Pre-operative treatment with DigiFab to prevent post-operative acute kidney injury (AKI) in patients at high risk for AKI undergoing cardiac bypass graft surgery

April 24, 2022

NCT # HP-00083553

1. Introduction

Acute kidney injury (AKI) occurs in up to 30% of patients undergoing coronary artery bypass graft (CABG) surgery[1]; consequently, renal replacement therapy is often required. In patients needing renal replacement therapy, mortality is as high as 60-70%[2]. There has been a trend to operate on higher risk patients[3], likely increasing the prevalence of AKI post CABG. With roughly four hundred thousand CABG surgeries performed annually in the US[4], this represents an enormous health care problem. Currently, there is no known therapy for AKI or way to prevent AKI occurrence in this patient population. Evidence suggests that elevated preoperative circulating endogenous ouabain (EO), a cardiac glycoside structurally similar to digoxin, leads to increased risk of AKI post CABG[5,6]. We propose to study the impact of giving preoperative DigiFab, a biological preparation approved by the FDA for the treatment of digitalis toxicity, as a means to reduce/prevent the incidence of CABG-evoked AKI. DigiFab is expected to have a beneficial and possibly unique utility in this scenario because the antibodies it contains have the side-property of also binding EO with high affinity (Hamlyn & Blaustein, unpublished).

2. Rationale

Postoperative AKI is one of the most clinically significant causes of morbidity and mortality in patients undergoing CABG.[3] Even minimal increases in serum creatinine are associated with increased mortality[7]. Similarly, acute renal failure is the strongest risk factor for death in patients undergoing cardiac surgery[8]. Clinical studies indicate that high pre-op plasma EO concentrations

are a marker for AKI in patients undergoing CABG surgery, and the highest plasma EO concentrations are associated with the highest risk and poorest clinical outcomes[5,6].

High plasma EO may directly cause renal damage in vivo and in vitro[5,9]. In cultured rodent renal cells, prolonged ouabain treatment has been shown to decrease nephrin expression (a protein involved in formation of the glomerular filtration barrier).[5] In vivo, prolonged ouabain administration decreases renal clearance, increases plasma creatinine, and increases proteinuria. Further, Rostafuroxin, a unique, first generation ouabain receptor antagonist, has been shown to attenuate the ouabain-evoked renal damage by preventing nephrin down regulation in rats[9,10]. Unfortunately, Rostafuroxin is not currently an FDA approved drug, and the commercial prospects for this agent seem minimal because it binds to its in vivo ouabain receptor with relatively low affinity[11].

The absence of a clinically-useful ouabain receptor antagonist with which to interfere/preempt AKI opens the door to other strategies, including the possibility of short-term in vivo immunoneutralization. In this regard, DigiFab is one of several biologicals approved for the treatment of digitalis toxicity worldwide. In the US, DigiFab (BTG, Intl.), which consists exclusively of monovalent Fab fragments, was preceded by the divalent Fab preparation, Digibind (Wellcome, Glaxo, Smith Kline). Digibind is no longer commercially available. Both Fab preparations bind and immunoneutralize digoxin as well as other digitalis-like entities, including ouabain, with high affinity[12,13], but differ in the rates at which their Fab fragments are cleared from the circulation. Like Digibind, DigiFab has proven to be safe and has negligible side effects and virtually no toxicity when used at approved doses.[14]

Furthermore, several trials using Fab fragment preparations have been conducted recently. Among pregnant women with severe pre-eclampsia, both Digibind and DigiFab significantly reduced progression of renal dysfunction[15,16]. The utility of these preparations in pregnant women with severe pre-eclampsia provides further evidence of safety as well as efficacy in minimizing the decline in renal function.

Based upon the aforementioned preliminary information, we will test the hypothesis that the ability to lower serum EO by immunoneutralization with DigiFab prior to CABG surgery will reduce the incidence of post-operative AKI, and thus, reduce post-operative morbidity and mortality.

3. Methods

We plan to conduct a randomized double-blinded study investigating the role of DigiFab in patients with elevated risk of acute kidney injury undergoing CABG surgery. Patients who are undergoing CABG, meet the inclusion criteria, and provide consent will be enrolled in this study and randomized to either DigiFab arm or the placebo (vehicle) arm. The number of patients to be consented will be enough to achieve two hundred and fifty evaluable participants. The number consented will not be more than 350 patients. The study involves a follow up period of 72 hours post CABG surgery.

