DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

Immediate Goals: To examine the mechanism of ischemic mitral regurgitation (MR) with the goal of designing and implementing more effective therapy to reduce adverse impact on patients. **Career Development Goals**: To provide sufficient time for mentoring and research activities. **Research Project**: Mitral valve function can be understood in terms of the force-balance concept in which tethering forces from the papillary muscles balance left ventricular valve closing forces. In ischemic MR, this force balance may be altered in ways that impair the ability of the mitral leaflets to close effectively at the annular level. This proposal uses a combined, parallel clinical and experimental approach to evaluate the mechanism, progression and therapy of ischemic MR, all relating to the **central hypothesis** that ischemic MR is caused by an abnormal relationship of the mitral valve to its supporting ventricular structures. These altered relationships involve both abnormal tethering forces due to displacement of the papillary muscles as well as reduced closing forces due to LV contractile dysfunction. Specific testable questions related to this hypothesis include: 1) The progression of mitral regurgitation in patients with acute myocardial infarction relates to abnormalities in the mitral valve-ventricular relationship; 2) These mechanisms also cause persistent MR despite coronary revascularization surgery, thereby impairing exercise capacity and raising pulmonary pressures; 3) Both an externally applied device and afterload reduction provide effective means of reducing ischemic mitral regurgitation by normalizing these relationships between the valve and the ventricle; cutting a minimum number of critically positioned strut chordae also has the potential to relieve tethering, and opens the way to potential minimally invasive percutaneous approaches. The aims of the mentored award will be met by allowing the PI to translate his experimental expertise to direct clinical studies of progression and functional outcome of ischemic MR, and to make the transition from mechanism to therapy in models reflecting the clinical situation, with the ultimate goal of patient applications.

PERFORMANCE SITE(S) (*organization, city, state*)

_____, _____,_____._____ _____

KEY PERSONNEL. See instructions on Page 11. *Use continuation pages as needed* to provide the required information in the format shown below.

Name	Organization	Role on Project
_____	_____ Hospital	Principal Investigator
_____	_____ Hospital	Senior Animal Technologist
_____	_____ Hospital	Senior Programmer/Analyst

Use this substitute page for the Table of Contents of Research Career Awards

Type the name of candidate at the top of each printed page and continuation page

RESEARCH CAREER AWARD

TABLE OF CONTENTS

(Substitute Page)

Page Numbers

Note: Type density and size for the entire application must conform to the instructions on page 6 of the general instructions.

*Include these items only when applicable.

CITIZENSHIP

[X] U.S. citizen or noncitizen national

[ ] Permanent resident of U.S.

[ ] If a permanent resident of the U.S., a notarized statement is included with the application

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory

An 800 sq ft animal laboratory is available with 2 full-time technicians experienced in open heart surgery and cardiopulmonary bypass, an 8-channel recorder, defibrillator, cardiopulmonary bypass equipment, fluoroscopy, radionuclide imaging, full chemistries, animal quarters and a sterile operating room. A Carolina Medical flow-meter with 3 specially designed (Yellin) intracardiac probes for mounting within the mitral annulus are available, as well as Transonic and thermodilution output devices.

Clinical

The inpatient and outpatient echocardiography laboratories have a combined area of approximately 6000 sq ft and include 8 examining rooms and two rooms for off-line review and computer analysis of studies. There are 9 clinical technologists and several are involved in ongoing clinical research studies.

Animal

(Please see Laboratory, above.) The Edwards 6 animal housing and care area contains 3800 sq ft for the housing of dogs, cats, primates, sheep, swine, and other large animals.

Computer

The Cardiac Computer Center (1800 sq ft) has a VAX 11/780 with 4 MB of main memory and 1.3 gigabytes of virtual memory, with 8 terminals in the echo lab, supporting the INGRES data base (>50,000 studies), RS/1, BMDP and SAS analysis packages, and IMSL math subroutines. A 3D echo reconstruction system has a dedicated programmer analyst, computer room, a SUN 486 data acquisition and analysis computers, Vista frame-grabbing boards, Silicon Graphics Indy and Indigo workstations with built-in video frame-grabbing and compression capabilities, and a Pinnacle Sierra 1.3 gigabyte magneto-optical disk drive.

Office

The echo lab facilities have office space for cardiac fellows, secretaries and staff members; with on-site laser printers, photocopiers, VAX terminals, and a variety of PC and word-processing computers.

Other

The _____ Hospital is a 933-bed, acute care facility with roughly 33,000 acute inpatient admissions/ year including several hundred with acute MI, >200,000 outpatient visits, 800 coronary bypass surgeries/year, and over 50 staff cardiologists active in patient care, research and teaching. 12 cross-sectional echocardiographic machines are available for imaging and research with combined 2D-echo and Doppler color flow mapping capability. These include 8 Hewlett-Packard Sonos 2500 and 5500 systems, with optical disk devices for storage and output of digital Doppler color flow maps in cine loops. Two of the machines have software that permits automated acquisition of 3D data sets using a rotational probe imaging at up to 1 degree intervals; acquisitions are gated to ensure consistent cardiac cycle lengths and phase in the respiratory cycle. The data sets covering either one point in the cardiac cycle or the entire cycle are rapidly stored to disk for transfer to our workstation. Four rotational (Omniplane) probes are available: one reserved for research work, and the other adapted for transthoracic scanning. A Vingmed system with onboard and off-line computers for digital analysis of Doppler color flow maps will soon be available. A portable 3D positional locating and reconstruction system is available, including spark gap (Science Accessories G6PD) and electromagnetic (Bird, Ascension Technologies) positional locators, and the Silicon Graphics workstation and optical disk drive noted above.

3. THE CANDIDATE

a. Candidate's Background: Commitment to a Career in Patient-Oriented Research –
Early positive experiences with role models in biomedical research at _____ and the _____ influenced the applicant in his high school and college years; these mentors included Drs. _____and _____, who introduced him to the biophysics of vision, and _____, later to win the Nobel Prize for solid-phase peptide synthesis. These experiences motivated him to spend a year at _____ University during medical school to study physiologic modeling, ranging from the basic physics of neural conduction with _____ to clinically relevant models of whole-animal physiology. As a medical student, he was strongly influenced by _____, Director of the fledgling Cardiac Ultrasound Laboratory at _____Hospital in _____, who stimulated his interest in using noninvasive techniques to address research challenges in patients. This led him to pursue an NIH Training Program in Noninvasive Cardiology at _____, directed by Dr. _____ and stressing the common principles of ultrasound, radionuclide, and magnetic resonance imaging techniques. In continuing postgraduate fellow-ship work, he applied ultrasound techniques to physiologic investigation, and over the past 13 years since completing this training, he has led independent research efforts in valvular heart disease.

In his career, *the candidate* has consistently drawn upon clinical observations to raise questions which are then addressed in patient studies and correlative in vivo and in vitro experiments and computer simulations. In his work, there is a dynamic interplay between hypothesis-driven physiologic investigation and the development of advanced techniques to permit noninvasive physiologic and hemodynamic studies in patients. He has benefited from a rich collaborative environment, including surgical, interventional, electrophysiologic, heart failure, and bioengineering investigators, and worked with Prof. _____ to build a program in valvular bioengineering at the _____. Mentoring junior clinical investigators has become integral to his career, based on personal satisfaction and the importance of broadening research efforts to span more than one individual or institution. *The candidate* has structured his career to give patient-oriented research highest priority, making specific choices in favor of research over increased clinical and administrative activities. At this juncture, however, committed funding as in the K24 award is necessary to continue this career path.

Ability and Potential to Conduct High-Quality Patient-Oriented Research - *The candidate* has demonstrated strong ability to conduct high-quality patient-oriented research which is rigorous and hypothesis-driven, with a strong record of publication in leading journals. He pioneered developments in three-dimensional echocardiographic reconstruction of the heart in order to test his hypothesis that the mitral valve and annulus have a non-planar saddle-like shape in the normal beating human heart. His findings, confirmed by several other groups, have led to a redefinition of mitral valve prolapse (MVP). This has eliminated the widespread diagnosis previously made in otherwise normal individuals based on an assumed planar annulus above which displacement would always be abnormal: in a side-to-side view through a saddle, the saddle leaflet surface will normally rise above the edges of the saddle without leaflet distortion.[24] Reducing the "epidemic" of diagnosis[67] and its attendant anxiety and antibiotic prophylaxis has also provided the specificity needed for studies of genetic linkage, begun in collaboration with Dr. _____ of the _____.

The candidate has also used three-dimensional echocardiography[29] as well as in vivo and in vitro modeling to demonstrate that a common principle relates abnormal motion of the mitral valve to abnormalities of its attachments to the left ventricle (LV) and papillary muscles (PMs). He first showed that anterior shift of the PMs in patients with hypertrophic cardiomyopathy creates systolic anterior motion (SAM) of the mitral valve with outflow tract obstruction.[68] His findings that SAM is really just an anterior form of prolapse (excessive leaflet motion directed anteriorly) have supported recent surgical techniques to prevent postoperative SAM in patients with MVP undergoing surgical repair.[69] Conversely, posterior shift of the PM attachments with myocardial infarction or dilated cardiomyopathy restricts the ability of the mitral leaflets to close effectively and causes mitral regurgitation (MR).[22] These insights have led to the design of new approaches to reposition the mitral valve attachments, for example, by plicating the infarct region to restore mitral leaflet tethering geometry toward normal,[70] or by the simpler techniques in this proposal, aiming toward practical clinical application.

In parallel efforts, *the candidate* has developed advanced noninvasive techniques for patient evaluation. He has shown that 3D echo increases the accuracy of chamber size and function evaluation[31-33] and can guide

surgical repair of congenital lesions and valvular heart disease. He has applied fluid mechanical principles to improve quantification of regurgitation using a new technique of integrated Doppler power (proportional to the cross-sectional area of flow) times velocity at the regurgitant jet origin. He has used these interrelated projects as opportunities to stimulate the creativity of beginning clinical investigators and later help them develop independent careers.

