AVICA - Acetaminophen Vs. Ibuprofen in Children with Asthma

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I. ABBREVIATIONS USED IN THIS PROTOCOL

AACD	Annualized number of asthma control days
AAP	American Academy of Pediatrics
ACD	Asthma control days
AE	Adverse event
AIMS	NHLBI/CARE Network Acute Intervention Management Strategies trial
API	Asthma Predictive Index
AVICA	Acetaminophen Versus Ibuprofen in Children with Asthma
BADGER	Best Add-on Therapy Giving Effective Response trial
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperresponsiveness
CLIC	Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid trial
COAST	Childhood Origins of Asthma birth cohort study
COX	Cyclooxygenase
CysLTs	Cysteinyl leukotrienes
ECP	Eosinophil cationic protein
EFD	Episode-free days
FeNO	Fraction of exhaled nitric oxide
FEF_{25-75}	Mid-expiratory flow rate at 25-75% of vital capacity
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GSH	Glutathione
HRV	Human rhinovirus
ICS	Inhaled corticosteroid
INFANT	Individualized Therapy for Asthma in Toddlers trial
ISAAC	International Study of Asthma and Allergies in Childhood
LTE_4	Leukotriene E4
LTRA	Leukotriene receptor antagonist
mAPI	Modified Asthma Predictive Index
MDI	Metered dose inhaler
MIST	Maintenance Intermittent Inhaled Steroids in Toddlers trial
NAEPP EPR-3	National Asthma Education and Prevention Program Expert Panel Report-3
NSAID	Non-steroidal anti-inflammatory drug
OTC	Over the Counter
PACT	Persistent Asthma Controller Therapy trial
PEAK	Prevention of Early Asthma in Kids
PGH_2	Prostaglandin H_2
PREVIA	Prevention of Viral Induced Asthma trial
RRA	Relative receptor affinity
RSV	Respiratory syncytial virus
RTI	Respiratory tract infection
SABA	Short-acting beta agonist
SAE	Severe adverse event
VHC	Valved holding chamber

II. PRINCIPAL HYPOTHESIS AND TRIAL SUMMARY

INFANT and AVICA are two separate, but linked, clinical trials that target preschool children, 12-59 months of age, who meet criteria for treatment with long-term, Step 2 asthma controller therapy.((EPR-3) 2007) These trials come together in a multi-center, prospective, randomized, double-blind factorial study. All children will be randomized in two processes: one to determine the sequence of controller therapy (INFANT), and the other to determine the analgesic-antipyretic medication (AVICA) to be used during the course of the study.

This protocol is for the clinical trial **AVICA** (**A**cetaminophen **V**ersus **I**buprofen in **C**hildren with **A**sthma). The AVICA study will test the primary hypothesis that in preschool children 12-59 months of age with persistent asthma on standardized asthma therapy, the number of asthma exacerbations requiring systemic corticosteroids will be more frequent in children randomized to receive acetaminophen as compared to those randomized to receive ibuprofen on an as needed basis for fevers and pain.

This is a randomized therapeutic trial involving two parallel treatment arms: 1) acetaminophen, and 2) ibuprofen. After a run-in period, participating children will enter the treatment portion of the study, where acetaminophen or ibuprofen will be administered as blinded active therapies to be used as needed per parental decision for fever and analgesia for a total duration of 48 weeks. During this time period, the enrolled children will have standardized asthma controller regimens as detailed in the INFANT protocol. The primary outcome of interest for AVICA is the number of asthma exacerbations per year, defined by a significant increase in asthma symptoms requiring treatment with systemic corticosteroids.

The AVICA study will address which is the most appropriate antipyretic-analgesic medication in young children with asthma, and will inform both clinicians and parents seeking to treat children with fever and pain. Given the high frequency of administration of these drugs, this study will have a significant impact on pediatric healthcare regardless of whether a differential effect is discovered as significant uncertainty currently exists as to whether acetaminophen use is associated with increased asthma symptoms.

The combination of the INFANT and AVICA studies will be beneficial as each study complements the other and is an efficient utilization of resources. INFANT will benefit the AVICA trial by providing a standardized protocol of asthma controller therapy for children randomized to acetaminophen or ibuprofen. AVICA will benefit the INFANT trial by standardizing the analgesic-antipyretic regimen to allow for equal uses of acetaminophen and ibuprofen in these young children. Since young children with asthma take both daily controller medications and frequently take as needed analgesic-antipyretics, it is important to evaluate the two medication interventions at the same time in the same population.

III. BACKGROUND AND RATIONALE

A. INTRODUCTION AND OVERVIEW OF THE CLINICAL PROBLEM

Analgesic-antipyretics (acetaminophen and ibuprofen) are very frequently used in preschool children. In fact, acetaminophen is the most commonly used medication for children with a weekly prevalence rate of 12%. Ibuprofen is the second most common childhood medication with a weekly prevalence rate of 7.9%. Overall, the weekly prevalence rate of either of these medications is 19% in children.(Vernacchio, Kelly et al. 2009) In the past decade, there has been growing observational evidence, suggesting an association between acetaminophen use and increased asthma symptoms.(Davey, Berhane et al. 2005; Beasley, Clayton et al. 2008) Furthermore, a post-hoc analysis of 1,879 children with asthma in a prospective acetaminophen and ibuprofen safety study of 84,192 children noted a marked increase in risk of unscheduled visits for asthma in the 4 weeks after acetaminophen use compared to ibuprofen.(Lesko, Louik et al. 2002) However, there are currently no published or ongoing prospective studies evaluating the relationship between acetaminophen or ibuprofen use and asthma exacerbations.

The AVICA study will therefore address this important unanswered question:
Is there a relationship between analgesic-antipyretic use (acetaminophen vs. ibuprofen) and the frequency of asthma exacerbations and asthma symptoms among young children with well-characterized asthma when evaluated prospectively in a randomized manner?

Given that acetaminophen and ibuprofen are the only two readily available, safe, and most widely used medications for fever and pain in young children, and the growing observational evidence that acetaminophen may increase risk of asthma, this trial will answer an important, real life question that parents and clinicians want to know. Does acetaminophen truly increase the risk of asthma exacerbations?

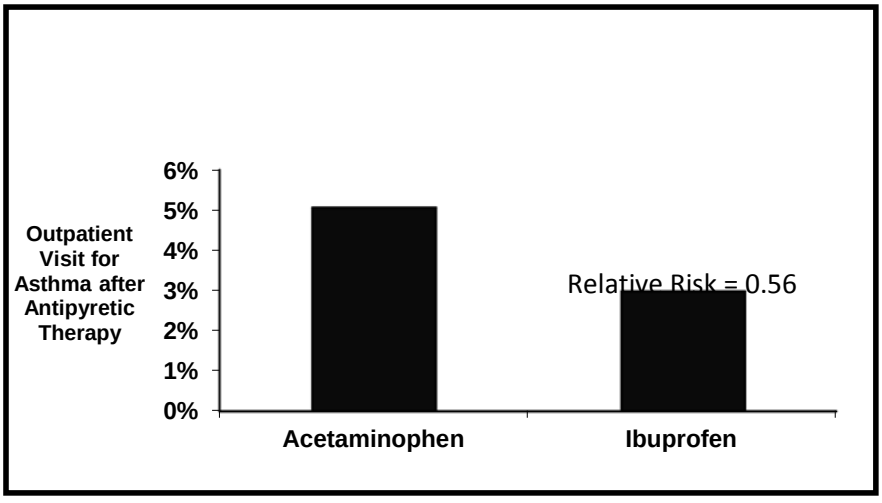
While the role of acetaminophen as contributing to the *development* of asthma is not the focus of this trial, it has been hypothesized that the change from aspirin to acetaminophen use, due to the association of Reye's syndrome in aspirin during the 1980's may have contributed to the rise in childhood asthma prevalence during the past few decades.(Varner, Busse et al. 1998; Johnson and Ownby 2011) Multiple observational studies have provided evidence that the risk of asthma may be increased with exposure to acetaminophen during prenatal periods,(Shaheen, Newson et al. 2002; Shaheen, Newson et al. 2005; Persky, Piorkowski et al. 2008; Rebordosa, Kogevinas et al. 2008; Garcia-Marcos, Sanchez-Solis et al. 2009; Perzanowski, Miller et al. 2010) infancy,(Beasley, Clayton et al. 2008; Wickens, Beasley et al. 2011) childhood,(Davey, Berhane et al. 2005; Beasley, Clayton et al. 2008) and adult life.(Shaheen, Sterne et al. 2000; Barr, Wentowski et al. 2004; Davey, Berhane et al. 2005; McKeever, Lewis et al. 2005; Shaheen, Potts et al. 2008; Thomsen, Kyvik et al. 2008) A few studies have suggested conflicting results.(Schnabel and Heinrich 2010; Scialli, Ang et al. 2010; Bakkeheim, Mowinckel et al. 2011) We acknowledge that a prospective birth cohort clinical trial evaluating the true role of prenatal acetaminophen use and the ultimate development of childhood asthma would be of interest; however, a study of this magnitude and required length of follow-up is beyond the scope of feasibility in AsthmaNet.

More relevant to this particular study, there is evidence that ***acetaminophen use in children who already have asthma may increase the risk of asthma symptoms***.(Lesko, Louik et al. 2002; Davey, Berhane et al. 2005; Beasley, Clayton et al. 2008) Data from the International

Study of Asthma and Allergies in Childhood (ISAAC) demonstrated a dose-dependent risk of current asthma symptoms with a 1.6- and 3.2-fold increased risk for moderate and high use of acetaminophen, respectively(Beasley, Clayton et al. 2008; Beasley, Clayton et al. 2011). Similarly, Davey et al found that current use of higher doses of acetaminophen was associated with a 1.9- fold increased risk of current wheeze(Davey, Berhane et al. 2005). The most striking evidence was found in the Boston Fever Study in which 84,192 children were prospectively randomized to blinded, matching acetaminophen and ibuprofen to determine ibuprofen safety in children. A post-hoc analysis of 1,879 of these children with asthma showed that those treated with ibuprofen for one febrile illness were almost half as likely to have a subsequent asthma exacerbation as compared to those treated with acetaminophen (adjusted relative risk = 0.56, 95% CI = 0.34-0.95)(Lesko, Louik et al. 2002). This study also showed that the risk of asthma exacerbation was reduced further (adjusted relative risk = 0.43, 95% CI = 0.24 – 0.79) if ibuprofen was given instead of acetaminophen in the specific setting of a respiratory infection (see Figure 1 below). While this was a prospective, randomized study, it was not specifically designed to answer the question of asthma symptoms or exacerbations. The results were determined by post-hoc analysis of all enrolled children who were identified to have asthma. As such, it represents only a fraction of all the children enrolled in the study. There are currently no prospective clinical trials published or ongoing to answer the question of whether this signal is real or not.