3A- *Recruitment*

All patients awaiting non-emergency CABG surgery will be considered for this study. Patients with high risk of developing AKI post CABG will be included in this study. High risk of AKI will be defined as one of the following: GFR >15 but below 60 (must be >36 hours after receiving any contrast, but within two weeks of signing consent) or a history of diabetes mellitus.[2] Diabetes will be defined as a past diagnosis or a current hemoglobin a1c of ≥6.5%.

3B. *Inclusion Criteria*

Each patient must meet all the following criteria to be enrolled in this study:

- Patient provided informed consent
- Male or female > 18 years of age
- Undergoing CABG and one of the following:
    - GFR (Cockcroft-Gault) less than 60 (should be > 36 hours after receiving any contrast, but within two weeks of signing consent)
    - Past diagnosis of Diabetes or current Hemoglobin A1c of ≥ 6.5%

3C . *Exclusion criteria*

A patient who meets any of the following criteria will be excluded from this study:

- AKI defined as:
    - Decrease in GFR > 25% of baseline based on RIFLE criteria[17]
- Received contrast within the past 36 hours of IP administration
- Prior kidney transplantation
- Prior adverse reaction to DigiFab
- Received Digoxin within the past 30 days

- Has allergy to papaya, papain, or sheep proteins
- GFR < 15 (within two weeks of signing consent and > 36 hours after receiving contrast)

Calculation of Creatinine Clearance (C_{Cr}; Cockcroft-Gault Formula)

C_{Cr}={((l 40–age) x weight)/(72xS_{Cr})}x 0.85 (if female)

C_{Cr} (creatinine clearance) = mL/minute

Age = years; Weight = kg

SCr (serum creatinine) = mg/dL

3D. Enrollment and Baseline

Patients awaiting surgery in the hospital or having elective surgery and initially seen as out-patients will be eligible for this study.

After a participant completes the consent process and is deemed eligible for this study, baseline parameters will be obtained. Laboratory tests—including basic metabolic panel (BMP, including serum creatinine), NT-pro-BNP (N-terminal prohormone of brain natriuretic peptide), and troponin—and hemodynamics – heart rate, blood pressure (systolic, diastolic, and mean arterial pressure)—obtained ideally within 24 hours before IP administration will be recorded. If multiple values exist within this time frame, the value closest to time of surgery will be used. Blood for EO concentrations, Proenkephalin A,[18] and TIMP × IGFBP7[19,20,21] will be drawn prior to investigational drug administration. Patient characteristics including age, sex, comorbidities (hypertension and diabetes) will be noted. Of note, ouabain concentrations take many days to return and will not be known prior to CABG.,

3E. *Randomization*

After ensuring eligibility, patients will be randomized by the investigational pharmacy. Stratified randomization will be employed using race and sex. Patients will be randomized approximately 24 hours prior to surgery. In the event that surgery is postponed to a later date, patients will be permitted to remain in the study. Digifab will be re-administered according to the usual protocol. If surgery is moved a second time and Digifab had been given, patients will be removed from the study.

3F. Intervention

BTG will provide DigiFab; placebo (saline) will be provided by the pharmacy. In-hospital study patients will be dosed intravenously with 80 mg Digifab or an equivalent volume of fluid the night prior to surgery (approximately 12-15 hours prior to surgery) and again 1 hour pre-operatively. The vials will be stored in the hospital investigational pharmacy. Patients being admitted to the hospital on the day of surgery will receive 80 mg Digifab or an equivalent volume of fluid approximately 1 hour prior to surgery.

3G. Post-operative data:

We will collect blood for endogenous ouabain, Proenkephalin A, and TIMP × IGFBP7 in the immediate post-operative period 0 to 4 hours post-surgery. The ouabain measurement at this time will determine the fraction that is free and that which is bound to DigiFab. This 4 hr post-op sample window provides the only opportunity to obtain critical information concerning the efficacy of the DigiFab immunoneutralization. Total serum ouabain, Proenkephalin A, TIMP × IGFBP7, and creatinine concentrations will also be obtained at 72 +/- 12 hours post-surgery.