The candidate's scientific abilities and judgment are recognized by his current membership on the editorial boards of leading patient-oriented research publications (Circulation, the Journal of the American College of Cardiology, and the Journal of the American Society of Echocardiography), and recent service as Program Director of the 1997 Scientific Sessions of the American Society of Echocardiography.

b. Career Goals and Objectives – *The candidate's* short- and intermediate-term goals are to continue to build a patient-oriented research program bringing scientific rigor and innovation to the field of noninvasive cardiology and cardiovascular hemodynamics. The maximum benefit of such a program, however, can be achieved only if it also involves training and mentoring of the next generation of investigators who combine a thorough understanding of the principles and pitfalls of noninvasive imaging with the ability to perform scientifically based and hypothesis-driven clinical research. More immediate goals include development and implementation of novel approaches to reduce MR in ischemic heart disease and dilated cardiomyopathy, to improve functional capacity and patient outcome. A parallel goal is to improve quantification of valvular regurgitation by practical implementation of the Doppler power-velocity approach. Longer-term career development goals are to explore basic mechanisms of heart failure in valvular regurgitation, answering the question of why MR can produce contractile dysfunction; to extend the same imaging principles to evaluate and improve repair of tricuspid and aortic valves; and to establish the genetic basis and diversity of idiopathic MVP, with the aim of guiding follow-up and potentially limiting progression in susceptible individuals by reducing valve stresses. The applicant hopes to accomplish these goals while advancing to Professor of Medicine, with potential administrative responsibilities directing research and research training.

Mentoring Commitment – *The candidate's* career has been profoundly influenced by role models in mentoring, such as _____, _____ and_____ at _____, who inspired him as they performed experiments one-on-one with him. Dr. _____, fresh from _____, where echocardiography was being introduced into physiologic research, first conveyed to him as a medical student the enthusiasm for integrating noninvasive imaging into clinical investigation. As a Clinical Cardiology Fellow at _____Hospital in _____, he was first given the opportunity by Dr. _____ to develop an original hypothesis regarding contrast echocardiography, plan the study, carry it out, and present it at a national meeting and as a first-author publication. Dr. _____, with his NIH Fellowship Training Program, provided a model of how allowing fellows to express their creativity in a supportive environment with a critical mass of people and resources could allow them to succeed and develop long-term careers, reflected in those of individuals such as Dr. _____, now Director of Imaging at the _____, and Dr._____, a distinguished coronary flow physiologist at the University of _____. Dr. _____ subsequent administrative role as Chief of Cardiology provided greater range for *the candidate* to develop an independent program with fellows and grant support. *The candidate's* commitment to mentoring therefore reflects what he has learned from these role models regarding the skills needed to help potential investigators appreciate their strengths, and the wisdom needed to help them balance individual success with strong teamwork. This commitment to mentoring in no small measure also reflects the legacy of his father, a respected educator who taught teachers how to teach, emphasizing skills of problem-solving and clear exposition, and the example of both parents working together to develop an effective curriculum for early childhood education.

Contribution of this Award to Attainment of Long-Term Career Objectives - This award mechanism recognizes the time-intensive nature of effective mentoring, reflected in the specific steps in the Mentoring Plan. *The candidate*, however, is an integral member of a busy clinical service, which has expanded over the past 13 years since the completion of his training from performing 4,000 to currently nearly 15,000 echocardiographic patient studies per year, with only a slight increase in physician staff because of progressively decreasing reimbursement. The complexity of the hemodynamic data derived from these studies has increased, with more time-intensive transesophageal and dobutamine stress studies, as well as studies in the

Operating Room and prolonged Catheterization Laboratory studies guiding device closure of shunt lesions. *The candidate's* clinical commitment has therefore increased to at least four days of clinical activity per week during the past two years, with frequent evening and weekend work. This has limited his ability to provide the necessary support to trainees seeking his mentoring; recently, in fact, he has unfortunately had to turn away several promising trainees who sought to work with him. Time constraints could therefore adversely impact his achieving his career goals, particularly in developing the next generation of patient-oriented investigators. He has also had to restrict severely time spent at a complementary program in cardiovascular fluid and valvular mechanics at the_____, which has until now provided opportunities for increased scientific depth, mentoring and recruitment.

The candidate's program also demands the time to support trainees' original research ideas that may not be reflected in productivity for his own research grants; and the time for collaboration and career development in the direction of studying basic mechanisms of heart failure in valvular regurgitation and the molecular basis of conditions such as familial MVP. The Midcareer Investigator Award is critically important to ensure that adequate time and effort can be provided to mentor trainees at all stages, but especially as they develop independence, without compromising *the candidate's* research programs and career evolution. This is particularly important for him in order to support Dr. _____ K23 Mentored Award. The K24 Award is consistent with recent findings that a >25% time commitment most strongly identifies effective role models in clinical teaching.[71] It recognizes that developing the necessary skills for innovation and independent hypothesis-driven research involves a process equally long as that for basic investigation.

Evidence of Ongoing High-Quality Patient-Oriented Research and its Relationship to This Program – The research plan in this application builds upon *the candidate's* prior work on ischemic MR. It has the dual aim of designing improved therapy based upon a more thorough understanding of mechanism; and of extending studies back to the clinical realm to demonstrate the natural history of ischemic MR and prove the benefit of its reduction in terms of quantifiable patient outcomes. The research plan has been adapted and extended from Dr. _____current R01 on ischemic MR because it is particularly relevant to the overall mentoring goal of the K24 Award. It uses a variety of investigational models to articulate studies of human physiology and pathophysiology with basic animal work, device development, and outcomes research approaches to evaluate therapeutic benefits rigorously. This interplay of research approaches can serve as a model for new investigators in patient-oriented and translational research.

Evidence of Monetary Support for Patient-Oriented Research - *The candidate's* research studies have received continuous funding as summarized below:

1987-90 PI, National AHA Grant-In-Aid: Ventricular Outflow Obstruction
1987-90 PI, Whitaker Foundation Grant. Fluid mechanics in valvular heart disease
1987-93 PI, NIH Grant R29 HL38176 – 01 to –05. FIRST Award: The Mechanism of Ventricular Outflow Obstruction in IHSS
1991-94 Consultant, NIH R01 HL45485: Fluid Mechanical Approaches to Valvular Regurgitation
1991-95 PI, National AHA Grant-In-Aid: Quantification of Valvular Regurgitation
1995-00 PI, NIH Grant R01 HL53702: Noninvasive Quantification of Valvular Regurgitation by the Flow Convergence Method (now on no-cost extension)
1998-03 PI, NIH Grant R01 HL38176-06 to –10: Integrated Mechanisms of Ischemic Mitral Regurgitation
2000-02 Joint PI with Dr. _____, Doris Duke Charitable Foundation Innovations in Medical Research Grant: Genetic Basis of MVP

The candidate's current R01 on ischemic MR will be supported by three further years of secure funding. He has also developed new scientific directions to renew his R01 on the quantification of valvular regurgitation.

c. Career Development / Training Activities: Mentoring Plan

Mentoring Experience and Evidence of Commitment – *The candidate* has extensive experience in mentoring individuals in patient-oriented research, both during their basic clinical training to motivate pursuit of an advanced track, and during postgraduate research fellowships. He consistently conveys the excitement of the creative process as well as the need for rigorously high standards. He identifies individual strengths and focuses on them in guiding career development. He has worked to create an environment that will support the development of independence by allowing fellows to implement their own ideas without having to re-create the necessary resources, which include a programmer to design the necessary 3D or digital data analyses; a superb surgical physiology team; and a high-quality clinical imaging service. As seen below, he has developed long-term mentoring relationships with his trainees, many of whom have been Young Investigator Award winners and finalists and gone on to become academic laboratory directors and service chiefs with independent grant support. Dr. _____, for example, a former fellow and current faculty member at MGH doing original work on the limitations of annular ring reduction as a therapy for ischemic MR, received an NIH National Research Service Award and a Grant-In-Aid of the American Society of Echocardiography, and has now submitted a K23 Mentored Patient-Oriented Research Career Development Award. Dr. _____, another former fellow who joined the faculty, has worked with *the candidate* since his PhD studies at _____ on mathematical analysis of pulmonary venous inflow and left heart filling, the basis of an AHA Minority Scientist Career Development Award; his current focus is device development for minimally invasive therapy of valvular heart disease, with the support of a Robert Wood Johnson Foundation Faculty Scholar Career Development Award developed together with the candidate.

Young Investigator Award Winners and Finalists
Mentored by Dr. _____

Year	Fellow	Award	Topic
1993	_____, MD	Young Investigator Award finalist American Society of Echocardiography	Echo can predict risk of complications in mitral valve prolapse
1993	_____, MD, PhD	Samuel A. Levine Clinical Young Investigator Award finalist, AHA	A new technique for quantifying valvular regurgitation: analyzing the proximal flow field
1995	_____, PhD	Young Investigator Award winner American Society of Echocardiography	Impact of valve shape by 3D echo on stenotic valve severity
1996	_____, MD	Young Investigator Award winner (Clinical) American College of Cardiology	3D echo/ physiology of valvular stenosis; stereolithography from 3D images
1996	_____, PhD, MD	Young Investigator Award winner (Physiology) American College of Cardiology	Mathematical analysis of pulmonary venous flow: Clinical insights
1997	_____, MD	Samuel A. Levine Clinical Young Investigator Award finalist, AHA	Pathophysiology of ischemic MR: Separating dysfunction and dilatation
1998	_____, MD	Daniel Kalmanson Award finalist International Cardiac Doppler Society	Annular role in functional MR
1998	_____, MD	Young Investigator Award winner (Clinical) American College of Cardiology	A new surgical therapy for ischemic MR: Infarct plication
1998	_____, MD	Young Investigator Award winner, Astra-Merck	The genetics of mitral valve prolapse
1999	_____, MD	Young Investigator Award winner (Clinical) American College of Cardiology	Novel regurgitant quantification using the Doppler power-velocity integral

1999	_____, MD	Young Investigator Award finalist European Cardiac Society	3D echo mapping of ischemic LV wall motion abnormalities
2000	_____, MD, MSc	Young Investigator Award finalist American Society of Echocardiography	Papillary muscle contractile dysfunction paradoxically decreases ischemic MR

Investigators Mentored by Dr. _____ to Develop Independent Careers With Ongoing _____ Collaborators