FIGURE 1:

Decreased Risk of Asthma Exacerbations 4 weeks after Treatment with Ibuprofen (as compared to Acetaminophen)



Any Fever:

RR = 0.56 (0.34 – 0.96)

Fever with Respiratory Infection:

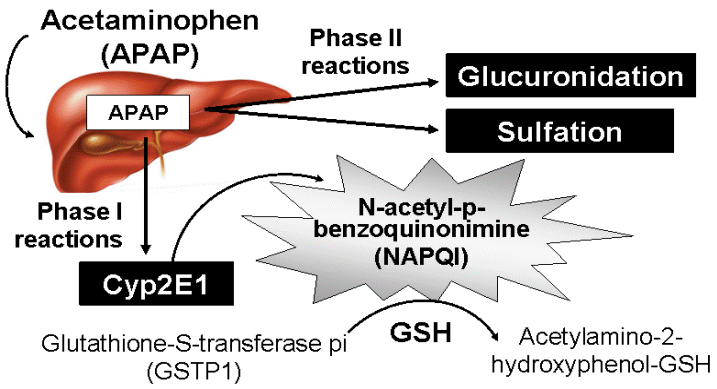
RR = 0.43 (0.24 – 0.79)

Lesko et al. Pediatrics, 2002

There are several plausible mechanisms that could explain the contribution of acetaminophen to worsening asthma. One possible mechanism is that acetaminophen decreases the amount of airway glutathione that may be protective for lungs. Glutathione a tri-peptide antioxidant that has a number of functions, including free radical scavenging and xenobiotic detoxification. In the case of acetaminophen, glutathione is essential for metabolism and excretion of the parent compound. After ingestion, acetaminophen undergoes detoxification in the liver by cytochrome p450 enzymes, primarily Cyp2E1, which oxidizes acetaminophen to form the highly reactive intermediate, N-acetyl-1,4-benzoquinonimine (NAPQI). NAPQI is then detoxified by glutathione-S-transferase pi1 (GSTP1)-mediated GSH conjugation(Dahlin, Miwa et al. 1984) (See Figure 2 below). While this process is important for detoxification of the parent acetaminophen compound, glutathione conjugation also irreversibly consumes glutathione stores (Chen, Richie et al. 1990; Kozer, Evans et al. 2003; Dimova, Hoet et al. 2005). In individuals with low glutathione reserves or glutathione depletion, acetaminophen conjugation may be impaired, leading to increased and prolonged circulation of highly reactive intermediates which may exert direct effects both systemically and in the airways.

FIGURE 2:

Metabolism of Acetaminophen



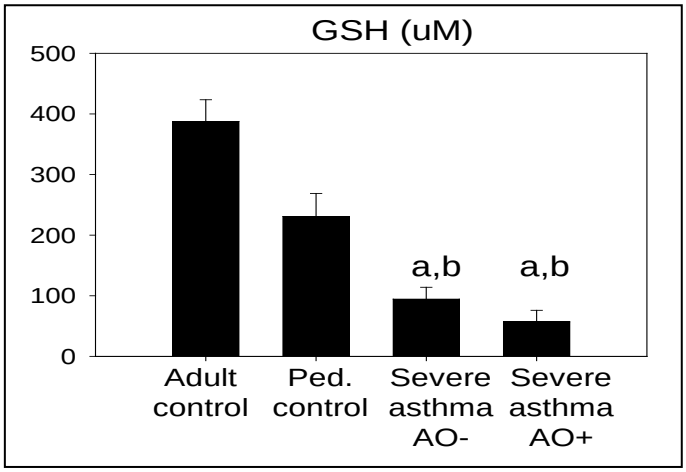
This may further decrease the amount of reduced glutathione in the lung, thereby impairing respiratory antioxidant defenses(Chen, Richie et al. 1990; Micheli, Cerretani et al. 1994; Dimova, Hoet et al. 2005; Fitzpatrick, Teague et al. 2009). Ultimately reduction of glutathione stores may contribute to epithelial damage(Freeman and Crapo 1982), smooth muscle contraction, bronchial hyper-responsiveness(Katsumata, Miura et al. 1990), and impaired β-receptor function. Furthermore, it has been demonstrated that children with certain genetic polymorphisms in the glutathione pathway are more susceptible to a causative association between acetaminophen and asthma.(Perzanowski, Miller et al. 2010)

In support of the acetaminophen-glutathione hypothesis, data from the Severe Asthma Research Program demonstrate significantly decreased levels of airway glutathione in severe asthma subjects when compared to pediatric and adult controls. Figure 3 below shows

glutathione levels from bronchoalveolar lavage (BAL) collected from 65 severe asthmatic children (with airflow obstruction, AO+), 6 children with chronic cough, and 35 healthy adult controls. Compared to controls, severe asthmatics had a significantly lower total GSH (adult control: 436 µM; pediatric control: 260 µM; severe asthma (AO-): 134 µM; severe asthma (AO+): 129 µM, $p < 0.001$)(Fitzpatrick, Teague et al. 2009). Given recent reports of increased disease severity in asthmatic children with frequent acetaminophen exposure (Beasley, Clayton et al. 2008; Beasley, Clayton et al. 2011), these findings warrant further investigation.

FIGURE 3:

Decreased Airway Glutathione in Children with Severe Asthma



Fitzpatrick et al. J Allergy Clin Immunol, 2009

Alternatively, acetaminophen may cause a shift away from Th1 to Th2 responses independent of glutathione reserves.(Peterson, Herzenberg et al. 1998; Dimova, Hoet et al. 2005) Ibuprofen may also have beneficial anti-inflammatory effects including a shift away from Th2 responses and thereby, decrease asthma symptoms.(Perez, Melo et al. 2002)

To date, there are no prospective randomized controlled trials evaluating the risk of asthma symptoms with analgesic-antipyretic use. ***Given the intriguing finding of a reduction in the risk of asthma exacerbations with ibuprofen as compared with acetaminophen, a prospective, randomized controlled trial is needed to determine whether analgesic-antipyretics differentially affect asthma control.*** The results of this trial will provide evidence which could answer the important real life question of whether there is indeed a difference between analgesic-antipyretic use in children with asthma.

B. REVIEW OF ADDITIONAL CLINICAL TRIALS RELEVANT TO THIS PROTOCOL

Acetaminophen

Efficacy and safety of acetaminophen for fever

Acetaminophen is the most commonly used antipyretic medication for children in the United States. Most previous studies have evaluated the efficacy of acetaminophen as compared to the efficacy of aspirin and ibuprofen; however, there are a few trials that have compared the antipyretic properties of acetaminophen versus placebo alone. In a double-bind, parallel-group, triple-dummy, single-dose study of 95 children (age 2 to 11 years) comparing acetaminophen, ibuprofen, and placebo, both acetaminophen (at 10 mg/kg) and ibuprofen (at 5 mg/kg and at 10 mg/kg) were found to be superior to placebo in regards to reduction of fever(Walson, Galletta et al. 1989). In this study, both drugs (at all doses studied) were well-tolerated and no significant clinical or laboratory adverse events occurred. A meta-analysis by Pierce and Voss(Pierce and Voss 2010) identified 31 articles that reported pediatric safety data on acetaminophen trials. Most of these studies compared acetaminophen to ibuprofen. There were no statistically significant differences in adverse events among acetaminophen, ibuprofen and placebo treatment arms. For acetaminophen, the time to peak plasma concentration is 30 minutes, the time to maximum temperature reduction is two hours and the duration of action is four to six hours.(Brown, Kearns et al. 1998)

Efficacy and safety of acetaminophen for analgesia

Acetaminophen has been studied for various causes of pain in children. These included sore throat, pain following myringotomy, orthodontic separator placement, musculoskeletal trauma, tooth extraction, vaccination, and headache. Acetaminophen at doses of 10 mg/kg or 15 mg/kg has been shown to be more efficacious than placebo and safe for the relief of migraine headaches in 4 to 15 year old children,(Hamalainen, Hoppu et al. 1997) sore throat in 2 to 12 year old children,(Bertin, Pons et al. 1991; Schachtel and Thoden 1993), and pain following adenoidectomy in 1 to 6 year old children(Viitanen, Tuominen et al. 2003).

Ibuprofen

Efficacy and safety of ibuprofen for fever

The Walson, et al. study referenced in the above section on acetaminophen was a 3-arm study with ibuprofen, acetaminophen, and placebo as the treatment arms.(Walson, Galletta et al. 1989) Ibuprofen at doses of 5 mg/kg and 10 mg/kg was superior to placebo at reducing fever and were well tolerated. The Pierce and Voss meta-analysis reference above identified 15 pediatric fever studies where the dose of 10 mg/kg of ibuprofen was used.(Pierce and Voss 2010) In all 15 studies, ibuprofen was superior to placebo for fever reduction and was well tolerated. For ibuprofen, the time to peak plasma concentration is 60 minutes, the time to maximum temperature reduction is three hours and the duration of action is four to eight hours.(Brown, Kearns et al. 1998) Furthermore, as referenced previously, the Boston Fever study demonstrated in 84,000 children that children's ibuprofen was safe and well-tolerated as an effective analgesic-antipyretic preparation in children and launched the availability and widespread use of pediatric ibuprofen use.(Lesko, Louik et al. 2002)

Efficacy and safety of ibuprofen for analgesia

The Pierce and Voss meta-analysis identified 9 pediatric pain studies in which the dose of 10 mg/kg of ibuprofen was used(Pierce and Voss 2010). In four of these studies, ibuprofen was shown to be superior to placebo. There was a trend for ibuprofen to be superior to placebo in two of the studies. This dose of ibuprofen was well tolerated in these studies.

Acute Effects of Acetaminophen and Ibuprofen on Asthma

Except for the known immediate adverse effects of ibuprofen and other NSAIDs in patients with aspirin-sensitive asthma in adults,(Babu and Salvi 2000) there are no large, published prospective clinical trials focused on the immediate, acute effects of ibuprofen or acetaminophen on a large group of children with asthma. In one small study of 25 children 8 to 18 years with asthma, challenge with aspirin (600 mg) and acetaminophen (600mg) on separate days resulted in FEV_1 decline >20% in four aspirin-challenged and two acetaminophen-challenged subjects(Fischer, Guilfoile et al. 1983). None of these subjects reported a history of aspirin-induced wheezing prior to enrollment into the study, likely due to concerns of Reye syndrome which minimized the aspirin exposure. Although the numbers of subjects in this study were very small, these data imply that only a minority of asthmatics will respond acutely to administration of acetaminophen, and does not address the role of frequency of asthma exacerbations or symptoms over time with repeated use.