Time spent on cardiopulmonary bypass will be noted. Seven days post-operatively, need for any type of renal replacement therapy will be recorded. Lastly, mortality within a seven-day period will be noted.

3H. Data collection

Each variable will be captured and stored in a HIPAA compliant and secure database.

3I. Primary endpoint: Changes in GFR will be compared in patients who receive placebo and intervention by ANOVA and, as appropriate, parametric or non-parametric tests. The primary endpoint will compare only patients with the 50% highest baseline (preoperative) concentrations of ouabain.

3J. Secondary endpoints:

1. Comparing GFR amongst all patients
2. Incidence of AKI comparing the groups (25% decrease from baseline)
3. Incidence of renal replacement therapy by 30 days
4. Comparing intraoperative and 30 day mortality for placebo vs DigiFab.
5. In patients on cardiopulmonary bypass for the longest time (top 50%).
6. Comparison of change in Proenkephalin A in patients receiving Digifab and placebo.
7. Comparison of change of TIMP × IGFBP7 in patients receiving Digifab and placebo.
8. Comparison of GFR in patients receiving one dose and patients receiving two doses of Digifab.

3K. Analysis

Mean comparisons will be made by ANCOVA and, as appropriate, parametric or non-parametric tests. Chi square will be used as indicated. We will control for GFR and sex.

3L. Power

We postulate that the average patient will have a GFR of 50 which correlates to a creatinine of 129 μmol/l. Previous studies have shown a post-operative mean increase of 48 μmol/l with a standard deviation of 76. Therefore, on average, post-operative creatinine should be 177 μmol/l, which is roughly a GFR of 35. A trial of 62 patients per group (top half of elevated ouabain concentrations) should have a power of >70% to detect a 36 μmol/l difference in plasma creatinine, i.e., a creatinine of 141 μmol/l (177-36). A creatinine of 141 μmol/l correlates to a GFR of 45—a ~25% increase in GFR.

4. Risks

The primary risk associated with this study includes all of the potential adverse effects of DigiFab including: anaphylactic and anaphylactoid reactions, delayed allergic reactions, and a febrile response to immune complexes formed. However, since the Fab fragment of the antibody lacks the antigenic determinants of the Fc region, this should pose a reduced immunogenic threat. Of note, no patients during the clinical trials experienced an anaphylactic event. Patients with allergies to sheep protein would be at particular risk. In a registry of 717 adult patients receiving DigiFab, 6 had a probable or possible adverse reaction to the medication. All were symptomatically treated successfully[22]. Study participants will be monitored closely for potential adverse effects in the perioperative, operative, and post-operative setting. If an anaphylactic

reaction occurs, epinephrine, steroids, an H2 blocker, and diphenhydramine will be given as appropriate. Each blood draw carries the risk of pain associated with the venous puncture. As this is the first use of DigiFab in this patient population, unknown side effects are possible.

Data Safety and Monitoring Board

There will be an independent Data Safety and Monitoring Board (DSMB) which will assess the safety of the trial after 62 patients (first 25% of patients are enrolled). An unblinded coordinator will provide the board with all adverse events and deaths to investigate. The DSM board will evaluate the cause of deaths and allergic reactions. The panel will consist of Dr. Libin Wang, Dr. Timm Dickfeld, Dr. Chaz Hong, and a statistician.

5. Benefits

Study participants may experience short and long-term benefits. Potential benefits include prevention of AKI, reduced hospital stay, protection from renal replacement therapy, and decreased mortality. In the long term, potential benefits include protection from: progression to chronic kidney disease, need for renal replacement therapy and early death.[23]

6. Confidentiality

All data collected will be de-identified once stored on the database.

7. Consent process

Study participants who meet inclusion criteria will be approached by a study coordinator or investigator. The consenter will review the risks and benefits to partaking in this trial. If the

participant expresses interest, a study consent form will be provided. Potential participants will have the opportunity to ask questions at any point during the explanation process.

8. Conflict of Interest

Drs. Gottlieb, Blaustein, and Hamlyn have a provisional patent and a patent pending for the use of DigiFab (and/or a ouabain antagonist) to prevent renal dysfunction in patients undergoing CABG. All investigators will be blinded to the treatment received until all data are finalized.