Mentored Fellow	Present Position and Career Development
_____, MD, PhD 1987-88	Director, Cardiac Ultrasound Lab, Hopital Boucicaut, Paris Director of Research and Chief of Clinic, Faculty Necker-Enfants Malades Faculty of Medicine V, University of Paris • Continuing work based on mitral valve studies in hypertrophic cardiomyopathy at MGH • Extending to independent studies: molecular genetics of familial hypertrophic cardiomyopathy • Echo studies of transgenic models and myoblast transplantation
_____, PhD 1988-93	Founding Director, Cardiac Dynamics Lab, Children's Hospital of Pittsburgh • Continuing work based on fluid mechanical studies of regurgitant and stenotic valves begun at MGH and Georgia Tech • Founded laboratory for exploring models of cardiovascular flow abnormalities, with independent training program and nationwide collaborations
_____, MD 1989-92	Assistant in Pediatric Cardiology, Children's Hospital, Nancy, France • Continuing work based on 3D echo studies of LV function at MGH with independent funding from the French government; developed a rotating 3D echo probe, now implemented commercially, and used collaboratively at MGH to analyze ventricular function in corrected tranposition and potential benefit of afterload reduction
_____, MD 1991-94	Associate Professor of Medicine, University of Nebraska, Omaha, Nebraska • Continuing 3D echo studies of LV, RV, and aneurysm quantification begun at MGH • Independent program developed to simplify algorithms for quantification & border detection as well as contrast echo for myocardial perfusion
_____, MD 1992-94	Director, Cardiac Ultrasound Lab, Ospedale Civile, Cento, Italy 7/00: Director, Cardiac Imaging, University Hospital, Ferrara, Italy • Continuing work based on regurgitant flow and 3D echo studies at MGH • Development of independent and collaborative program with novel techniques for rapid quantifi-cation of LV function by 3D echo; development of new technologies for quantitative assessment of LV regional wall motion abnormality; collaboration with Dr. Schwammenthal (below) to improve quantification of valvular regurgitation • Program support from GE/VingMed
_____, MD 1992-94	Associate Professor of Medicine, Tel Aviv University Director, Cardiac Ultrasound Lab and Valvular Clinic, Beilinson Hospital, Petah Tiqvah, Israel • Extending work on valvular heart disease begun at MGH • Developed independent clinical service and patient-oriented research program with computerized data base • Independent program for noninvasive evaluation of aortic atherosclerosis and coronary artery disease risk, as in patients with homocysteinemia

_____, MD, PhD 1992-94	Senior Lecturer, Tel Aviv University, Israel • Extending work on valvular regurgitation from MGH • Independent grant support from Israel Heart Association: Clinical Investigator Award • Independent program of innovative research in valvular heart disease: Role of left atrial compliance in mitral stenosis; Dobutamine as guide to severity of aortic stenosis in patients with LV dysfunction
_____, PhD 1994-96	Assistant Professor of Engineering Fluid Dynamics & Turbo Machinery Department, Penn State University • Extending work on mitral valve mechanics begun with Dr. Levine at MGH and Georgia Tech; development of independent laboratory for flow modeling studies • Collaborative studies of backscattered Doppler power for regurgitant flow rate quantification, with submission of NIH R01 (Dr. Levine consulting)
_____, MD 1994-96	Senior Lecturer, Hadassah University Director, Cardiac Ultrasound Lab, Hadassah University Hospital, Jerusalem • Extending work on 3D echo and valvular heart disease begun at MGH • Independent projects on tissue velocity imaging and 3D echo
_____, MD 1994-96	Instructor in Medicine, Harvard Medical School; Staff Cardiologist, MGH • Collaborative patient studies of the mechanism of functional aortic insufficiency in aortic aneurysm and dissection, with aim of improved therapy • Collaborative clinical studies of physical basis of imaging artifacts • Independent international registry of aortic dissection
_____, MD 1995-97	Assistant Professor of Medicine, Kagoshima University, Japan Co-Chief of the Ward, Department of Internal Medicine • Extending work on ischemic MR begun at MGH • Independent fellowship training program director, with patient-oriented research on the role of annular function in MR and clinical impact of ischemic MR
_____, MD 1995-97	Associate Professor of Medicine, Ulsan University Director, Cardiac Ultrasound Lab, Asan Medical Center, Seoul, Korea • Extending work on 3D echo reconstruction for quantification of intracardiac flows begun at MGH • Independent laboratory director, with internationally recognized program of Ergonovine– echo stress testing for coronary artery disease
_____, MD 1995-97	Instructor in Anesthesia, Harvard Medical School; Staff Anesthesiologist, MGH and BWH • Continuing intraoperative studies of 3D echo to guide MVP repair begun at MGH • Collaborative program to improve therapy of ischemic MR in patients
_____, MD 1995-98	Instructor in Anesthesia, Beth Israel-Deaconess Medical Center Now Assistant Professor of Anesthesia, Rhode Island Hospital, Providence, RI • Collaborative work on preventing LV outflow obstruction by the mitral valve after mitral valve repair: publication of simplified clinical algorithm; collaborative work on improved methods of assessing aortic stenotic valve area intraoperatively
_____, MD 1996-98	Director, Cardiac Ultrasound Lab, Soroka Hospital Ben Gurion University, Beersheva, Israel • Extending work begun at MGH on mechanism of ischemic MR • Collaborative studies of mechanistic and prognostic factors in patients with ischemic MR

_____, MD 1996-98	Assistant Professor of Medicine, Yale University Medical School • Project with Dr. Levine and Framingham Heart Study led to epidemiology training and NIH Research Fellowship at Framingham • Continuing collaborative studies of genetic basis of mitral valve prolapse, population prevalence and natural history
_____, MD 1996-98	Instructor in Medicine, Harvard Medical School; Staff Cardiologist, MGH **Please see page 26, paragraph 1**
_____, MD 1997-99	Instructor in Medicine, The Jewish General Hospital, McGill University • Extending work on ischemic MR begun at MGH • Collaborative studies of innovative devices to reduce ischemic MR • Independent program of pharmacologic management of ischemic MR
_____, MD 1997-99	Assistant Professor of Medicine, University of Essen, Germany • Extending work on backscattered Doppler power–velocity integration for regurgitant flow rate quantification begun at MGH • Collaborative patient studies to implement and validate this technique vs. MRI • Independent program extending this technique to valvular stenosis • Independent grant funding: the Emmy Noether Award of the Deutscheforschungsgemeinschaft for Faculty Career Development
_____, MD, PhD 1997-99	Instructor in Medicine, Harvard Medical School; Staff Cardiologist, MGH **Please see page 26, paragraph 1**
_____, MD, MSc 1999-01	Current Research Fellow, Cardiac Ultrasound Lab, MGH • Collaborative funding from Parke Davis Pharmaceuticals for role of angiotensin converting enzyme inhibitors in reversing ventricular remodeling

Former Mentees and Advisees include: _____, MD (1987-88), now Director, Cardiac Ultrasound Lab, Greek Naval Hospital, Athens; _____, MD (1987-88, shared supervision), now Director of Clinical Cardiology, Boston Medical Center, Associate Professor of Medicine, Boston University School of Medicine; _____, MD (1988-89), now Associate Director, Noninvasive Imaging, Assistant Professor of Medicine, University of Pennsylvania; _____, MD (1988-90), now Medical Director, Echocardiography, Cleveland Clinic Foundation; _____, MD (1989-90), Assistant Professor of Medicine, University of Massachusetts Medical School, Director, Cardiac Ultrasound Lab, St. Vincent's Hospital & Fallon Clinic, Worcester, MA; _____, MD (1992-93, shared supervision), now Director, Echocardiography; Assistant Professor of Medicine, University of New Jersey Medical School, Newark, New Jersey; _____, MD (1989-91; shared supervision), now Director, Cardiovascular Training Program, Cleveland OH; _____ MD (1990-92), now Director, Research Center, Hospital La Fe, Valencia, Spain; _____, MD, now Director, Cardiac Ultrasound Lab, Sir Charles Gairdner Hospital, Queen Elizabeth II Medical Centre, Nedlands, Australia; _____, MD (1990-92, shared supervision), now Director, Cardiac Ultrasound Lab, Leuven, Belgium; _____, MD (1991-93), Chief Physician, Cardiovascular Rehabilitation Center, Clinique de Genolier, Geneva, Switzerland; _____, MD (1993-94), Director, Cardiovascular Institute, Chief, Cardiac Unit, University Hospital "Virgen de la Salud", Toledo, Spain; _____, MD (1993-94), now Graduate Assistant in Medicine, MGH (Affiliated Staff); _____, MBBS (1994-96, shared supervision), now Staff Cardiologist, Dunedin, New Zealand; _____, MD (1996-98, shared supervision), now Director, Cardiac Ultrasound Lab, University Hospital, Basel, Switzerland; and _____, MD (1997-99), now Director, Cardiac Ultrasound Laboratory, Dunedin, New Zealand.

Recruitment of Trainees - Trainees will be recruited from the Clinical Cardiology Fellowship Program at _____, the _____ Hospital, and other area, national and international teaching programs. The majority will be fellows trained in clinical cardiology and interested in developing research careers. An anesthesia fellow, Dr. _____, has also trained with *the candidate* and subsequently pursued an investigative career in cardiac anesthesia. Recruiting will be based upon contacts made by fellows them-selves as well as discussions with program directors here and abroad, including former fellows of the laboratory. Criteria will include commitment to a career in patient-oriented research; strong clinical training and evidence for critical and independent thinking; capacity for the teamwork required for patient-oriented research; and secondarily,

interest or experience in the major areas of focus (valvular heart disease, ultrasound advances). Typically, there are 5-10 trainees in the Laboratory at any time, distributed over 2-3 mentors, with some sharing of responsibilities. Consideration will be given to balancing the need for a critical mass of investigators with that for individual attention; the K24 mechanism will permit a larger team (3-5 fellows working mostly or in part with *the candidate*) with preserved attention to individual needs. Current fellows include Dr. _____ (Table, above), Dr. _____ (combined 3D echo-radionuclide displays to study viability), and Dr. _____. Recruited fellows in the "pipeline" to strengthen new lines of study include Dr._____, experienced in studying the basic mediators of apoptosis, to study heart failure in MR; and Dr. _____, trained by Dr. _____ in Ultrasound Physics at _____ University, to move 3D LV function quantitation to the next higher level.

Mentoring Program - Although specific activities for each trainee will be individualized based upon prior experience, long-term goals, and individual abilities, there will be a common basic structure that has proved effective. Mentoring will involve a combination of formal and informal training, both one-on-one and in laboratory sessions, as well as taking advantage of the formal didactic and seminar structure established as part of the _____ Clinical Research Program and Center for _____. The core experience will be the one-to-one relationship between the trainee and PI. Meetings with fellows will involve daily contact for discussion of experimental progress and problems; a more formal weekly session with each fellow, scheduled to review progress and analyze primary data as well as prepare presentations and publications; a weekly research conference including the entire group and reviewing progress; a weekly in-depth noninvasive cardiology seminar, including Journal Club sessions and a faculty lecture series covering the major areas of noninvasive cardiology with contributions from radionuclide and magnetic resonance techniques as well as ultrasound; and discussions every 3-4 months focusing on career development and long-term planning.

