle #: 2

Date Due	Cycle Day	Date Given	Carboplatin	VinCristine	Studies/Comments
	0				a, b, c, e
	7**				a
	14**				a
	21**				a
	28				
	35				
	41				d

Start next cycle on day 42 or when count criteria have been met.

Cycle #: 3

Date Due	Cycle Day	Date Given	Carboplatin	VinCristine	Studies/Comments
	0				a, b, c, e
	7**				a
	14**				a
	21**				a
	28				
	35				
	41				d

Start next cycle on day 42 or when count criteria have been met.