There are a few trials in patients with cystic fibrosis that demonstrate attenuation of lung function decline with high-dose ibuprofen.(Konstan, Byard et al. 1995; Konstan, Schluchter et al. 2007; Lands and Stanojevic 2007) In one of these studies, 20-30 mg/kg of ibuprofen (with adjusted peak serum concentrations of 50-100 µg/mL) administered to children 6-18 years with cystic fibrosis and mild lung disease (FEV_1 >60% predicted) demonstrated a significant reduction in the rate of decline of FVC, but not in FEV_1, compared to placebo(Lands and Stanojevic 2007). However in a similar study of longer duration, high dose ibuprofen did result in a slower annualized rate of decline in FEV_1(Konstan, Byard et al. 1995). A similar observation was noted in a 7-year observational study of children 6-17 years of age with cystic fibrosis and mild airway disease(Konstan, Schluchter et al. 2007). Although these studies focused on school-age children and utilized significantly higher dosages of the ibuprofen compared to this trial, these findings suggest that the anti-inflammatory effects of ibuprofen may have important clinical benefits when administered long-term. However, the pathophysiological mechanisms underlying cystic fibrosis are considerably different from those underlying asthma. Therefore, these findings cannot be directly generalized to the preschool children with persistent cough and wheeze who are the focus of this study.

C. SELECTION OF INTERVENTIONS FOR THIS TRIAL

The primary rationale for use of acetaminophen and ibuprofen is that both drugs are widely accepted as safe and effective analgesics.(Berde and Sethna 2002) **No other oral, non-narcotic medication is commonly administered to children aged 1-5 years for either analgesia or antipyresis in the US.**

Acetaminophen: Acetaminophen (or paracetemol) is a fever reducer and pain reliever used to treat many conditions such as headaches, post-vaccine pain and fever, muscle aches, arthritis, toothaches, colds, and other minor aches and pains. It is the most commonly utilized analgesic-antipyretic used and comprises 5% of all medications used by children.(Vernacchio, Kelly et al. 2009) It is also commonly included as an ingredient in many routine over-the-counter cold/cough preparations. Unlike aspirin, acetaminophen is not associated with an increased risk of Reye's syndrome.

The onset of analgesia is approximately 11 minutes after oral administration of acetaminophen and its half life is 1–4 hours(Moller, Sindet-Pedersen et al. 2005) with a duration of action of 4-6 hours.(Sullivan and Farrar 2011) Acetaminophen, aspirin, ibuprofen, and other NSAIDs all act by a similar mechanism (inhibition of prostaglandin synthesis) and all show varying levels of analgesic, anti-inflammatory, antipyretic, and antiplatelet actions.

The mechanism of action of acetaminophen is considered to possibly be the inhibition of cyclooxygenase (COX) with recent findings suggesting that it is selective for COX-2. Because of its selectivity for COX-2 it does not significantly inhibit the production of the pro-clotting thromboxanes(Hinz, Cheremina et al. 2008).

Acetaminophen is metabolized primarily in the liver. Three metabolic pathways are notable:

- Glucuronidation is believed to account for 40-65% of the metabolism of acetaminophen.
- Sulfation (sulfate conjugation) may account for 20–40% of the metabolism.
- N-hydroxylation and rearrangement, then GSH conjugation, accounts for less than 15%. The hepatic cytochrome P450 enzyme system metabolizes acetaminophen, forming a minor yet significant alkylating metabolite known as NAPQI (*N*-acetyl-*p*-benzo-quinone imine). NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione.

Ibuprofen: Ibuprofen is a NSAID used for fever reduction and as an analgesic with anti-inflammatory properties as well. Ibuprofen is known to have an antiplatelet effect, though it is relatively mild and short-lived when compared with that of aspirin or other better-known antiplatelet drugs. Ibuprofen also generally acts as a vasodilator, having been shown to dilate coronary arteries and some other blood vessels.

Ibuprofen is considered a non-selective COX inhibitor—that is, it inhibits two isoforms of cyclooxygenase, COX-1 and COX-2. The analgesic, antipyretic, and anti-inflammatory activity of NSAIDs appears to be achieved mainly through inhibition of COX-2, whereas inhibition of COX-1 would be responsible for unwanted effects on platelet aggregation and the gastrointestinal tract(Rao and Knaus 2008). However, the role of the individual COX isoforms in the analgesic, anti-inflammatory, and gastric damage effects of NSAIDs is uncertain and different compounds cause different degrees of analgesia and gastric damage(Kakuta, Zheng et al. 2008). Ibuprofen appears to have the lowest incidence of digestive adverse drug reactions of all the non-selective NSAIDs(Rao and Knaus 2008).

Ibuprofen is generally well tolerated in the toddler/preschool age group. Common adverse effects include: nausea, dyspepsia, gastrointestinal ulceration/bleeding, raised liver enzymes, diarrhea, constipation, epistaxis, headache, and dizziness.

D. RATIONALE FOR SELECTED STUDY POPULATION

The AVICA study will enroll preschool children 12-59 months of age who are concurrently enrolled in the INFANT study. The target study population for INFANT is preschool children of this age group who meet criteria for treatment with long-term “Step 2” asthma controller therapy, as defined by the NAEPP EPR-3 guidelines((EPR-3) 2007). This is an ideal age group to study analgesic-antipyretics given the frequent use of these medications in young children. As previously noted, acetaminophen and ibuprofen are the most commonly used medications in children with the highest rates for children under the age of 3 years.(Vernacchio, Kelly et al. 2009) Thus, the frequent use of antipyretics-analgesics in all preschool children coupled with the lack of evidence-based management of asthma, necessitates further studies in this age group.

E. SELECTION OF STUDY MEDICATIONS, DOSAGES, AND DURATION

Acetaminophen Dosing Strategy: The standard dosing for fever in children ages >1 month to 12 years is 10 to 15 mg/kg/dose every 4 to 6 hours as needed (maximum: 5 doses in 24 hours). To ensure safety in this protocol, **acetaminophen will be administered at a dose of 15 mg/kg every 6 hours as needed** (maximum: 4 doses in 24 hours and 60 mg/kg/day). The rationale for every 6 hour dosing is to maintain subject blinding since ibuprofen should not be administered every 4 hours. Additionally, this dosage schedule is within the range that was recently approved by the American Academy of Pediatrics (AAP) which recommended a dose of 75 mg/kg/day or a maximum dose of 90 mg/kg/day limited to less than 3 consecutive days(Sullivan and Farrar 2011). Dosages will be weight-adjusted at all visits throughout the study to adjust for a child's changing weight through the approximately one year study period. Subjects will be instructed to seek medical attention immediately if any of these severe side effects occur: severe allergic reactions (rash, hives, itching, difficulty breathing, tightness in the chest, swelling of the mouth, face, lips, or tongue), dark urine or pale stools, unusual fatigue, or yellowing of the skin or eyes (see safety monitoring). Acetaminophen overdose can lead to liver failure; however, therapeutic doses are not associated with liver injury in children(Lavonas, Reynolds et al. 2010).

Ibuprofen Dosing Strategy: Recently, the AAP recommended the administration of ibuprofen at a dose of 10 mg/kg/dose every 6 hours as needed (maximum: 40 mg/kg in 24 hours) for children greater than 6 months of age.(Sullivan and Farrar 2011) To ensure safety in this protocol as well as equivalent volumes of medications between treatment groups, **ibuprofen will be administered at a slightly lower than maximum allowable dose of 9.4 mg/kg every 6 hours** (maximum: 4 doses in 24 hours and 37.6 mg/kg/day). Dosages will be weight adjusted at all subjects visits throughout the study to adjust for a child's changing weight through the almost one year study period. Subjects will be instructed to seek medical attention immediately if any signs of an allergic reaction such as rash, hives, itching, difficulty breathing, or swelling. An overdose of ibuprofen may lead to symptoms including abdominal pain, nausea, vomiting, drowsiness, dizziness, headache, tinnitus, and nystagmus.

Blinded Dosing Strategy: Based on the doses as noted above, we have standardized the doses of both medications to the same volume. We will be using the standard concentrations of 160mg / 5mL for acetaminophen and 100mg / 5mL for ibuprofen. Therefore, **the dosing of each medication will be exactly equivalent in volume (0.47 mL / kg)**. This will allow for double blinding. After using the weight and the dose (0.47 mL / kg), the final calculated dose will be rounded down to the nearest 0.5 mL and will be weight adjusted at each visit.

Rationale for Not Including a Third Study Arm (Placebo or Alternative Drug):

While we acknowledge that our current study design will only answer the question of differential response between acetaminophen and ibuprofen, we discuss in this section why a placebo arm in children is not feasible.

Analgesic-antipyretics are the most commonly used drugs by children(Vernacchio, Kelly et al. 2009). No other analgesic-antipyretic besides acetaminophen or ibuprofen is routinely administered to children in home, childcare, or school settings in the US. Furthermore, a recent Clinical Report from the AAP, "Fever and Antipyretic Use in Children" discusses only two drugs for the treatment of fever in children, acetaminophen and ibuprofen.(Sullivan and Farrar 2011). Aside from illnesses that may be associated with fever, acetaminophen and ibuprofen are both frequently used to reduce pain and discomfort for many reasons such as for pain associated with immunizations in children aged 1-5 years(Taddio, Manley et al. 2007).

Because acetaminophen and ibuprofen are readily available medications for pain, it would be conidered unethical to withhold pain medication from young children by including a third, placebo arm. Furthermore, it is unlikely that any parent would enroll a young child in a year long trial with no option for rescue from pain or fever. Other clinical trials comparing acetaminophen and ibuprofen for other conditions besides asthma have not included a placebo arm, including one describing treatment for arm fracture pain in children(Drendel, Gorelick et al. 2009) and one for the treatment of musculoskeletal trauma(Clark, Plint et al. 2007).

Other analgesic-antipyretic therapies were considered as a third comparison arm. Naproxyn is primarily used for pain in conditions such as rheumatoid arthritis and too similar to ibuprofen as a non-steroidal antinflammatory agent, blocking both cyclooxygenase-1 and 2, and would not provide any additional information. The only other available consideration was a cyclooxygenase-2 selective inhibitor, celecoxib, an FDA drug approved for children age 2 and above for juvenile rheumatoid arthritis. Several considerations made this medication an unviable option. Celecoxib has significant safety concerns, including potential for adverse cardiovascular effects for both adults and children(Young 2007) and lack of safety data in children under 2 years of age, with a recent FDA advisory for cautious use in children. Furthermore, there is lack of data using celecoxib as an acute, as needed medication. Celecoxib is administered with daily to twice daily dosing and demonstrates different pharmokinetics, longer half lives and time to peak levels compared to acetaminophen and ibuprofen, which would alter the interpretation and results of the trial. Celecoxib is also not available in a liquid preparation, which would complicate blinding. Finally, it is unlikely that any parent would enroll their young child in a trial with the potential of randomization to a celecoxib arm for analgesic/antipyretic treatment with its associated safety concerns.