Major steps in the **mentoring process** will include the following:

(1) The process will begin with presentations by the mentor and other laboratory staff of **key topics** in our patient-oriented research and basic research techniques, including principles of noninvasive imaging, biostatistics, experimental physiology, and integration of noninvasive data into the clinical examination. Trainees will be **challenged to think critically and creatively** at this stage to design research projects. ***Example****:* In reviewing use of the proximal regurgitant jet (vena contracta) as a measure of lesion severity in aortic insufficiency, Dr._____ recognized this technique had not yet been applied to mitral regurgitation. His clinical validation of standardized measures of the vena contracta have provided a convenient technique for routine clinical evaluation.[52]

(2) Trainees will typically choose or be assigned to work on a **combination of projects at different stages**. A **beginning, educational project**, generally involving participation in ongoing efforts, will provide an introduction to the team and to research methods. A relatively straightforward independent clinical study or data base review may also be performed by those with some prior training, providing early positive reinforcement. Guided by early progress and long-term goals, a **major project theme will be selected**, often involving correlative patient and in vivo experimental studies.

(3) As a step toward independence, trainees will be encouraged to develop their own ideas, providing a potent stimulus to their research efforts. Creativity is stimulated through journal review and the challenge of independent thinking posed by questions such as the following: *What are the gaps in the current literature? What is the next step in this research?* and *How can this research be used to help patients?* ***Example****:* Dr. _____, while participating in animal studies of the proximal flow convergence technique for quantifying valvular regurgitation, observed the same flow convergence pattern in patients with mitral stenosis. This led him to conduct a study, published in ***Circulation***, using this technique to measure flow rate and mitral stenotic valve area in patients, providing a technique which is now widely accepted for use in patients in whom simple orifice planimetry is not technically possible.[72] (3[a]) Creativity is also encouraged through cross-disciplinary interactions involving Cardiovascular Surgery, the Cardiac Catheterization Laboratory, the Electrophysiologic Laboratory, and collaborating programs such as the Biofluid Dynamics Laboratory at the Georgia Institute of Technology and the Framingham Heart Study, with whom we share a staff member. ***Example***: Dr. _____, interested in studying the impact of new criteria for MVP developed by our laboratory, sharpened her skills in biostatistics and epidemiology through coursework at the Harvard School of Public Health, and worked

together with the Framingham Heart Study to produce a landmark paper showing the population prevalence of MVP to be 2.4 percent, not the 5 to 15 percent previously reported, with a far more benign presentation than in previous referral-based series **(please see her NEJM article, Appendix[72])**. **Example***:* Dr. _____developed the concept of chordal cutting to relieve ischemic MR (see proposal, below) based on clinical observations and participation in in vivo experiments; he then worked the procedure out in vitro with our collaborating program at _____ before implementing it in vivo **(please see his paper, Appendix).**

(4) Trainees will be **introduced to the resources available for research**, especially members of the research team who create a supportive environment, including _____, Senior Animal Physiologist and Surgeon, essential to the design of physiologic experiments and device development; _____, Senior Programmer and Analyst, critical to the design of image processing algorithms and 3D reconstruction techniques to answer specific questions, as well as directly guiding data acquisition; and other colleagues, including Drs. _____, _____, and _____. Working with this team helps trainees learn how to translate theoretical hypotheses into workable experiments and protocols. ***Example****:* Dr. _____, as a consequence of her clinical studies demonstrating the inadequacy of annular ring reduction for eliminating ischemic MR because of persistent tethering to the ventricular wall,[41] organized a collaborative discussion with the research team, including Luis Guerrero and _____, leading to the development of a plication procedure which eliminates the infarct bulge and repositions the papillary muscles to reduce tethering and MR **(please see her Circulation article, Appendix[70]).** Such innovations result from synergistic interactions of mechanistic knowledge and surgical expertise. (4[a]) _____ of the Cardiac Computer Center and Dr. _____ of the Clinical Information Management Unit provide other resources, teaching trainees to use clinical data bases for retrospective analysis and to help with power calculations.

(5) Critical review of concepts and research will take place in weekly research **conferences** and in preparation for scientific meetings. Trainees will be expected to attend and to present their work at national meetings (AHA, ACC, Echo Society), as well as Harvard Medical School postgraduate courses. Trainees, in preparing for **presentations**, will practice skills of gaining the attention of the audience; marshalling support for conclusions; preparation for critical questioning; and developing confidence in presentation.

(6) The principles of **scientific paper writing** will be taught by reviewing models of how papers should be written, as well as critical review of existing literature and the trainees' own writing. Emphasis will be on, for example, a clear statement of hypothesis and significance of the work; optimal presentation of physiologic data and primary images; clear exposition of how the data support the hypothesis, along with limitations; and the importance of giving credit to other investigators' work.

(7) There will be direct teaching of **how to write a grant** as well as encouragement of trainee grant writing and critique of the resulting documents. Points to be emphasized include: a clear statement of a central hypothesis; step-by-step exposition of methods; emphasis on the significance of the studies and their relevance to the clinical situation; power analysis; regulatory issues and institutional approval; and appropriateness of the study design and statistical tests. ***Example****:* The PI has worked with Dr. _____on a K23 Mentored Patient-Oriented Research Career Development Award, using it as a vehicle to go through the major steps of hypothesis formulation and grant-writing, teaching both by critique of the trainee's writing and by providing models. Staff of the _____ Clinical Research Program, including Drs. _____ and _____, were also invaluable in grant writing and organizing an Advisory Committee.

(8) Developing independence and a research career will be encouraged through support of trainees' original ideas and their concurrent development into research projects and careers, if appropriate. As trainees advance, they will be given opportunities to work with and supervise fellows as members of a research team, commensurate with their development of independence. Monthly Fellow Seminars of the Clinical Research Program will also cover a range of topics from career paths to mentoring and developing scientific collaborations. Continuing relationships with the mentor will be developed as trainees become independent investigators and direct their own laboratories, to be achieved through collaborative work, support of their career development and grant funding, and promotion to receive invitations to speak at local, national, and international meetings, and to participate in editorials and other publications. Such

collaborations and how they build upon the fellowship work at MGH are detailed in the above table of trainees.

Overall, these efforts at mentoring are furthered through direct demonstration of mentor commitment, devo-tion of time, and enthusiasm; and by providing trainees with a sense of positive achievement, encouraging their ability to accept suggestions, and fostering a sense of collaboration in a larger effort involving multiple past and present trainees with important scientific goals to improve patient care. Emphasis is placed on review of primary data, and teaching fellows how to think clearly and solve problems. Throughout the process, the PI and trainee will work to build a long-term career best suited to the trainee's own strengths and interests.

Didactic sessions: The _____ Clinical Research Educational Unit offers a series specifically designed to train clinical research investigators. The series includes: Introduction-Clinical Investigation (12 sessions), Design and Conduct of Clinical Trials (14 ss), Scientific Writing (4 ss), Grant Writing (2 ss), Biostatistics (4 ss), and Introduction to Physiologic Investigation (9 ss). In addition, there are seminar series for fellows and junior faculty interested in a clinical investigative career. The seminars of the Center for Innovative Minimally Invasive Therapy are also helpful in translating mechanistic insights into therapies for patients. **Instruction in the Responsible Conduct of Research**: Under the direction of _____ administration, yearly sessions in **ethics** are held. Attendance is required. Topics include ethical principles of human and animal studies, conflict of interest and data management. _____ "Faculty Policies on Integrity in Science," "Honor In Science" (Sigma XI), and a bibliography are distributed. Attendees are encouraged to continue discussion within their own units. Human subjects in research, appropriate representation of women and of minorities, and obligations to patients and volunteers are also part of the _____ Clinical Research Program courses on Physiologic Investigation and Clinical Trials.
Biostatistics. Coursework includes an intensive four-session course by Dr. _____, Statistician to the_____ General Clinical Research Centers; Seminars in Biostatistics for Physiologic Investigators, as part of the _____ Clinical Research Program, as well as sessions in the Clinical Trials courses.

4. Statements by Consultant(s), and Collaborator(s)

5. Environmental and Institutional Commitment to the Candidate

a. Description of Institutional Environment

The Cardiology Division at _____ provides a strong, well-established research environment for career devel-opment. The hospital itself has a long, rich, and diverse tradition of excellence in clinical research that continues to expand. Extensive resources for training clinical investigators include an integrated full-service primary, secondary, and tertiary care hospital network, as well as over 600,000 square feet of research space and a wide range of colleagues in clinical investigation. In addition, the _____ Clinical Research Program (CRP) was established in 1996 to improve the environment for clinical investigation through academic enrichment programs and direct support services, thereby creating a culture in which all forms of clinical research can flourish. Its mandate is to foster "translational" (bench-to-bedside) research, train clinical investigators, increase clinical trial activity and support outcomes and disease management initiatives; as well as coordinate joint efforts with the _____ Hospital, the _____, and _____. The hospital's allocation of resources (13 FTEs) to fund the CRP demonstrates the deep institutional commitment to developing clinical research.

The _____ Cardiology Division has over 50 staff cardiologists active in patient care, research, and teaching. The Cardiac Ultrasound Laboratory is extremely active, performing nearly 15,000 echocardiographic studies per year, and has an outstanding research and clinical program, training 5-10 clinical and research fellows per year, many of whom are now laboratory directors at other institutions. Faculty members include Drs. _____, _____, _____ and _____, and are dedicated to the clinical and research program, having trained many of the leaders in the field of echocardiography. Clinical activities and research have been closely integrated in the laboratory since its inception, and the research program is closely articulated with other laboratories and services. Sources of support include research grants from the NIH and American Heart Association, industrial support for developing and testing ultra-sound equipment and both contrast and inotropic agents for use in ultrasound studies, private endowments for equipment support, and individual postdoctoral and faculty fellowships, as well as institutional support.