9. HIPAA Compliance

All authors have completed HIPPA training in accordance with the University of Maryland.

10. Stopping Rules

The study will be halted if the independent data monitoring determines that known or unknown side effects of DigiFab are occurring with unacceptable symptoms for the patient and/or mortality. The DSMB will determine the specific rules.

11. Timeline

1. Patient consent and enrollment

2. Prior to administering the first dose of DigiFab, draw blood for NT-pro-BNP, troponin, proenkephalin A, TIMP × IGFBP7, and endogenous ouabain (ideally within 24 hours of IP administration. Record BMP (including creatinine) values, demographics, and hemodynamics.

3. For hospitalized patients, approximately 12-15 hr before surgery: Administer DigiFab 80 mg or placebo if > 36 hours post contrast. If necessary to meet entry requirements, only immediate pre-op dose will be given.

4. One hr pre-op: Administer a second dose (or first dose for same day admissions) of DigiFab 80 mg or placebo

5. Surgery

6. 0-4 hr Post-op: Draw blood for free and bound ouabain, proenkephalin A, TIMP × IGFBP7 determinations. Record BMP (including creatinine) values

7. 72 ± 12 hr post-op: Draw blood for total ouabain, proenkephalin A, TIMP × IGFBP7. Record BMP values (including creatinine).

8. 30 days-Contact patient, next of kin, or hospital records to ascertain whether patient is alive and whether the patient has undergone renal replacement therapy.

References

1. Lagny MG, Jouret F, Koch JN, Blaffart F, Donneau AF, Albert A, et al. Incidence and outcomes of acute kidney injury after cardiac surgery using either criteria of the RIFLE classification. *BMC Nephrol*. 2015;16:76.

2. Rosner MH, Portilla D, and Okusa MD. Cardiac surgery as a cause of acute kidney injury: pathogenesis and potential therapies. *J Intensive Care Med* 23: 3-18, 2008.

3. Schopka, S., Diez, C., Camboni, D., Floerchinger, B., Schmid, C., and Hilker, M. Impact of cardiopulmonary bypass on acute kidney injury following coronary artery bypass grafting: a matched pair analysis**.** *J Cardiothorac Surg*. 2014; 9: 20

4. Alexander JH and Smith PK. Coronary-artery bypass grafting. *N Engl J Med* 2016; 374:1954-1964

5. Bignami E, Casamassima N, Frati E, Lanzani C, Corno L, Alfieri O, Gottlieb S, Simonini M, Shah KB, Mizzi A, Messaggio E, Zangrillo A, Ferrandi M, Ferrari P, Bianchi G, Hamlyn JM, and Manunta P. Preoperative endogenous ouabain predicts acute kidney injury in cardiac surgery patients. *Crit Care Med* 41: 744-755, 2013.

6. Simonini M, Lanzani C, Bignami E, Casamassima N, Frati E, Meroni R, Messaggio E, Alfieri O, Hamlyn J, Body SC, Collard CD, Zangrillo A, and Manunta P. A new clinical multivariable model that predicts postoperative acute kidney injury: impact of endogenous ouabain. *Nephrol Dialysis Transplant* 29: 1696-1701, 2014.

7. Lassnigg A, Schmidlin D, Mouhieddine M, Bachmann LM, Druml W, Bauer P, Hiesmayr M: Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study. *J Am Soc Nephrol* 15: 1597-1605, 2004.

8.Chertow GM, Levy EM, Hammermeister KE, Grover F, Daley J: Independent association between acute renal failure and mortality following cardiac surgery. *Am J Med* **104**: 343–348, 1998

9.Villa L, Buono R, Ferrandi M, Molinari I, Benigni F, Bettiga A, Colciago G, Ikehata M, Messaggio E, Rastaldi MP, Montorsi F, Salonia A, and Manunta P. Ouabain Contributes to Kidney Damage in a Rat Model of Renal Ischemia-Reperfusion Injury. International journal of molecular sciences 17: 2016.

10. Ferrandi M, Molinari I, Rastaldi MP, Ferrari P, Bianchi G, and Manunta P. Rostafuroxin protects from podocyte injury and proteinuria induced by adducin genetic variants and ouabain. *J Pharmacol Exp Therap* 351: 278-287, 2014.