While we acknowledge that the placebo arm would help answer whether or not acetaminophen is harmful versus ibuprofen is beneficial in children with asthma, this trial will still answer a question of significant important clinical relevance by determining whether or not there is indeed any differential effect between the two most commonly utilized analgesic/antipyretic medications used worldwide in children with asthma.

F. PRIMARY OUTCOME MEASURE

The primary outcome will be the number of asthma exacerbations per subject per year (as calculated from the study treatment duration). An asthma exacerbation will be defined by a significant increase in asthma symptoms requiring treatment with systemic corticosteroids (PO/IV/IM).

In order to account for any analgesic-antipyretic that may have been given during the run-in period, the first two weeks of the study period will not be formally counted for the primary outcome of asthma exacerbations. Therefore the study period that will be analyzed for the primary outcome will be 46 weeks. We will be allowing parents to administer their choice of antipyretics-analgesics during the run-in period. As a result, we will not be counting the first two weeks after randomization to account for any antipyretic-analgesic medications that may have been given during the run-in period.

G. RESEARCH QUESTIONS

This study will provide evidence to guide asthma management in children under the age of 5 years by answering the following question: **Should either acetaminophen or ibuprofen be the preferred analgesic-antipyretic for children with asthma less than 5 years of age?**

IV. HYPOTHESES TO BE TESTED BY THIS TRIAL

A. PRIMARY HYPOTHESES

In young children with mild persistent asthma on standardized asthma therapy, the number of asthma exacerbations requiring systemic corticosteroids will be more frequent in children randomized to receive acetaminophen as compared to those randomized to receive ibuprofen on an as needed basis for fevers and pain.

B. SECONDARY HYPOTHESES AND EXPLORATORY ANALYSES

Hypotheses for the pre-stated secondary analyses are listed below. In young children with mild persistent asthma on standardized asthma therapy, when comparing acetaminophen versus ibuprofen, those randomized to receive acetaminophen will have:

Increased asthma impairment as measured by:

- Fewer asthma control days
- A higher frequency of use of asthma rescue medications

Increased asthma risk as measured by:

- Increased unscheduled physician visits for asthma, emergency department visits for asthma or hospitalizations for asthma

Additionally, we hypothesize that the incidence of adverse events will not differ between treatment groups.Exploratory analyses will examine relationships between baseline concentrations of glutathione and related metabolites (i.e., opthalamic acid) in the plasma and erythrocytes of participating children and the primary and secondary outcome measures. We hypothesize that children with lowest baseline total glutathione concentrations will have: 1) fewer asthma control days, 2) a higher frequency of use of asthma rescue medications, and 3) increased healthcare utilization for asthma.

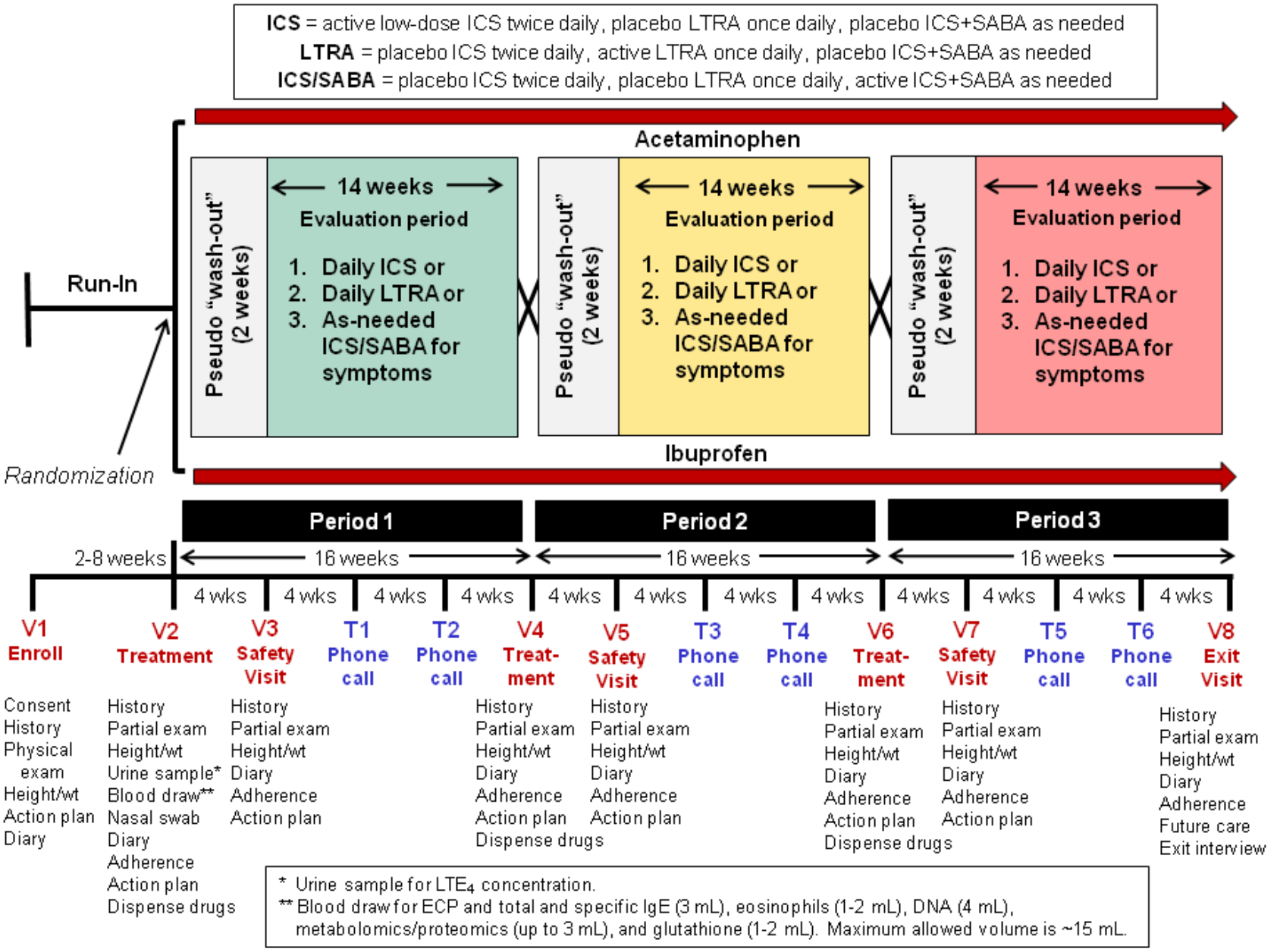
V. STUDY PROTOCOL OVERVIEW AND DESIGN

This trial is a multi-center, prospective, randomized double-blind factorial study. A total of 294 eligible subjects, ages 12-59 months, who meet NAEPP EPR-3 criteria for treatment with long-term, Step 2 asthma controller therapy will be enrolled. Subjects will undergo a run-in period of 2-8 weeks according to their symptom presentation and prior medication exposure. During the run-in period, subjects will be allowed to use open label, over-the-counter acetaminophen or ibuprofen as needed per parental choice. The choice and amount of analgesic-antipyretics used during the run-in period will be monitored and documented. After the run-in is completed, subjects will enter the treatment portion of the study. All children will be randomized in two processes: one to determine the sequence of controller therapy (INFANT), and the other to determine the analgesic-antipyretic medication (AVICA) to be used during the year-long study.

For the AVICA portion of the study, children will be randomized to a blinded analgesic-antipyretic after the run-in period. During the 48 week treatment portion of the study, acetaminophen or ibuprofen will be administered as blinded active therapies to be used as needed per parental decision for fever and analgesia. During this time period, the enrolled children will have standardized asthma controller regimens as detailed in the INFANT protocol. The primary outcome of interest for AVICA is the number of asthma exacerbations (calculated as exacerbations per subject per year), defined by a significant increase in asthma symptoms requiring treatment with systemic corticosteroids. In order to account for any analgesic-antipyretic that may have been given prior to the study and during the run-in period, the first two weeks of the study period will not be counted for the primary outcome of asthma exacerbations. Therefore the study period that will be analyzed for the primary outcome will be 46 weeks. We will be allowing parents to administer their choice of antipyretics-analgesics during the run-in period. As a result, we will not be counting the first two weeks after randomization to account for any antipyretic-analgesic medications that may have been given during the run-in period.

The estimated total study duration is 2 years and 3 months. This takes into account an estimation of one year for the 9 centers and associated participating sites to enroll the required number of subjects. Adding the run in period and the 48-week study duration, we expect that the total study duration will be slightly longer than 2 years from study initiation until the last subject completed.

The study diagram is shown on the following page. Please see INFANT Section V, Page 79 for the table of study procedures.


ICS = active low-dose ICS twice daily, placebo LTRA once daily, placebo ICS+SABA as needed
LTRA = placebo ICS twice daily, active LTRA once daily, placebo ICS+SABA as needed
ICS/SABA = placebo ICS twice daily, placebo LTRA once daily, active ICS+SABA as needed
Acetaminophen
Run-In
Pseudo "wash-out" (2 weeks)
14 weeks
Evaluation period
1. Daily ICS or
2. Daily LTRA or
3. As-needed ICS/SABA for symptoms
Pseudo "wash-out" (2 weeks)
14 weeks
Evaluation period
1. Daily ICS or
2. Daily LTRA or
3. As-needed ICS/SABA for symptoms
Pseudo "wash-out" (2 weeks)
14 weeks
Evaluation period
1. Daily ICS or
2. Daily LTRA or
3. As-needed ICS/SABA for symptoms
Randomization
Ibuprofen
Period 1
Period 2
Period 3
2-8 weeks
16 weeks
16 weeks
16 weeks
4 wks
V1 Enroll
V2 Treatment
V3 Safety Visit
T1 Phone call
T2 Phone call
V4 Treat-ment
V5 Safety Visit
T3 Phone call
T4 Phone call
V6 Treat-ment
V7 Safety Visit
T5 Phone call
T6 Phone call
V8 Exit Visit
Consent
History
Physical exam
Height/wt
Action plan
Diary
History
Partial exam
Height/wt
Urine sample*
Blood draw**
Nasal swab
Diary
Adherence
Action plan
Dispense drugs
History
Partial exam
Height/wt
Diary
Adherence
Action plan
History
Partial exam
Height/wt
Diary
Adherence
Action plan
Dispense drugs
History
Partial exam
Height/wt
Diary
Adherence
Action plan
Dispense drugs
History
Partial exam
Height/wt
Diary
Adherence
Action plan
History
Partial exam
Height/wt
Diary
Adherence
Future care
Exit interview
* Urine sample for LTE4 concentration.
** Blood draw for ECP and total and specific IgE (3 mL), eosinophils (1-2 mL), DNA (4 mL), metabolomics/proteomics (up to 3 mL), and glutathione (1-2 mL). Maximum allowed volume is ~15 mL.