The applicant has a proven track record of developing young scientists. He is an internationally recognized leader in the field of mitral valve disease and has pioneered the use of quantitative three-dimensional echo-cardiography, proximal flow convergence techniques and power-velocity analysis to address mechanistic and physiologic questions. *The candidate* has been an Established Investigator of the American Heart Association, has been involved for over 15 years in NIH-funded research, and is currently the Principal Investigator of 2 R01 awards. He has over 200 publications, including 145 original contributions as well as reviews and contributions to textbooks. He has a joint appointment in Bioengineering at the _____, working with Professor _____. He is currently on the Editorial Boards of Circulation, and the Journals of the American College of Cardiology (ACC) and American Society of Echocardiography (ASE), having served on the ASE Board of Directors and as its Scientific Program Chairman. His recent trainees have included a series of Young Investigator Award finalists and winners of the AHA, ACC and ASE, and many who have developed independent research careers and academically productive clinical laboratories both here and abroad. As a mentor, he has continued to provide support as independence develops, helping to obtain the necessary resources, including a K23 Mentored Career Development Award and a Robert Wood Johnson Faculty Scientist Career Development Award at MGH, and a comparable Emmy Noether Award in Germany.

Resources available for career development include the full patient care activities of the Cardiology Division; the Cardiac Computer Center, with full-time staff (_____) available for data-base searches; research- level equipment for noninvasive imaging in echocardiography, radionuclide scanning, and MRI; and an active research program in three-dimensional echocardiography, with a full-time programmer and analyst, _____, essential to tailoring software for addressing hypotheses on advanced Silicon Graphic workstations.

The Cardiac Surgical Research Center, directed by Dr. _____, Associate Professor of Surgery, and _____, Technical Supervisor, is a 500 sq ft animal laboratory with 3 full-time technicians experienced in open-heart surgery and cardiopulmonary bypass. There is potential for collaboration with an active Device Development Laboratory (Dr. _____) and with our Heart Failure and Molecular Genetics groups (Drs. _____, _____, _____, _____, _____, and _____) to study basic mechanisms and innovative therapy of ventricular remodeling and heart failure. In addition, there are weekly divisional conferences, allowing for collaboration among members of the division. The Center for Innovative Minimally Invasive Therapy (CIMIT) at _____ also provides a forum and framework for encouraging innovative collaborations. The therapeutic techniques in this proposal are also being considered for minimally invasive implementation by the PI together with Dr. _____, Director of the Laser Center, and Dr. _____, Director of CIMIT. Other resources and personnel available for the research program include the following: **Fluid Mechanics**: _____, Regents Professor of Chemical and Mechanical Engineering, _____, and _____, MD, DrEngrSci, a rheologist and engineer. **Imaging**: Drs. _____ and _____, Nuclear Cardiology, and Drs. _____ and _____, Cardiac MRI. **Interventional Ultrasound:** Dr. _____, Cardiac Catheterization Laboratory.

b. Institutional Commitment to Candidate's Research Career Development

Please see Dr. _____and Dr. G. _____ letters under Letters of Reference and Institutional Commitment to the Candidate, above.

6. RESEARCH PLAN (adapted from current R01 and other grants as relevant to the overall mentoring program)

a. STATEMENT OF HYPOTHESIS AND SPECIFIC AIMS

Ischemic mitral regurgitation (MR) is a common complication of ischemic heart disease that conveys adverse prognosis after both myocardial infarction and coronary revascularization, more than doubling the risk of late death.[1-4] Both its mechanism and therapy, however, are controversial. Patients with MR lesions of all varieties have recently benefited from surgical techniques for valve *repair* as opposed to replacement, with improved LV function and decreased complications of anticoagulation and endocard-itis. The most

vexing form of MR to repair remains that due to ischemic heart disease, with uncertainties regarding mechanism and inadequacy of repair techniques.

We therefore plan a combination of clinical studies and clinically-oriented experimental investigations relating to the mechanism, progression, and therapy of ischemic MR. The **central hypothesis** of these complementary studies is that development and progression of ischemic MR can be understood on the basis of altered relationships between the mitral valve leaflets and their supporting ventricular structures. This hypothesis is based on the fundamental physical principle that leaflet behavior is determined by the **balance of forces** acting upon them **(Fig. 1).** This force balance can in principle be altered in several ways, alone or in combination: (1) Increased tethering of the leaflets away from effective coaptation by displacement of their papillary muscle (PM) and annular attachments, thus restricting their ability to move toward closure; and (2) left ventricular (LV) contractile dysfunction per se, decreasing the force acting to close the leaflets, particularly when tethering is increased. These mechanisms have practical implications for therapeutic interventions to normalize the force balance and alleviate MR.

The *clinical arm* of this proposal addresses gaps in our current knowledge regarding the mechanism of ischemic MR. Tools have previously been lacking for comprehensive quantitative analysis of the mitral complex, and evaluation of mitral regurgitant volume and orifice area have been limited. Noninvasive techniques, however, are now available for three-dimensional analysis of the mitral valve complex and for quantitative assessment of MR volume and orifice area. These techniques put us in a better position to study the mechanism of ischemic MR.

Ultimately, we expect that increased understanding of mechanism will improve our ability to design specific therapy. Work to date by the PI using 3D echo analysis of the mitral valve complex in experi-mental models of MI has helped us understand the limitations of current annular ring reduction therapy: In the face of persistent posterior tethering by displaced PMs, the mitral leaflets still cannot close effectively at the annular level, and the anterior excursion of the posterior leaflet to meet its anterior counterpart is particularly restricted. Therefore, in addition to studying the mechanism underlying ischemic MR in patients, we plan complementary experimental studies aimed at alleviating MR by addressing the primary problem of increased tethering. Pilot studies in our laboratory have shown that this tethering can be addressed in reality;[70] the proposed studies will test **simplified and practical** surgical methods of achieving this goal successfully. These studies will test the hypothesis that external fixation of the PM region, by reducing the distance over which the mitral leaflets are tethered from PM to annulus, or alternatively, cutting a small number of critically positioned tethering chords, can restore restricted mitral leaflet coaptation toward normal.

In summary, by measuring variables that determine components of the proposed force balance, and relating their changes to changes in MR, we will test **specific questions** related to the central hypothesis and the mitral valve-ventricular relationship. Restoring this relationship toward normal, either with an external device simply applied or chordal cutting should alleviate ischemic MR without importantly compromising LV function.

The research aims of the K24 Award will be met in two ways: first, by allowing the PI to translate his expertise in imaging and mitral valve physiology, gained in the experimental laboratory, to direct clinical studies; and second, by allowing him to move from mechanism to therapy in models reflecting the clinical situation, with the ultimate goal of application to the human condition.

b.. Background, Significance and Rationale

Ischemic mitral regurgitation (MR) is a common complication of both acute myocardial infarction (MI) and chronic coronary disease (CAD). It is a major predictor of morbidity and mortality in ischemic heart disease[1-3], more than doubling mortality following revascularization.[4] Despite its prevalence and clinical importance, the basic mechanism remains unclear, with multiple conflicting mechanisms proposed. In one of the earliest proposals, Burch et al. suggested that papillary muscle (PM) contractile dysfunction caused

MR[5,6]. However, subsequent models in which isolated PM dysfunction was created showed that no MR resulted unless the myocardium underlying the PMs is also injured.[7-10] Ogawa[11] and Godley[12] have proposed that ischemic MR results from apical displacement of the mitral leaflets in a pattern referred to as incomplete mitral leaflet closure, attributing this to dyskinesis of the underlying myocardium **(Fig. 1).** Izumi et al.[13] and others[14] proposed that asynergy of the PM or LV and enlargement of the mitral annulus are the dominant factors causing ischemic MR. In contrast to localized myocardial changes, Kono and Sabbah[15-18] have suggested that global change in ventricular shape, reflected by the sphericity of the ventricle, is the major determinant of MR. In an alternate proposal, Kaul[19] and Dent[20] suggested that global LV dysfunction with insufficient LV forces acting to close the valve, not PM or myocardium dysfunc-tion, is the primary cause of ischemic MR. More recently, Kisanuki et al.[21] observed a significant decrease in fractional PM shortening in patients with prior myocardial infarction; moderate or severe MR was more frequent in such patients than in those with normal PM function. Thus, a confusing variety of often contradictory results from different clinical and experimental settings continues to support conflicting mechanistic proposals.

The continuing controversy regarding ischemic MR may in part be due to difficulty testing the proposed mechanisms. Because LV dysfunction is almost always accompanied by LV dilatation and geometric changes in the mitral valve complex, it has been difficult to separate dysfunction from dilatation to deter-mine their relative contributions to ischemic MR. Otsuji et al.[22] in the PI's group separated these factors in an experimental model of functional MR. First, they produced global LV dysfunction by infusing esmolol and phenylephrine, but limited LV expansion by increasing pericardial restraint with sutures. Despite severe LV dysfunction, with pericardial restraint, only trace MR developed. When the sutures were released, moderate to severe regurgitation developed as the LV dilated. Three-dimensional echocardiography (3D echo) demonstrated that the tethering distance over which the mitral leaflets were stretched from PM to anterior mitral annulus was the most important predictor of MR in this model when compared with mitral annular area, LV ejection fraction, or dP/dt **(please see Appendix)**. Dysfunction was also separated from dilatation in the chronic sheep infarct model of Llaneras and Edmunds,[23] in which regurgitation was absent acutely following occlusion of several left circumflex coronary artery branches, but did develop 8 weeks later as the LV remodeled and dilated. 3D echo in that model also confirmed the role of tethering changes in causing MR even when decreases in global LV function were mild.

Recent technical developments employed in these studies have made it easier to examine the complex 3D geometry of the mitral valve apparatus, overcoming the prior limitations of standard 2D imaging. 3D echo reconstruction has been applied in experimental and patient studies, and validated extensively for quantitative accuracy against implanted sonomicrometers.[22,24-36] To date, although mitral geometric relations have been derived from 2D echo images in patients with dilated and hypertrophic cardiomyopathy,[14,37] 3D methods have not been applied extensively to analyze the mechanism of MR in patients with CAD. In patient studies, 3D echo can provide detailed insights into the mitral apparatus and direct in situ measure- ment of PM function and spatial relations (see Preliminary Studies). The proposed work would extend our prior experience in 3D echo to address mechanistic questions and explore integrated cardiovascular physiology, with the aim of developing improved therapy. Recent advances in the noninvasive quantifi-cation of MR will also permit more accurate quantification of this key variable.