11. Song H, Karashima E, Hamlyn JM, and Blaustein MP. Ouabain-digoxin antagonism in rat arteries and neurones. *Journal of Physiology* 592: 941-969, 2014.

12. Pullen MA, Brooks DP, and Edwards RM. Characterization of the neutralizing activity of digoxin-specific Fab toward ouabain-like steroids. *J Pharmacol Exp Therap* 310: 319-325, 2004.

13. Pullen MA, Harpel MR, Danoff TM, and Brooks DP. Comparison of non-digitalis binding properties of digoxin-specific Fabs using direct binding methods. *J Immunol Methods* 336: 235-241, 2008.

14. Adair CD, Buckalew VM, Kipikasa J, Torres C, Stallings SP, and Briery CM. Repeated dosing of digoxin-fragmented antibody in preterm eclampsia. *J Perinatol* 29: 163-165, 2009.

15 Adair CD, Buckalew VM, Graves SW, Lam GK, Johnson DD, Saade G, Lewis DF, Robinson C, Danoff TM, Chauhan N, Hopoate-Sitake M, Porter KB, Humphrey RG, Trofatter KF, Amon E, Ward S, Kennedy L, Mason L, and Johnston JA. Digoxin Immune Fab Treatment for Severe Preeclampsia. *Am J Perinatol* 27: 655-662, 2010.

16. Lam GK, Hopoate-Sitake M, Adair CD, Buckalew VM, Johnson DD, Lewis DF, Robinson CJ, Saade GR, and Graves SW. Digoxin antibody fragment, antigen binding (Fab), treatment of preeclampsia in women with endogenous digitalis-like factor: a secondary analysis of the DEEP Trial. *Am J Obstet Gynecol* 209: 119 e111-116, 2013.

17. Srisawat N, Hoste EE, and Kellum JA. Modern classification of acute kidney injury. *Blood Purification* 29: 300-307, 2010.

18. Hollinger A, Wittebole X, François B, Pickkers P, Antonelli M, Gayat E, Chousterman BG, Lascarrou JB, Dugernier T, Di Somma S, Struck J, Bergmann A, Beishuizen A, Constatin JM, Damoisel C, Deye N, Gaudry S, Huberlant V, Marx G, Mercier E, Oueslati H, Hartmann O, Sonneville R, Laterre PF, Mebazaa A, Legrand M. Proenkephalin A 119-159 (Penkid) Is an Early Biomarker of Septic Acute Kidney Injury: The Kidney in Sepsis and Septic Shock (Kid-SSS) Study. *Kidney Int Rep*. 2018 Aug 22;3(6):1424-1433.

19. Cummings JJ, Shaw AD, Shi J, Lopez MG, O'Neal JB, Billings FT. Intraoperative prediction of cardiac surgery-associated acute kidney injury using urinary biomarkers of cell cycle arrest. *J Thorac Cardiovasc Surg*. 2018 Sep 26. pii: S0022-5223(18)32484-X. doi: 10.1016/j.jtcvs.2018.08.090. [Epub ahead of print]

20. Fan W, Ankawi G, Zhang J, Digvijay K, Giavarina D, Yin Y, Ronco C. Current understanding and future directions in the application of TIMP-2 and IGFBP7 in AKI clinical practice. *Clin Chem Lab Med*. 2018 Sep 4doi: 10.1515/cclm-2018-0776. [Epub ahead of print]

21. Johnson ACM, Zager RA. Mechanisms Underlying Increased TIMP2 and IGFBP7 Urinary Excretion in Experimental AKI. *J Am Soc Nephrol*. 2018 Aug;29(8):2157-2167. doi: 10.1681/ASN.2018030265. Epub 2018 Jul 6.

22. Hickey AR, Wenger TL, Carpenter VP, Tilson HH, Hlatky MA, Furberg CD, Kirkpatrick CH, Strauss HC, Smith TW. Digoxin Immune Fab therapy in the management of digitalis

intoxication: safety and efficacy results of an observational surveillance study. *J Am Coll Cardiol*. 1991 Mar 1;17(3):590-8.

23. O'Neal JB, Shaw AD, Billings FT, Ftt B. Acute kidney injury following cardiac surgery: current understanding and future directions. *Crit Care* 20(1):187, 2016.