VI. PROTOCOL

A. INCLUSION CRITERIA

This study will enroll preschool children 12-59 months of age who meet criteria for treatment with long-term, Step 2 asthma controller therapy, as defined by the NAEPP EPR-3 guidelines,((EPR-3) 2007) that fulfill inclusion/exclusion criteria for INFANT. To ensure adequate representation, we will enroll at least 33% racial minorities. Our recruitment goal is to also enrich our sample with children less than 3 years of age (~50%).

Flow charts detailing decision-making with regard to enrollment are provided in the Appendix of the INFANT protocol (Flowchart A and Flowchart B).

Inclusion criteria include the following:

If the child *is not* currently taking long-term asthma controller therapy (meaning that the child takes no ICS or LTRA medication whatsoever), then at least one of the following criteria must be met:

1. Daytime asthma symptoms more than two days per week (average over the past 4 weeks)
2. At least one nighttime awakening from asthma (over the past 4 weeks)
3. Two or more asthma exacerbations requiring systemic corticosteroids in the previous 6 months
4. Four or more wheezing episodes in the previous 12 months (note: one wheezing "episode" is equal to 24 hours or more of symptoms).

These children will be treated with placebo during the run-in (2 weeks). Children will qualify for randomization if they demonstrate symptoms (i.e., impairment) during the run-in, or if they continue to meet the criteria for the number of exacerbations and wheezing episodes (i.e., risk). For children who meet criteria for exacerbations and wheezing episodes, the purpose of the run-in is to demonstrate adherence and not to elicit symptoms or standardize therapy.

If the child *is* currently taking long-term asthma controller therapy (meaning that the child takes daily or intermittent ICS or LTRA), then at least one of the following criteria must be met:

1. Taking ICS or LTRA for more than 3 months (or more than 90 days) out of the previous 6 months (or 180 days)
2. Two or more asthma exacerbations requiring systemic corticosteroids in the previous 12 months
3. 4 or more wheezing episodes in the previous 12 months (note: one wheezing "episode" is equal to 24 hours or more of symptoms).
4. Daytime asthma symptoms more than two days per week (average over the past 4 weeks)
5. More than one nighttime awakening from asthma (over the past 4 weeks)

Children who are treated intermittently with ICS or LTRA will be treated with placebo during the run-in. The run-in can be extended up to 8 weeks total. If children do not qualify for the study based on wheezing episodes or

exacerbations (i.e., risk), the purpose of the run-in is to elicit symptoms during an observation period of up to 8 weeks. However if children do qualify based on wheezing episodes or exacerbations (i.e., risk), the purpose of the run-in is to demonstrate adherence and not to elicit symptoms or standardize therapy.

Children who are treated daily with either ICS or LTRA will be treated with open-label study medication (fluticasone or montelukast) during the run-in period (up to 4 weeks total). For these children, the purpose of the run-in is to demonstrate adherence and acceptable symptom control (and not to elicit symptoms or standardize therapy).

In addition, children must be up to date with immunizations, including one dose of varicella vaccine (unless the subject has already had clinical varicella). If the subject has not received one dose of varicella vaccine, this will be arranged with the primary care physician and must be received prior to randomization. Willingness to provide informed consent by the child's parent or guardian is also required for enrollment.

We do anticipate that some children potentially eligible for this trial will have received asthma controller medication for periods of time during the year prior to enrollment and thus will be less symptomatic. Some children may also have their asthma controller therapy discontinued under medical supervision. The inclusion criteria and the run-in for this study were developed with this in mind, with careful and ongoing evaluation of symptom burden to ensure the safety of participating subjects.

B. EXCLUSION CRITERIA

Exclusion criteria at the screening visit (V1)

Participants who meet any of the following criteria are **NOT eligible for enrollment** in this study (and may **not** be re-evaluated at a later date):

1. Allergic sensitization or history of anaphylaxis to the study medications or any component of the study drugs, including (but not limited to) urticaria, rash, angioedema, or hypotension following delivery. This includes a history of allergic sensitization or anaphylaxis to either of the AVICA medications (acetaminophen or ibuprofen) and/or any of the asthma controller medication used in the INFANT protocol.

2. Chronic medical disorders that could interfere with drug metabolism/excretion (for instance chronic hepatic, biliary, or renal disease).

3. Chronic medical disorders that may increase the risk of drug-related injury, including (but not limited to):
    a. Osteogenesis imperfecta (increased risk of bone demineralization/fracture with corticosteroid therapy),
    b. Chron's disease, ulcerative colitis, juvenile rheumatoid arthritis, clotting disorders, or Factor deficiency (increased risk of bleeding with corticosteroid therapy),
    c. G6PD deficiency (increased risk of hemolytic anemia with acetaminophen use),
    d. Phenylketonuria (potential for aspartame exposure with study interventions),

   e. Seizure disorder treated with anticonvulsants (risk of acetaminophen toxicity with carbamazepine), and
   f. History of clotting disorders or Factor deficiency (increased risk of bleeding with corticosteroids).
4. Co-morbid disorders associated with wheezing including (but not limited to) immune deficiency disorders, cystic fibrosis, aspiration, clinically-relevant gastroesophageal reflux, tracheomalacia, congenital airway anomalies (clefts, fistulas, slings, rings), bronchiectasis, bronchopulmonary dysplasia, and/or history of premature birth before 35 weeks gestation.
5. Significant developmental delay/failure to thrive, defined as <5th percentile for height and/or weight (on the WHO chart for children >2 years, CDC charts for children 2 years or more) or crossing of two major percentile lines during the last year for age and gender.
6. History of a near-fatal asthma exacerbation requiring intubation or assisted ventilation.
7. Participating, or planning to participate in, another therapeutic drug trial (aside from INFANT-AVICA).
8. Evidence that the family may be unreliable or poorly adherent, or may move from the clinical center area before trial completion.

Participants who meet any of the following exclusion criteria **may be re-enrolled at a later date** if the criteria are resolved:

1. No primary medical caregiver (e.g., a nurse practitioner, physician assistant, physician, or group medical practice such as a hospital-based clinic) whom the subject can contact for primary medical care.
2. Immunizations not up-to-date.
3. Three or more hospitalizations in the previous 12 months for wheezing or respiratory illnesses.
4. Treatment with 5 or more courses of systemic corticosteroids (oral, intramuscular or intravenous) in the past 6 months.
5. Current use of **higher than step 2** NAEPP asthma guideline therapy (e.g. medium-high dose ICS alone or combination therapy of low-medium-high dose ICS + LABA, montelukast, theophylline or cromolyn).
6. If receiving allergy shots, change in the dose within the past 3 months.

Exclusion criteria at the randomization visit (V2)

Participants will be ineligible for randomization if any of the following are documented:

1. Inadequate adherence (< 75% of the diaries records completed, and, if applicable, <75% of the expected medication doses taken).
2. Asthma exacerbation requiring systemic corticosteroids (may be re-enrolled at a later time if the subject was not hospitalized)
3. Daily asthma symptoms over the past two weeks if not taking asthma controller therapy
4. Daily asthma symptoms more than two days per week if taking asthma controller therapy
5. More than one nighttime awakening from asthma over the past 2 weeks
6. Exclusion criteria assessed at V1 are no longer met.

Flow charts detailing decision-making with regard to randomization are provided in the Appendix of the INFANT protocol (Flowchart C and Flowchart D).

C. STUDY TREATMENTS

Acetaminophen and ibuprofen will be provided in liquid form, double-blinded with identical flavoring, taste, and smell. The strength of the created suspensions will be identical to the current over-the-counter formulations for "children's" preparations of both acetaminophen (160 mg / 5 mL) and ibuprofen (100 mg / 5 mL). Standard dosing devices will be provided and parents will be instructed on proper use. Clear verbal and written instructions on the blinded bottle will inform parents to use this medication for pain and fever only no more than every 6 hours as needed only. In addition to extensive instructions, parents will be strongly encouraged that the AVICA is their only medication that should be used for antipyresis and analgesia during the study period. Parents will also be instructed to fill out electronic diaries as to their usage. Dose will be based on weight at the clinic visits and the dose will be adjusted throughout the duration of the study to match any changes in weight. To standardize dosing and volume between the 2 medications, **our study will use 15 mg/kg/dose of acetaminophen every 6 hours and 9.4 mg/kg/dose ibuprofen every 6 hours** as needed. This will ensure that the dose of acetaminophen and the dose of ibuprofen will be supplied by the exact same volume of blinded study medication. This will ensure proper dosing and optimal blinding. An example to show that this is feasible in the standard concentrations provided is depicted below.

Acetaminophen.

Our Study Dose:	15 mg/kg/dose every 6 hours
Suspension (how supplied):	160 mg / 5 mL
For a 21.5 kg child:	320 mg every 6 hours
	= 10 mL (2 tsp) every 6 hours

Ibuprofen.

Our Study Dose:	9.4 mg/kg/dose every 6 hours
Suspension (how supplied):	100 mg / 5 mL
For a 21.5 kg child:	200 mg every 6 hours
	= 10 mL (2 tsp) every 6 hours

Blinded Dosing Strategy: Based on the doses as noted above, we have standardized the doses of both medications to the same volume. Therefore, **the dosing of both AVICA medications will *exactly equivalent in volume* (0.47 mL / kg)**. This will allow for double blinding. All dosing calculations will be based on volume. After using the weight and the dose (0.47 mL / kg), the final calculated dose will be rounded down to the nearest 0.5 mL and will be weight adjusted at each visit.

Parents will be given a specific amount of blinded study drug at each clinic visit. The amount of study drug distributed will be equivalent to approximately 30 study doses. This will provide an adequate amount of study drug to be used intermittently over the time period between each clinic visit. Dosages will be weight adjusted at all subjects' visits throughout the study to adjust for a child's changing weight through the almost one year study period. At every follow up visit (in person and by telephone), caregivers will be asked how much of the study medication was used by the subject. In addition to monitoring the quantity of AVICA study medications, diaries and questionnaires will track to timing and reasons (fever, pain, URI, other) of the AVICA medication use. At each return clinic visit, parents will return their old AVICA medication

supply and will receive a new supply. The old, returned AVICA medication bottle will be weighed and measured to determine the amount of study drug that was consumed since the last visit. This will allow for monitoring of adherence. Additionally, the approximate amount of study drug used will be assessed monthly during the phone interviews. If children have used their entire supply of the AVICA medication prior to an in-person visit, a new bottle will be mailed to the family home. This will allow for ample supply to be available and will increase adherence.