There is often reluctance to address ischemic MR at the time of revascularization, which may relate to several factors: 1) Uncertainty as to its precise mechanism; 2) Concern regarding the inadequacy of current repair techniques; and 3) Concern regarding the risk/benefit ratio of the additional cardiopulmon-ary bypass time required for these techniques, which focus on annular ring insertion through an atrial incision. Because of its **dynamic nature**, ischemic MR may also appear mild in the operating room under general anesthesia, dissuading the surgeon from repair, but it may then revert to moderate severity with normal activity. These limitations provide the motivation to pursue our understanding of mechanism and design specific therapies with improved efficacy and reduced need for cardiopulmonary bypass.

c. Preliminary Studies and Results

Clinical Studies

The PI's group have applied 3D analysis in two preliminary studies. The first addressed the observation that some patients with inferior MI have important MR while others with infarcts of comparable size and location

do not. Liel-Cohen et al.[38] performed transthoracic 3D echo studies in 22 patients with inferior MI selected to have equal numbers with moderate to severe MR versus trace to no MR. LV volume, mitral annular area and PM-to-mitral annulus tethering distance were measured. They found no signifi-cant difference in infarct size, LV volumes, ejection fraction or mitral annular area between patients with and without MR. Those with MR had more visible inferior wall bulging with significantly higher PM-to-mitral annulus tethering distances (39.5±3.0 vs. 29.8±2.0mm for the medial PM, p<0.002). Tethering distance was the only independent predictor of regurgitation. The importance of tethering distance was also shown in the second preliminary study by Otsuji et al.,[39] who examined mitral valve geometry by intra-operative 3D transesophageal echo in patients prior to coronary artery bypass surgery. Two consecutive groups of patients were studied, 10 with moderate to severe MR, and 10 with trace or no MR. Patients with MR had greater tethering distances than those without MR (sum of both PMs = 79±8 vs 67±5mm, p=0.002). Differences in LV ejection fractions (40±11 with MR vs. 47±16%) and annular area were not significant in this pilot study.

Dr. _____ and the PI, however, showed that the *entire force balance*, not only tethering, needs to be considered **(Appendix)**.[40] Ischemic MR typically varies throughout systole, with early and late systolic peaks. These phasic changes in MR, measured by the Doppler proximal flow convergence method, were compared in 30 patients with phasic changes in potential contributory factors, including mitral annular area (MAA) and tethering distance, and found to relate most strongly to variations in the closing force generated by the LV. This force = MAA x (LVP-LAP), where the pressure difference is obtained from continuous wave Doppler velocities as $4v^2$ by Bernoulli's equation. Therefore, although mitral annular dilation and tethering are important in "setting the stage" for MR, the closing force generated by the LV is essential to driving the leaflets closed, so that MR is greatest in early and late systole when tethering is unopposed.

Experimental models and 3D analysis. The PI's pilot studies take advantage of his experience with the chronic sheep model of Llaneras and Edmunds,[23] which is used as a surrogate for the human situa-tion for several reasons: 1) As the initial authors found, the coronary anatomy is relatively constant, providing a better-defined substrate to reduce clinical hetereogeneity; 2) The inferoposterior MI distribu-tion is comparable to that in most patients with MR from segmental ischemia; 3) The model incorporates chronic and evolving changes in the LV, an important part of the clinical spectrum: occluding obtuse marginal branches 2 and 3 of the left circumflex (LCX) coronary artery produces no MR acutely, but moderate MR develops over 8 weeks as the LV dilates and remodels, while occluding marginals 2 and 3 and the posterior descending artery (a continuation of the left circumflex in sheep) can in contrast cause MR acutely; 4) The finger-like sheep PMs protruding into the LV cavity resemble the human, while other species have shorter PMs (pigs) or PMs joined to the posterior LV wall (dogs); and 5) The animal model allows controlled prospective testing of the safety and efficacy of new interventions for which direct human study is too premature.

The evolution of progressive ischemic MR was therefore studied in 7 sheep after ligation of the second and third circumflex obtuse marginal branches, with LV remodeling initially limited by the early stage of infarction, and then 8 weeks later with prominent remodeling. The 3D geometry of the mitral apparatus was reconstructed from rotated echocardiographic views, as described under Data Collection and Ana-lysis, below. The results can be understood in the format presented in **Figure 2,** in which the three-dimensional mitral apparatus is viewed en face from the apex to provide a convenient two-dimensional map; this highlights the tethering distance from each PM tip to the anterior mitral annulus. MR volume was calculated as LV ejection volume by 3D echo minus forward aortic stroke volume. In this model, MR was initially only trace with limited LV dilatation (26±7 vs 18±5 ml at end-systole, p=0.01), but became moderate over 8 weeks as the LV dilated (44±8 ml, p=0.01 vs acute), without change in ejection fraction (39±3% to 36±7%). The only independent predictor of MR was the increase in tethering distance (r^2 = 0.86; tethering distance =31±4mm vs. 24±1mm at baseline for the ischemic medial PM, p<0.01; **Fig. 3**).

Limitations of ring annuloplasty. The sheep model was used by Dr. _____ and the PI to investigate why mitral ring annuloplasty, the standard surgical therapy, frequently leaves the patient with import- ant residual MR despite reducing annular area. Our clinical experience over 6 years shows approximately 55% of patients receiving a ring for ischemic MR have moderate persistent regurgitation.[41] One of the potential limitations of annuloplasty is that it does not address the ventricular end of the problem, namely, LV

dilatation leading to PM displacement and increased tethering. We therefore studied 7 sheep using 3D echo to examine mitral valve geometry, comparing baseline with infarction, and then following placement of Physio mitral annular rings intentionally smaller than the baseline annulus.

Four sheep were studied 8 weeks after ligation of LCX marginals 2 and 3, and 3 studied with acute MR induced by ligating marginals 2 and 3 and the posterior descending artery. Moderate to severe MR developed in all sheep, with increased mitral annular area and PM tethering distance. **With ring inser-tion, however, significant regurgitation remained in 6/7 sheep despite a 50% reduction in mitral annular area**. Of note is that ring insertion shifts the posterior annulus anteriorly, while the ischemic PM remains posterior, which restricts the anterior excursion of the posterior leaflet to meet its anterior counterpart, thereby limiting coaptation.[42]

Pilot data: new approaches to reduce tethering. These data have directed us toward two potential solutions to address the tethering end of the problem. **One approach** repositions the displaced PM toward the mitral annulus using **a balloon contained within a Dacron patch which is sewn externally over the infarcted myocardium and PM (Fig. 5)**. Instilling saline into the balloon can therefore push the myocardium and PM inward and toward the annulus to reduce tethering. Initial pilot studies of the patch device by Dr. _____ and the PI in two sheep studied 8 weeks into the chronic infarct model are encouraging. Balloon inflation repositioned the ischemic PM toward its normal position, decreasing MR from moderate-to-severe to trace **(Fig. 6)** as tethering distance decreased from 33 to 22 mm for the ischemic medial PM, without an important change in global LV size at end-systole (40 to 38 ml) or systolic function (EF = 40% vs. 35%). Such an approach has the advantages of directly addressing ventricular tethering while potentially avoiding additional cardiopulmonary bypass time, which often deters repair after coronary revascularization. Like recent procedures that re-shape the LV by muscle excision or myoplasty with the aim of improving loading conditions and LV ejection,[46-50] this external device also has the potential to limit LV expansion in remodeling.

The **second procedure** for reducing tethering involves **cutting several critically positioned tethering chords** in a way that will not produce prolapse, based on valve anatomy. This idea resulted from the observation that increased tethering exerts its greatest effect on the configuration of the basal portion of the anterior mitral leaflet near the annulus **(Fig. 7)**. This portion of the leaflet is held nearly rigid and tented toward the LV apex by what is referred to as a primary, strut, or **basal chord** inserting closest to the leaflet base (the mitral annulus). The more distal leaflet pivots around this “knee,” but only its tip can then meet the posterior leaflet, decreasing the coaptational surface needed to ensure an effective seal. We proposed that cutting these critically positioned chordae symmetrically (the most central one on each of the medial and lateral sides of the valve) could allow the body of the anterior leaflet greater excursion, with less tenting and more effective coaptation, but without prolapse. Dr. _____ first verified this with excised porcine mitral valves in a physiologic pulsatile flow simulator in collaboration with _____, and has now confirmed this in 4 pilot sheep with acute ischemic MR from circumflex artery branch ligation **(Fig. 8)**, with a decrease in regurgitant fraction from 28±3% to 4±1.7% ($p<0.001$). This procedure appears to work because the chords that are cut normally buttress the anterior leaflet body, but, with PM displacement, they exert a dominant and maladaptive role in distorting the leaflet configuration to limit the effectiveness of coaptation. Cutting these chords in a relatively simple manner restores the anterior leaflet toward its normal configuration without a sharp angulation near its base. The leaflet body can then approach the annulus more closely and meet the posterior leaflet more effectively to form a coaptational seal.

Further work is necessary to observe mitral valve function for longer times after this procedure, and to observe its effects with more severe MR. As an initial approach, chords were cut under direct observa-tion through an atrial incision; this procedure, however, opens the door to potential minimally invasive therapy, with a percutaneous snare approach guided by ultrasound imaging, similar to ultrasound-guided percutaneous closure of a patent foramen ovale or atrial septal defect.

d. Research Design and Methods

The **central hypothesis** of this proposal is that the development of ischemic MR can be understood on the basis of altered relationships between the mitral valve leaflets and their supporting ventricular structures. These altered relationships involve an abnormal balance of increased tethering forces due to PM displacement and annular dilatation, as well as reduced closing forces due to LV dysfunction. The clinical and experimental arms of the proposal provide opportunities to test this central hypothesis in several contexts. In the experimental arm of the proposal, guided by mechanistic insights from the clinical arm, we will explore surgical/interventional therapies aimed at reducing MR.

CLINICAL STUDIES:

Data collection. Echocardiographic imaging will be performed in the left lateral decubitus position using an HP 5500 machine. Standard 2D parasternal and apical views will be obtained using a 2.5 MHz trans-ducer with harmonic imaging for optimal penetration and image quality. 3D echo data to quantitate LV and mitral valve geometric relationships will be collected with a 4 MHz transthoracic multiplane probe from a fixed imaging position at the apex, with the probe positioned to align the axis of rotation through the center of the mitral valve, parallel to the LV long axis. The scanner contains 3D software which rotates the ultrasound array to record images at regular angular increments (10°) from 0-180°, with ECG and respiratory gating to record images from beats of consistent length at a consistent end-expiratory point in the respiratory cycle. We have successfully imaged patients in this manner in the Preliminary Studies described above;[38,39] however, we can also to take advantage of harmonic imaging to maximize image quality in the largest number of patients by using a 2.5 MHz transducer with harmonic capabilities rotated within a cylindrical holder by a TomTec computer interfaced with the ultrasound scanner. Images will be recorded on videotape and magneto-optical disk as digital data for transfer to a Silicon Graphics workstation for analysis.

Data analysis

1. **LV measures**. LV volumes will be obtained by 3D echo, using endocardial borders from 6 planes at equal angular intervals and a validated surfacing algorithm.[29] The extent of ischemic wall motion abnormality (WMA) will be measured by tracing the hinge points between normally and abnormally contracting muscle, creating a 3D endocardial surface map (validated against sonomicrometer crystals in vivo) which quantifies the extent of WMA and the location of the infarct centroid circumferentially (relative to the aorta) and axially.

2. **MR**. MR volume will be calculated as the difference between LV ejection volume by 3D echo and forward aortic stroke volume, which equals the velocity-time integral of forward flow at the annulus times aortic annular area. This value correlates well with independent measures of MR volume, and applies even with aortic stenosis or insufficiency (included in both ejection and aortic stroke volumes, and therefore cancels out). Doppler color flow mapping will also provide instantaneous regurgitant flow rates and orifice areas throughout systole by the proximal flow convergence technique applied by Dr. _____ in her preliminary clinical studies,[40] as validated by _____ and the PI.[51]
   Proximal MR jet size will be measured in parasternal long- and short-axis views by the method of Mele et al.[52] as a simple reflection of lesion size as used in clinical practice. **Regurgitant orifice area (ROA)**, which corrects MR volume for varying driving pressures, will be calculated as MR volume / the time-velocity integral of MR orifice velocity by continuous wave Doppler.

3. **3D analysis of the mitral valve complex.** This analysis aims to identify PM displacement relative to the annulus, which can increase tethering and potentially impair coaptation. As reference frame, we will take the least-squares plane of the mitral annulus (plane with the least deviation of annular points about it). Using this reference, we will correlate MR with a series of *uniquely three-dimensional measurements that cannot be made in any two-dimensional view* **(Fig. 9)**. Displaying intersecting views simultaneously enhances spatial appreciation (upper left). The ventricular borders of the mitral leaflets will be traced, and their tips closest to the cardiac base and anterior annulus determined by reviewing several adjacent images. An endocardial surface color-coded for adjacent structures (lower left) will be generated, and spatial relations of the mitral apparatus established (lower right).

The tethering length over which the mitral leaflets and chordae are stretched between the PMs and the relatively fixed fibrous anterior portion of the annulus will be measured from each PM tip to the medial trigone of the aortic valve (medial junction of aortic and mitral annuli); this point is selected because the line connecting it with the mitral annular centroid roughly bisects the line connecting the PM tips, so that symmetric outward PM displacements appear symmetric in this reference frame (see **Figure 2**, which views 3D relations from the apex.) Changes in these tethering distances relative to baseline will be measured, as well as changes in their 3 components: x (mediolateral PM shifts in a broader LV), y (posterior PM shifts) and z (shifts toward the apex). Changes will also be measured in the PM tip side-to-side separation, and in the projected PM tip displacement beyond the closest point on the annular ring (PM DIS, as shown in **Figure 4**), which expresses the limitation the PMs place on anterior excursion of the posterior leaflet toward coaptation. **Validation**. These 3D measurements have correlated and agreed well with distances measured by sonomicrometer crystal array (Sono-metrics, London, Canada), both in vivo (four PM and annular crystals, two times/ beat, several hemodynamic stages, n=36) and in a ventricular phantom (8 crystals, n=28); $y=0.99x+02$, $r^2=0.99$, SEE=0.7mm, $P<10^{-10}$, mean difference=0.08 ± 0.7mm (not significant vs. 0).

4. **Mitral annular area (MAA) and leaflet closing force**. MAA will be measured from the 3D reconstruction when largest (before atrial systole) and smallest (mid-LV systole); % change will be calculated. Peak leaflet closure force will be calculated as the peak mid-systolic transmitral pressure gradient times the corresponding MAA, where gradient = 4 (continuous wave Doppler transmitral velocity)^2 by Bernoulli's equation; mean force = mean gradient times mean MAA (mean of extremes).

Hypothesis testing. The central hypothesis that ischemic MR relates to alterations in the mitral valve-ventricular relationship will be tested in this clinical study by relating MR volume and regurgitant orifice area to variables that determine the force balance acting on the mitral valve, including tethering distance and its 3 components; PM tip separation; mitral annular area and its % change; and leaflet closing force. These relations will specifically be tested by univariate and stepwise multiple linear regression analysis, as well as LV end-diastolic and end-systolic volumes, ejection fraction (EF) and infarct size, expressed as % involved endocardial surface area from 3D echo. PM-to-annulus tethering distance and its components will be entered into the analysis for each PM alone, and as the summated changes for both. Variables will be entered in the order suggested by the multiple regression model based on the F to enter or remove at $p<0.05$.

Potential limitations. Although our Preliminary Studies[38,39] indicate the feasibility of clinical 3D reconstruction of the mitral apparatus, which is particularly well seen from the apex (leaflets perpendicular to the beam, ideal for reflecting ultrasound), we also have the possibility of imaging with a low-frequency harmonic transducer for optimal image quality in the vast majority of patients.

Power considerations. Although reported frequencies of ischemic MR vary,[1-3,53-57] a reasonable estimate is 30% with important MR. In order to have an 83% chance of detecting a difference between patients with and without MR that is .75 x the standard deviation (for example, an important difference of 3 mm in tethering length with a standard deviation of 4 mm, conservatively), we will have to accrue **75 patients**. In actuality, our study should have even greater power because we will use the extent of MR rather than just its presence or absence. This should be achievable at our institution within 1.5-2 years.

EXPERIMENTAL STUDIES

The experimental therapeutic studies complement the clinical, providing further opportunities for testing the central hypothesis by demonstrating the resolution of MR with normalization of one or more force balance components towards normal. To reflect salient features of the clinical spectrum of CAD leading to MR, the therapies will be applied to both acute and chronic models of both segmental and global LV dysfunction, allowing for the possibility of a differential response of the ventricle to reshaping maneuvers in the acute versus the chronic infarct with scarring.

MODELS AND INTERVENTIONS

Segmental ischemic LV dysfunction

Acute studies. 6 Dorsett sheep (30-40 kg) will be anesthetized with thiopentothal sodium (0.5 ml/kg), intubated and ventilated at 15 ml/kg with a 2% isoflurane and oxygen mixture. All animals will receive 1 dose of glycopyrrolate (0.4 mg IV) and prophylactic vancomycin (0.5 g IV). A surface ECG will be monitored and a sterile left thoracotomy performed with the pericardial incision. A high-fidelity Millar micromanometer-tipped catheter will be placed into the LV via the carotid artery. To provide continuous assessment of LV volumes and function, an array of 24 sonomicrometer crystals (Sonometrics, London, Canada) will be placed over the LV epicardium from base to apex; the PI's group has had extensive experience with this array in models of coronary occlusion, and it provides a continuous LV volume output for constructing pressure-volume loops (difficult to calculate frame-by-frame by 3D echo). After baseline echo imaging (see below), the second and third obtuse marginal branches of the left circumflex coronary artery as well as its continuation into the posterior descending artery will be ligated at their origins. All animals will receive lidocaine (3 mg/kg IV followed by 2 mg/min) 10 minutes before coronary ligation. Echo imaging will monitor the development of MR, which occurs usually within 30-60 minutes following ligation, following which hemodynamic measurements and imaging will be repeated, with analysis of LV end-systolic and end-diastolic pressure-volume relationships (ES and EDPVRs) as described below. Subsequently, either the patch with inflatable balloon device will be placed epicardially without bypass, or two basal chords will be cut.

As in the pilot studies **(Figs. 5-6)** of the **balloon-patch device**, the edges of the patch will be sewn onto the myocardium over the region of infarction (visible by alterations in color and bulging motion pattern) using interrupted sutures, and the balloon inflated with 10-20 ml saline. Patch placement and balloon inflation will be guided in situ by echo to reduce MR and normalize leaflet seating. This permits immediate adjustment of the device if necessary. Once the device has been properly positioned, imaging and hemodynamics will be repeated along with LV function studies. For each intervention, a total of 24 sheep will be studied as follows:

	Acute MR	Chronic MR
Segmental ischemia	n = 6	n = 6
Global ischemia	n = 6	n = 6

As an initial approach, **chordal cutting** will be performed under direct observation to test the efficiency of the procedure itself (as opposed to the feasibility of any subsequent less invasive implementation). Cardiopulmonary bypass will be instituted with caval and femoral artery cannulation and hypothermic cardioplegia; after left atrial incision, the anterior mitral leaflet will be everted through the mitral annulus, and the two most centrally attaching basal chordae cut **(Figs. 7-8, Appendix)**. After repair of the atrial incision, the heart will be warmed and defibrillated, normal circulation restored, and, if necessary, hemo-dynamics adjusted with saline infusion to achieve cardiac output and LV pressure comparable to pre-bypass values.

Chronic segmental dysfunction. Anesthesia, medications, surgical preparation, hemodynamic record-ings, and echo measurements will be performed as above under sterile conditions. Circumflex obtuse marginals 2 and 3 only will be ligated, and measurements repeated. The thoracotomy will be closed and the animals returned to an animal care facility for 8 weeks. Following this, a second sterile thoracotomy will be performed for therapeutic interventions, as described above. The sonomicrometer crystal array will be placed only during this therapeutic thoracotomy to evaluate LV function before and after interven-tion. To test whether intervention provides persistent relief of MR, or alternatively permits recurrent remodeling with return of MR, and to evaluate LV function longer after intervention, the animals will be returned to the animal care farm, with sonomicrometers removed, for an additional 8 weeks, after which thoracotomy will be performed for imaging, hemodynamics, and LV function studies. Such observations extending beyond the acute repair are essential prior to contemplation of human studies, and this overall protocol design has been applied in the PI's laboratory without post-repair complications or attrition.