In addition to receiving the blinded study medication, parents will receive a detailed instruction sheet regarding the proper use of the AVICA medication. The instruction sheet will describe to parents that use of this medication is not necessary, but may be used to help alleviate fever or pain. The parental instruction sheet will also strongly encourage that the AVICA study medication is their only medication that should be used as an antipyretic or analgesic. If used properly and at the instructed doses, the AVICA medication will provide proper antipyresis and analgesia, and therefore eliminate the need for use of OTC medications. Additionally, parents will be given a letter to be shown to any treating medical care provider. This detailed letter will describe the fact that the subject is in a study and has been given a blinded regimen or acetaminophen or ibuprofen to be used on an as needed basis. This letter will encourage the medical provider to keep the subject on the study medication as directed. If it is deemed medically necessary for the subject to get analgesic-antipyretic medication outside of the blinded medication, the letter will ask that the medical provider contact our study staff to report the deviation. To allow for maximal adherence and cooperation, this letter will be designed with the assistance of primary care pediatricians. Additionally, we will provide a similar letter explaining the study medication in more simple terms that can be used for non-medical caretakers, such as daycare providers, babysitters, or family relatives.

D. VISIT SPECIFIC PROCEDURES

Overall, there are 6 types of scheduled study visits or contacts as follows. These visits are illustrated in the study diagram shown above. Additionally, these visits are described in detail in the section. AVICA-specific details are noted with ******* prior to the information. The rest is identical to INFANT.

1. Enrollment visit (V1).
2. Randomization visit (V2) – 4 weeks following V1.
   ******* Initiation of AVICA drug therapy.
3. Clinic visits to initiate INFANT drug therapy (V4, V6), 16 weeks after receiving the previous randomized study drug.
4. Clinic “safety” visits 4 weeks after the initiation of INFANT drug therapy to assess interim responses (V3, V5, V7).
5. Treatment telephone calls 4 weeks (T1, T3, T5) and 8 weeks (T2, T4, T6) after each safety visit to ensure compliance with study procedures and assess interim responses. A final telephone call (T7) will occur 4 weeks after the study close-out visit to ensure safety and appropriate medical follow-up.
6. Study close-out visit (V8) 48 weeks after V2 (randomization), or 14 days after Study Failure (see section on Study Failure).

Enrollment visit (V1), Study Week -2 to -8

- Informed consent obtained
- Eligibility determined based upon inclusion and exclusion criteria
- Medical history obtained
- Physical examination including height and weight performed
- An Action Plan provided and explained, to include standard education about wheezing, use of the action plan, avoidance of allergens and irritants.
- Provide and teach Preschool Asthma Diary completion.
- Dispense rescue medications (albuterol, prednisolone).
- Dispense spacer with face mask.
- Provide education for appropriate medication and spacer use.
- Review current long-term asthma controller medication use and discontinue if appropriate, or dispense open-label study medication if criteria are met (see Flowchart A and B in the INFANT Appendix).
- ******* Discuss the proper use of over-the-counter acetaminophen and ibuprofen.
- ******* AVICA-specific diaries given to monitor the use of over-the-counter analgesic-antipyretics to be used during the run-in period.

Randomization visit (V2), Study Week 0

- Diaries reviewed and evaluated for adherence – subjects must demonstrate at least 75% adherence to diaries.
- Informed consent reviewed.
- Inclusion and exclusion criteria reviewed.
- Review of asthma symptoms and medical history, including healthcare utilization.
- Brief physical exam including height and weight performed.
- Administer questionnaires.
- Blood sample for total and allergen-specific IgE, ECP, eosinophil count, genetic analyses, proteomics, metabolomics, and glutathione and related metabolites.
- Urine sample for LTE_4
- Nasal swab sample for respiratory viruses.
- Action plan administered and reviewed.
- Provide education for appropriate medication and spacer use.
- ******* Dispense AVICA study medications – Subjects randomized to acetaminophen or ibuprofen arm and blinded analgesic-antipyretic given to family.

Clinic "INFANT drug initiation" (cross-over) visits, V4 – Study Week 16; V6 – Study Week 32

- Diaries reviewed.
- Review of asthma symptoms and medical history, including healthcare utilization.
- Brief physical exam including height and weight performed.
- Administer questionnaires.
- Action plan reviewed.
- Dispense study medications
- Provide education for appropriate medication and spacer use.
- ******* Review diaries related to amount/frequency of use of AVICA study medication
- ******* Review indications and proper usage of AVICA study medication

- ******* Adjust AVICA study medication dose based on any change in weight
- ******* Give new supply of AVICA study medication based on adjusted weight

Clinic "safety" visits, V3 – Study Week 4; V5 – Study Week 20; V7 – Study Week 36

- Diaries reviewed.
- Review of asthma symptoms and medical history, including healthcare utilization
- Brief physical exam including height and weight performed.
- Administer questionnaires.
- Action plan reviewed.
- ******* Review diaries related to amount/frequency of use of AVICA study medication
- ******* Review indications and proper usage of AVICA study medication
- ******* Adjust AVICA study medication dose based on any change in weight
- ******* Give new supply of AVICA study medication based on adjusted weight

Follow-up telephone calls (T) – 4 and 8 weeks after V3, V5, V7 [Study Weeks 8, 16, 24, 28, 40, 44]

- Assess respiratory symptoms, albuterol use, healthcare utilization since previous visit
- Confirm diary completion
- Encourage medical follow-up if need and not already been done.
- Study procedures, action plan, and medication adherence reviewed.
- ******* Review diaries related to amount/frequency of use of AVICA study medication
- ******* Review indications and proper usage of AVICA study medication

Study close-out visit, V8 – Study Week 48

- Diaries reviewed.
- Review of asthma symptoms and medical history, including healthcare utilization
- Brief physical exam including height and weight performed.
- Administer questionnaires.
- Exit interview performed (critique of study experience; permission to be contacted for future studies).
- Action plan reviewed.
- Treatment recommendations given.
- ******* Review diaries related to amount/frequency of use of AVICA study medication

E. OUTCOME VARIABLES

Primary Outcome Measures

The primary outcome for AVICA will be the number of asthma exacerbations per subject per year (as calculated from the study treatment duration). An asthma exacerbation will be defined by a significant increase in asthma symptoms requiring treatment with systemic corticosteroids (PO/IV/IM). In order to account for any analgesic-antipyretic that may have been given during the run-in period, the first two weeks of the study period will not be formally counted for the primary outcome of asthma exacerbations. Therefore the study period that will be analyzed for the primary outcome will be 46 weeks.

Secondary Outcome Measures

Specific secondary measures to be obtained in this study include the following: 1) asthma control days (calculated from the INFANT study), 2) frequency of asthma rescue medication use, and 3) unscheduled physician visits, emergency department visits or hospitalizations for asthma. Additional secondary analyses will investigate rates of drug-related side effects.

Exploratory analyses

We also anticipate that children with the lowest baseline concentrations of plasma and erythrocyte glutathione will have: 1) fewer asthma control days (calculated from the INFANT study), 2) increased frequency of asthma rescue medication use, and 3) increased unscheduled physician visits, emergency department visits or hospitalizations for asthma.

F. RANDOMIZATION

Patients who satisfy all the eligibility criteria at the V1 and V2 visits will be randomized to study treatment arms of INFANT and AVICA after all data collection has been completed. Treatment assignment will be performed according to a double-dummy, double-blind randomized parallel group design, with stratification by clinical center.

G. CRITERIA FOR STARTING RESCUE THERAPY

Rescue algorithms for fever and/or pain

Subjects will receive either ibuprofen or acetaminophen for fever and pain control. The medications will be blinded. Parents will be instructed to use only the blinded study medication for fever and/or pain control for the duration of the study. Parents may administer the blinded acetaminophen or ibuprofen as frequently as every 6 hours as needed. Parents will be instructed to call the child's healthcare provider for any signs or symptoms that are concerning to them as they normally would outside of study participation. Regardless of other signs or symptoms, if the fever persists after three days, parents will be instructed to contact the child's primary care provider for guidance and alert the study team to this occurrence and the primary care provider's guidance. In the event of a seizure, the child's caretaker should call 911 immediately.

Persistent fever. In the event of persistent fever, parents will be instructed to consult the children's primary care provider for treatment options. Persistent fever despite antipyretic therapy will be defined as a fever lasting for more than three days with continuous temperatures of 100.4 F or more.

Insufficient analgesia. Parents may administer the blinded study medication to which their child was randomized per their own discretion for pain/discomfort as frequent as every 6 hours. If the parents feel that the child's pain is not adequately controlled by the blinded study medication, parents will be instructed to consult the children's primary care provider for emergency evaluation and treatment.

We do acknowledge that there may be extenuating circumstances where fever and pain may not be fully controlled by the single AVICA study medication. We will give instructions to both parents and primary care physicians regarding recommendations for this scenario. In this case, we will ask the family to follow up with their primary care provider for further evaluation of high persistent fever and uncontrolled pain. If deemed necessary by the primary care provider, we recommend that the child's AVICA medication not be used, but instead may be replaced with open label acetaminophen and/or ibuprofen. We will provide detailed verbal and written instructions for the subjects' families regarding this procedure. Additionally, we will provide a written letter for the families to bring to the primary care provider that explains our recommendations if this situation occurs.

Availability of AsthmaNet Clinical Center personnel

The AsthmaNet Clinical Center personnel will be available for discussion with families 24 hours/day should uncertainty or questions arise. Parents will be asked to contact the AsthmaNet Center as outlined in the INFANT protocol. Specific to the AVICA study, parents will also be instructed to contact the AsthmaNet Center:

- If an open label analgesic-antipyretic was used (i.e., the caregiver gave the child an OTC medication that contains either acetaminophen or ibuprofen)
- If the parent sought medical advice for fever or pain from a provider outside the AsthmaNet Clinical Center.
- At any time should they have specific questions or concerns about the study.

Symptoms requiring immediate medical attention during

Parents will be instructed and directed by an asthma action plan to seek emergent care immediately (e.g. Urgent Care or emergency department) as outlined in the INFANT protocol. Criteria for immediate medical evaluation specific to this study include any of the following:

- Jaundice (yellowing of the skin or eyes)
- Unexplained bruising
- Severe allergic reactions (rash, hives, itching, difficulty breathing, swelling of mouth, face, or tongue) after taking the study drug
- dark urine or pale stools
- Bleeding that will not clot

Parents will be instructed to call the AsthmaNet Clinical Center or after-hours nurse triage center to inform the study personnel that emergency care was sought, after the child's status has improved. If the AsthmaNet Clinical Center confirms the occurrence of any of these criteria, the parent will be directed to seek immediate medical care (AsthmaNet Clinic, Urgent Care, or emergency department). We will also assess criteria that indicate the need for immediate medical attention at all study visits and direct the family to seek emergency care if not already obtained.

H. CRITERIA FOR TREATMENT FAILURE

Treatment failure will occur if ANY of the following criteria develop during the course of the study:

- Febrile seizure

- New onset hepatic, renal or biliary disease that interferes or potentially interferes with pharmacokinetics of the study interventions
- Jaundice
- Clinical signs or findings consistent with hepatitis or liver disease.
- If any of the above occur, subjects will be referred for emergency medical evaluation and treatment.

I. CRITERIA FOR STUDY FAILURE IN INFANT

For INFANT, study failure will occur if ANY of the following criteria develop during the course of the study:

1. Four courses of prednisolone are required after randomization,
2. Hospitalization >24 hours is required for an acute asthma exacerbation, or
3. If a child steps moves forward to the next treatment arm due to recurrent exacerbations (protocol-defined) two times during the course of the study

J. NON-STUDY DRUGS

Other drugs considered necessary for the child's welfare may be given if they are not specifically contraindicated for this study, although these will be recorded specifically. The exception would be if the AsthmaNet physician feels these drugs are necessary for other medical reasons. These reasons would be documented and discussed with the Data Coordinating Center.

K. RECRUITMENT

Each clinical center (9 total) involved in the AsthmaNet was chosen, in part, based on documentation for participant availability in clinical trials with similar entry criteria. Because the total sample size for AVICA is 294 subjects, each center will randomize 33 subjects. Satellite clinics are established for some AsthmaNet Clinical Centers to aid in recruitment. The specific plans for recruitment at each center are summarized in the INFANT protocol.

L. DRUG SUPPLIES

Active blinded acetaminophen and active blinded ibuprofen will be will be manufactured by a private research drug manufacturing company. Negotiations with the vendor are ongoing.

M. ADHERENCE

Patients will be continuously encouraged to adhere to their AVICA study medication. Additionally, detailed instructions will be provided and repeated at all study visits. Parents will receive a detailed instruction sheet regarding the proper use of the AVICA medication. The instruction sheet will describe to parents that use of this medication is not necessary, but may be used to help alleviate fever or pain. The parental instruction sheet will also strongly encourage that the AVICA study medication is their only medication that should be used as an

antipyretic or analgesic. If used as instructed, the AVICA medication will provide proper antipyresis and analgesia, and therefore eliminate the need for use of OTC medications. We also will provide parents with guidelines and recommendations for treating fevers, pain, viral symptoms, and upper respiratory infections. This will include supportive measures such as fluid hydration, nasal saline washes, rest, and a list of allowable medications that can be used if needed. Additionally, parents will be given a letter to be shown to any treating medical care provider. This detailed letter will describe the fact that the subject is in a study and has been given a blinded regimen or acetaminophen or ibuprofen to be used on an as needed basis. This letter will encourage the medical provider to keep the subject on the study medication as directed. If it is deemed medically necessary for the subject to get analgesic-antipyretic medication outside of the blinded medication, the letter will ask that the medical provider contact our study staff to report the deviation. To allow for maximal adherence and cooperation, this letter will be designed with the assistance of primary care pediatricians. Additionally, we will provide a similar letter explaining the study medication in more simple terms that can be used for non-medical caretakers, such as daycare providers, babysitters, or family relatives.

Use of study medication and adherence will be monitored closely. Subjects' caregivers will complete diaries documenting study medication use (amount, frequency, timing, and reason for use). At each in-person visit, old medication bottles will be returned and the volumes of these will measured. Finally, diaries and questionnaires will track the use of any OTC, open-label antipyretics or analgesics that may be used in an emergent situation. We will track the amount, frequency, and reason for this non-adherence to the AVICA medication. To assess compliance, we will quantifying the number of times an OTC medication was used, which OTC medication was used, and for what reason (fever, pain, etc). We will also monitor the reason that and OTC medication was used and not the AVICA drug. For the entire study population, we will be monitoring what percentage of subject used OTC medications and what percentage of the total doses of acetaminophen or ibuprofen were AVICA medications as compared to OTC medications. We will pro-actively monitor compliance with the AVICA medications during the trial. We will also pro-actively monitor reasons for any non-compliance and use of OTC medications containing acetaminophen or ibuprofen. If warranted, we will adjust our instructions and recommendations during the course of the study to improve compliance.

N. EDUCATION

Standardized education about the management of fever and pain will be made in accordance with published position statements from the American Academy of Pediatrics. We will also educate parents on the proper use of study medications at each visit.

O. RETENTION

Because this is a year-long study (48 weeks), some attrition is possible. Therefore retention efforts will focus on ease of visits and informational rewards (such as the asthma education). Visits will be at times convenient to the parents, many of whom work (thus, hours after day care and preschool will be available). We will make every effort to minimize parking problems and other general inconveniences. A monetary incentive will be given for each visit, with a bonus at the end of the study for completion of all visits. Study staff will be available to answer questions about asthma and how to use the action protocol. A study physician will be available by phone during off-hours to aid in management of wheezing illnesses.

P. MONITORING FOR ADVERSE EFFECTS

Assessment of adverse events will be made at each study visit. This will include a review of all the criteria for treatment failure and study failure as noted above.

Q. SPECIAL STUDY TECHNIQUES

The special study techniques are well documented in the INFANT protocol and include venipuncture, urine collection, and nasal sampling. At the same time that venipuncture is performed in the INFANT study, one additional tube of blood will be obtained for the AVICA study. This is described below (and also in the INFANT protocol).

Venipuncture for glutathione and related metabolites (i.e., opthalamic acid)

At the same time that blood is obtained in the INFANT protocol (i.e., at the randomization visit), one additional tube of blood will be obtained for plasma and erythrocyte measures of total glutathione and related metabolites (i.e., opthalamic acid). The blood volume necessary for these analyses is approximately 1-2 mL. Detailed methods for blood collection, sample processing, storage, and shipment appear in the accompanying manual of procedures.

A table outlining the blood collection procedures, including the order in which samples are to be drawn, also appears in the INFANT protocol on page 49.

AVICA Diaries

Diaries specific for the AVICA protocol will be developed. Subjects will be asked to record AVICA medication use and the reason prompting use of the study medications. Diaries for the INFANT protocol will document respiratory symptoms as well as INFANT study medication use on a daily basis.

R. RISKS/BENEFITS

AVICA compares the effect of acetaminophen and ibuprofen on asthma exacerbations and symptoms. Specific to this study, potential risks include side effects from analgesic-antipyretic medications administered. The medications used in this trial have been demonstrated to be safe and are FDA-approved for the age group studied. Safety algorithms have also been carefully constructed to ensure the safety of participating children to the best extent possible. Potential benefits from participation include intensive education and support for the management of fever and pain as well as the potential benefit of the study interventions resulting in less severe wheezing illnesses and less child and family morbidity.

S. ANTICIPATED RESULTS

The purpose of AVICA is to determine what, if any, is the most appropriate analgesic-antipyretic in preschool children with persistent asthma. In AVICA, we anticipate that ibuprofen will significantly reduce the frequency of wheezing episodes relative to acetaminophen. We further expect that the number of exacerbations requiring systemic corticosteroids will be

increased in subjects randomized to take acetaminophen vs. ibuprofen for fevers and pain. If this study demonstrates that ibuprofen is indeed effective in reducing rates of exacerbations, this will provide an important evidence base for clinical practice. Alternatively, a negative result would lend support to discount emerging concerns about acetaminophen use in young preschool children with asthma.

VII. ADVERSE EVENTS

A. DEFINITION OF AN ADVERSE EVENT

An adverse event (AE) shall be considered any detrimental change in the patient's condition whether it is related to an exacerbation of asthma or to another unrelated illness. The International Conference on Harmonization guidelines further define an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits or telephone interviews or by a patient presenting for medical care. Unanticipated AEs and severe adverse events (SAEs) will adhere to federal and local IRB reporting mandates as well as International Conference on Harmonization Guidelines for Good Clinical Practice.

B. MONITORING OF ADVERSE EVENTS RELATED TO STUDY MEDICATION

Although serious allergic reactions (e.g., angioedema, anaphylaxis, Stevens Johnson Syndrome, toxic epidermal necrolysis) are rare with the drugs to be used in this study, fatalities are possible if the allergic reaction is very severe. If an allergic reaction occurs, the study drugs will be immediately discontinued and the appropriate therapy initiated. Patients will be advised to: 1) discontinue use immediately, 2) seek emergency medical care, and 3) contact the AsthmaNet Clinical Center if signs of an allergic reaction occur. This caution will be listed specifically in the informed consent document. Similarly, because all the study drugs are metabolized in the liver and excreted in the urine/bile, we will exclude children with chronic renal, hepatic, or biliary disorders that may interfere with pharmacokinetics of the study drugs. Other drug-specific adverse effects are listed below.

Acetaminophen

Acetaminophen is an analgesic-antipyretic that is FDA approved for the treatment of fever and pain in children. When used in small doses, no common side effects have been reported with Acetaminophen Elixir. Subjects will be instructed to seek medical attention right away if any of these severe side effects occur when using acetaminophen elixir: severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); dark urine or pale stools; unusual fatigue; yellowing of the skin or eyes.

Because acetaminophen may cause severe hepatic injury with overdose, the amount of study drug given to parents will be limited to approximately a 30 dose supply. Because several case reports of acetaminophen-associated hemolytic anemia have been reported in children with G6PD deficiency, these children will be excluded. Similarly, because certain seizure medications such as carbamazepine may increase the risk of hepatotoxicity, we will exclude

children with seizure disorders. We will also exclude children with known elevations in liver transaminase enzymes.

Ibuprofen

Ibuprofen is a NSAID that is FDA-approved for the treatment of pain, fever, and inflammatory disorders in children. Common adverse effects include dyspepsia, nausea, vomiting, and gastrointestinal bleeding. To minimize these adverse effects, parents will be instructed to administer the drug with food or milk. Food or milk may decrease the rate but not the oral absorption. Because overdose is possible, the amount of study drug given to parents will be limited to approximately a 30 dose supply. Children with clotting disorders or Factor deficiencies will be excluded from enrollment due to the increased risk of bleeding with NSAIDs that is augmented with oral prednisolone use.

C. ADVERSE EVENTS UNRELATED TO RESPIRATORY SYMPTOMS / ASTHMA

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the patient is no longer able to effectively participate in the study or meets pre-specified criteria for study failure (e.g., hospitalization for an acute asthma exacerbation). Subjects experiencing minor illnesses may continue in the study if the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are recorded. Examples of minor illnesses include skin disorders such as atopic dermatitis and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician. Patients will be asked to report to the clinical center the use of any prescription medication other than study medications so that appropriate adjustments can be made in coordination with the prescribing physician.

Documentation of an adverse event unrelated to asthma will be recorded on an Adverse Event Report Form and will include the following information:

- Description of the illness
- Dates of the illness
- Treatment of the illness and dates
- Whether emergency treatment or hospitalization was required
- Treatment outcome

D. ADVERSE EVENTS RELATED TO RESPIRATORY/ASTHMA EXACERBATIONS

The inclusion criteria require that all participants have persistent asthma with evidence of impairment (symptoms) or risk (previous exacerbations requiring systemic corticosteroids). Thus we are likely to see similar asthma symptoms and exacerbations during the course of this study. All children in the trial will receive SABA for rescue. All children will have action plans available, which include criteria for initiating prednisolone (see Criteria for Initiating Rescue Therapy in INFANT protocol). AsthmaNet physicians are also available 24 hours a day for guidance.

VIII. STATISTICAL DESIGN AND ANALYSIS

A. OVERVIEW AND ANALYSIS PLAN

In the AVICA study, the primary outcome is the number of exacerbations per subject per year. Because participants will possibly receive over-the-counter acetaminophen or ibuprofen according to their personal preference during the run-in, the first two weeks of data after randomization and initiation of blinded AVICA therapy will be excluded from data analysis. For the purpose of determining sample size, the choice of relative rate of exacerbations in the ibuprofen group as compared to the acetaminophen group was guided by the previously discussed Lesko et al study.(Lesko, Louik et al. 2002) In that study, the relative risk was 0.56 for ibuprofen use as compared to acetaminophen use.(Lesko, Louik et al. 2002) The choice of expected overall exacerbation rate utilized for sample size calculation was guided by the results of the PEAK and AIMS studies from the CARE network.(Guilbert, Morgan et al. 2006; Bacharier, Phillips et al. 2008)

Data recording and data management

Recording of all data including informed consent and assent, history, physical examination, adverse events, confirmation of medication dispensation, and initial data entry will be done at each Clinical Center and forms will be forwarded to the Data Coordinating Center (DCC) for confirmatory entry. Results from all tests and compliance will be transmitted electronically to the DCC where all data will be stored and analyzed.

Each Clinical Center will have a computer configuration that includes a PC, a printer, and a modem. This will give each clinical center the capability of logging directly into the AsthmaNet web site with the modem as a back-up if the connection is not possible. Though this set-up is installed primarily to allow for distributed data entry into a centralized and secure database at the AsthmaNet web site, menu options will also include sending electronic mail, downloading study documents such as forms and reports, and viewing a calendar of AsthmaNet events. A sophisticated security system will limit access to qualified personnel and prevent corruption of the study database.

The DCC will be responsible for generating the data collection forms based on input from the clinical centers. Once the data collection forms have been completed and reviewed, the Clinic Coordinator will log into the AsthmaNet Network web site and enter the data within 3 days of the patient visit. The advantage of this distributed data entry system is that the Clinic Coordinators will review the data a second time as they are entering it, which serves as another level of quality control. However, the Clinic Coordinators will not be able to query their own data. The data base management system will have range checks and validity checks programmed into it for a second level of quality control. Forms will then be forwarded to the DCC for the second data entry and filing, which will be performed within 3 days of receipt. The DCC will be responsible for identifying problem data and resolving inconsistencies. Once the quality control procedures are complete, new study data will be integrated into the primary study database.

Randomization

Randomization will be performed to determine which parallel treatment arm (blinded acetaminophen arm or blinded ibuprofen arm) that each subject enters and will be stratified by clinical center and INFANT treatment sequence. The target sample size is 294 randomized

participants, 147 in each treatment. Each of the nine clinical centers and their associated satellite sites will randomize approximately 33 participants.

When a child at a particular Clinical Center is deemed eligible for the study, the Clinic Coordinator will authenticate into the AsthmaNet server and indicate to the system that a participant requires randomization. After entering the pertinent information with respect to Clinical Center and eligibility criteria, the Clinic Coordinator will be asked to verify that all of the entered information is correct. If so, the Clinic Coordinator will be given a packet number, from which all medication for that child will be dispensed. In order to maintain security of the randomization schedules, the data manager of the DCC will receive automatically a notice from the AsthmaNet Network server that a child has been randomized. If no follow-up information is forthcoming on the child, then the data manager will contact the Clinic Coordinator about the status of the child.

Masking

To minimize the bias due to possible knowledge of the sequence assignment, the study will be double-blinded. Thus, the investigators and the children, along with their caregivers, will not know which analgesic-antipyretic treatment (acetaminophen or ibuprofen) is being received during the course of the study.

Statistical analyses

The run-in period is considered the baseline evaluation period. The initial statistical analysis will focus on summarizing the baseline characteristics of the study participants. Descriptive statistics (means and standard deviations, or medians and inter-quartile ranges) will be calculated for continuous baseline measures such as current age and asthma symptom severity. Frequency tables will be generated for categorical baseline measures such as sex and prior medication history.

Primary outcome measure. The primary outcome measure is the number of exacerbations per subject per year (calculated over the last 46 weeks of the randomized treatment period). The primary analysis will utilize maximum likelihood estimation based on the log-linear regression model for outcomes following the negative binomial distribution. This analysis incorporates the actual follow-up time so that rates can be estimated appropriately when the observed number of exacerbations for a given subject follows a Poisson distribution and allows for variation across subjects in the expected number exacerbations, also described as over-dispersion. The primary analysis will be performed under the intent-to-treat paradigm whereby the treatment effect corresponds to randomized treatment assignment regardless of any information about possible non-compliance. In addition to treatment effect, these models will also incorporate covariates including clinical center and INFANT treatment sequence.

Secondary outcome measures. Secondary analyses will examine the effect of treatment on: 1) asthma control days (calculated from the INFANT study), 2) frequency of asthma rescue medication use, and 3) unscheduled physician visits, emergency department visits, or hospitalizations for asthma. Additional secondary analyses will investigate the incidence of adverse events.

For outcome variables that are measured as counts (i.e., unscheduled physician or emergency department visits or hospitalizations for asthma), a similar log-linear model maximum likelihood

analysis will be applied. Standard ANOVA will be applied for outcomes that are measured on a roughly continuous scale (i.e., asthma control days and frequency of use of asthma rescue medications averaged over the last 46 weeks of the randomized treatment period). For any continuous outcomes that are not approximately normally distributed, appropriate transformations will be applied prior to ANOVA.

Exploratory analyses. Exploratory analyses will focus on baseline plasma and erythrocyte concentrations of total glutathione and related metabolites and their associations with the secondary outcomes listed above.

Handling of missing data. Because of missed visits and the possibility of drop-outs there will be some missing data. The statistical models and analyses planned for the primary and secondary outcomes assume that the data are "missing at random" (MAR). Because likelihood-based methods of analysis will be applied, MAR data still yield valid estimates. Although not expected, if it appears that the MAR assumption is not reasonable, pattern-mixture models for non-ignorable missing data will be applied.

Assessment of compliance. The primary analysis will be done under the intent-to-treat paradigm, but compliance data will be collected. Analyses of compliance will utilize multiple imputation methodology to assess the possible effects of non-compliance on the primary analysis results (see description below) (Taylor and Zhou 2009). The most likely type of non-compliance will be use of open-label antipyretics or analgesics. Because of the nature of the AVICA study and treatments being used, it is less likely that there will be non-compliance in the form of under- or over-using the study treatments. The blinded AVICA treatments represent the most likely open-label treatments. That is, if the participant uses an open-label treatment, it will almost certainly be either acetaminophen or ibuprofen. Thus, non-compliance may or may not represent a deviation from the randomized treatment. A participant who is randomized to the ibuprofen arm, but uses open-label ibuprofen, is actually getting the randomized treatment. Whereas a participant who randomized to the acetaminophen arm, but uses open-label ibuprofen, is actually getting the same treatment as someone randomized to the ibuprofen arm. This situation is commonly called cross-over non-compliance because the participant is effectively crossing over to the opposite treatment arm. If this analysis indicates potential significant biasing of the primary analysis results due to non-compliance, this will be reported along with the primary analysis result. If this occurs, it will be a major limitation on the conclusions of the primary analysis results.

Assessment of interaction with INFANT. Given the factorial nature of the INFANT and AVICA studies, some interaction is possible. For assessment of interaction between INFANT and AVICA treatments, outcomes will be analyzed in longitudinal models according to the three INFANT treatment periods so that assigned INFANT treatment can be included as a time dependent covariate. As described above, different model structures will be applied depending on the type of outcome (eg, count or continuous). All of the models can be adapted to a longitudinal framework by including random effects and applying a correlation structure that does not assume independence between observations.

Assessment of dose-dependent relationships. Cumulative doses of AVICA study medications and the timing of AVICA study medication administration will also be quantified during the study period. This will allow for dose- and time-dependent relationships to be evaluated between analgesic-antipyretic use and asthma outcomes.

B. SAMPLE SIZE JUSTIFICATION

The target total sample size for this protocol is 294 randomized subjects and is the same sample size for INFANT. The overall exacerbation rates, across all treatment arms, for these studies were approximately 1.0 per year for PEAK(Guilbert, Morgan et al. 2006) and 0.94 per year for AIMS.(Bacharier, Phillips et al. 2008) Assuming that the expected overall exacerbation rate for this study, across both AVICA treatment arms and across all INFANT treatment periods is 0.97 per year, and assuming that the relative risk for the AVICA treatment effect is 0.56, then 190 total subjects (95 per treatment arm) are required to achieve 90% power using a two sided test for $\alpha = 0.05$ based on the Poisson distribution. Assuming a more conservative relative risk of 0.65, it is expected that there would be 1.18 exacerbations per year in the acetaminophen group and 0.76 exacerbations per year in the ibuprofen group. Under this scenario, 244 total subjects (122 per treatment arm) are required for 90% power using a two sided test for $\alpha = 0.05$. Accounting for a 25% drop out rate, the required number of randomized subjects is 294 (147 per treatment arm).

C. INTERIM ANALYSES AND DATA MONITORING

There will be no formal interim analysis of efficacy for the AVICA study. However, interim statistical analyses to evaluate the safety of the two treatments will be presented to the AsthmaNet Data and Safety Monitoring Board (DSMB) semi-annually for review. Based on the results of these interim analyses, the DSMB will recommend to the NHLBI the continuation or discontinuation of the AVICA trial. In addition, the DSMB will be monitoring all of the safety data throughout the course of the AVICA trial and will be notified within 72 hours of any SAE that occurs. Finally, we will pro-actively monitor compliance with the AVICA medications during the trial in the manner as previously described. We will also pro-actively monitor reasons for any non-compliance and use of OTC medications containing acetaminophen or ibuprofen. These data will be shared with the DSMB regularly. Our intention is to provide this information to the DSMB along with regular study updates/reports related to adverse events, recruitment milestones, and protocol execution (e.g., violations and deviations). If warranted, we will adjust our instructions and recommendations during the course of the study to improve compliance.

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