Global ischemic LV dysfunction
In principle, the same devices should be able to reposition the PMs to eliminate MR in global ischemic LV dysfunction as well, which has been reproduced in the PI's laboratory. In this model, anesthesia, medications and animal care will be as above. After left thoracotomy (sterile for the chronic studies), imaging and baseline hemodynamic measurements will be performed, and global LV dysfunction pro-duced by injecting polystyrene latex microspheres (77-102 microns; Polysciences, PA) into the left cir-cumflex (LCX) and then, 20 minutes later, into the left anterior descending (LAD) coronary artery during subselective catheterization by the method of Sabbah et al.[16,18,60,61] Two ml of the microsphere suspen-sion (800,000 microspheres) will be injected into each artery after vortex mixing. Initial 3D echo images and Doppler traces will be obtained 30 to 60 minutes after embolization, with acute LV dysfunction and MR, following which interventions will be performed in acute studies. In the chronic studies, the thoraco-tomy will be closed and the animals cared for over 8 weeks, with weekly transthoracic echo monitoring of LV function and MR. In order to maintain LV dysfunction, which otherwise tends to resolve,[61] the micro-sphere injections will be repeated every two weeks in the closed-chest animals under fluoroscopic guid-ance and general anesthesia,[60,61] using a 4F catheter inserted over a 0.14-mm guidewire through an 8F guiding catheter to achieve selective LAD and LCX injections. After 8 weeks, a second sterile thoraco-tomy will be performed, with hemodynamic measurements and echocardiographic imaging. The patch will be placed or chords cut as described above. Sonomicrometer studies of LV function and pressure-volume relations before and after therapeutic maneuvers will be performed. The sonomicrometer crystals will then be removed, the thoracotomy closed, and the animals cared for over 8 weeks, followed by a final thoracotomy for reevaluation of LV and mitral valve function.

Data collection and analysis for the experimental studies. Heart rate, EKG, LV pressure and dP/dt will be recorded on a multi-channel physiologic recorder. 2D, Doppler, and 3D echo data will be collected in the same way as for the clinical studies above, using a high-frequency (3.5-5 MHz) multiplane probe to image the heart through a water bath and obtain 45 rotated images of the mitral valve and LV at angular increments (4°) from 0-180°, with ECG gating and suspended respiration during the data acquisition for most accurate 3D reconstruction.

Measures of LV volume, EF, and extent of wall motion abnormality will be obtained by 3D echo as described under Clinical Studies, above. MR volume will be calculated most precisely as the difference between LV ejection volume by 3D echo and forward aortic stroke volume by Transonic **flowmeter** placed firmly around the ascending aorta just above the coronary ostia. Regurgitant orifice area will be calcula-ted from MR volume and the time-velocity integral of Doppler MR orifice velocity, as before. 3D analysis of the mitral valve complex and annular area will be the same as in the clinical studies, with calculation of leaflet closure force from annular area times LVP-LAP, obtained by Bernoulli's equation or, for open-chest studies, from LA and LV Millar catheters.

Detailed measures of LV function. Pressure-volume loops will be constructed by feeding continuous tracings of LV volume and Millar micromanometer pressure into the display program of the Sonometrics device that controls the sonomicrometer crystal array. LV volume will be calculated from the 3D positions of the 24 crystals using a standard validated surfacing geometric algorithm comparable to that used in 3D echo. (3D echo could be used, but is not currently practical for frame-by-frame volumes.) The end-systolic pressure-volume relationship (ESPVR), a relatively load-independent measure of LV contractility, will be obtained by transiently occluding the inferior vena cava with umbilical tape, thereby rapidly producing beats with varying systolic pressures and LV volumes. End-systole is defined as the maxi-mum ratio of LVP to LV volume. ESPVR will be fit to a linear equation and its slope taken as a measure of contractile state, using Dr. _____ expertise from her basic science work; end-systolic volume will be solved for at a matched LV end-systolic pressure common to all time points. End diastole will be defined by the trough in LVP after atrial contraction. The end-diastolic pressure-volume relationship (EDPVR) data from caval occlusion will be fitted to an exponential equation $LVP=A_0 + Be^{Cx}$,[64] where A_0 is the intercept of the LVP value, B and C are curve-fitting parameters and x is the LV volume. End-diastolic volume will be solved for at a matched LVP common to all time points. The time constant of LVP fall, tau, will be calculated as the negative of the inverse slope of the ln(LVP) vs. time relationship.[65]

Hypothesis testing. 1) The efficacy of the proposed therapeutic approaches will be tested by 2-way analysis of variance of MR volume and orifice area among stages (baseline, acute MI, chronic MI if pertinent, therapy-acute, therapy-chronic if pertinent). Significant differences will be explored by paired t-test, and are protected by Fisher's F-test criterion for multiple comparisons. Other key variables, such as hemodynamics (HR, EF, dP/dt) and mitral valve geometric measures, will also be compared among stages and sheep by ANOVA. 2) The **central hypothesis** that ischemic MR relates to alterations in the mitral valve-ventricular relationship will be tested by relating changes in MR with each therapy to the corresponding changes in variables that determine the balance of tethering and closing forces acting on the mitral valve, as detailed under the clinical studies, above (univariate and step-wise multiple linear regression analysis). 3) **Intermediate-term efficacy** of the interventions will be evaluated as part of the ANOVAs under point #1, above, checking for lack of signifi-cant LV dilatation or redevelopment of MR over 8 weeks in the chronic models following therapy. Effects of interventions on LV function will be tested by comparing the ESPVR slope, EDPVR, and tau by ANOVA at stages with MI and MR before therapy, just after therapy, and 8 weeks later in the chronic models.

Potential limitations and future directions

1. The devices primarily address the ventricular-PM end of the tethering process, not the annular end, because of our clinical observations that addressing the annular end only frequently fails. Nevertheless, the patch device can be readily extended if necessary to reduce annular size as well, by elongating it toward the base of the LV; and, in pilot studies, chordal cutting reduced annular area from 6.9 ± 0.2 to 5.9 ± 0.2 cm^2 ($P<.003$), perhaps because of decreased MR or changes in the entire complex.

2. More than two basal chords may need to be cut to reduce greater degrees of MR due to more chronic and severe LV dilatation; so long as the marginal chords to the leaflet edges are intact, prolapse should not result, as in our pilot studies **(Appendix)**.

3. It is possible that the balloon-patch device will reduce LV diastolic compliance, thereby shifting the diastolic LV pressure-volume (P-V) curve upward. Nevertheless, the actual **LV filling pressures** may be the same or even lower, because removing the MR can decrease LV diastolic volume and therefore shift the ventricle to a lower-pressure point along the P-V curve.

ALTERNATIVE APPROACHES. The two interventions described (balloon patch and chordal cutting) were selected because they are relatively straightforward in their approach to tethering. The external device does not appear to require cardiopulmonary bypass, favoring its clinical implementation. The PI's R01 proposes several other techniques for consideration, and they provide alternatives, particularly should results with the two interventions described above not meet the expectations of the pilot studies.

One alternative is specifically suited to the bulging inferior wall MIs that commonly cause ischemic MR.[38,70] Three rows of mattress sutures are used to **plicate** or reduce the size of the visible infarct bulge, thereby reducing the diameter of the LV at the PM level, and bringing the infarcted PM tip closer to the anterior mitral annulus **(Fig. 10; Appendix)**. This decreases tethering length and reduces or eliminates MR. Over time, the epicardium and the endocardium form a smooth layer over the folded myocardium, and no thrombi form. Further work needs to be done to evaluate LV end-systolic and end-diastolic pressure-volume relationships with this intervention, and determine whether it is effective for global ischemic dysfunction as well. Ultimately, selecting approaches for patient use will be based on maximal benefit and ease of application, with least risk or impairment of LV function.

The chronic models of ischemic MR present an opportunity for **career development** through collaboration with other investigators at _____ (Drs. _____, _____, _____, _____, and _____) and the vicinity (Dr. _____) dealing with **the basic science of remodeling**. The K24 Award is essential to provide the time necessary to develop collaborations and advance this work to a molecular level.

SUMMARY. The central hypothesis that ischemic MR relates to alterations in the mitral valve-ventricular relationship will be tested by examining changes in mitral valve geometry as they relate to MR in patients.

Furthermore, demonstrating that restoration of the normal mitral valve-ventricular relationship with devices can alleviate MR strengthens the case for this central hypothesis. Experimental work will aim toward implementing potential improved therapies in patients toward the latter stages of this award or in subsequent work. These approaches have the potential to interrupt the cycle of remodeling and MR, which can limit functional capacity and adversely affect prognosis. A tentative timetable involves a focus on the clinical study of MR in years 1 to 3. Years 1-4 will also have time devoted to the experimental work, with the ultimate goal of clinical application toward the end of the award cycle.

Gender and Minority Inclusion. Patients will reflect the full range of gender and minorities seen in patients at this hospital, which serves as a general community hospital for substantial portions of Boston, Charlestown and Cambridge, as well as a referral center for the metropolitan area and beyond. The immediate geographic area from which inpatient samples will be drawn has the following gender and minority distributions: 47.8% male, 52.2% female, 59% Caucasian, 24% Black, 10% Hispanic, 1% Indian, 5% Asian, and 1% other. Patients will be recruited for this study from this population in a consecutive fashion. Clinical outreach and research newsletters to all the Partners-affiliated Community Health Centers will also encourage minority participation in these studies.

e. HUMAN SUBJECTS

Protocols #95-7468 and 98-09030

1. In patients with CAD, the question frequently arises whether additional effort should be directed toward ischemic MR, depending on its severity and mechanism. Over the course of the proposal, we plan to study at least 100 consecutive patients with CAD with ischemic MR and no organic mitral valvular disease (rheumatic, degenerative, or infectious). Such patients typically range in age from 35 to 80 years old, with a mean of 60 years.

   Patients will reflect the full range of gender and minorities seen in patients at this hospital, which serves as a general community hospital for substantial portions of _____, _____, and _____ as well as a referral center for the metropolitan district area and beyond. (The immediate geo-graphic area from which this sample will be drawn has the following gender and minority distribu-tions: 47.8% male, 52.2% female, 59% Caucasian, 24% Black, 10% Hispanic, 1% Indian, 5% Asian, and 1% other.)

2. Data: The data to be obtained are views comprising a transesophageal or transthoracic echocardio-graphic study. The TEE studies involved are currently being routinely requested at the time of bypass grafting or other surgery to monitor LV function and assess MR; therefore, no additional procedure will be needed, only collection and subsequent analysis of registered data for 3D reconstruction.

2. Recruitment: If TEE is used, informed consent will be obtained by the cardiologist or anesthesiologist obtaining consent for the routine TEE study, the only difference being a concerted 3D acquisition of views. The clinical need for the study and attendant risks and benefits (below) will be explained. Written informed consent will be obtained prior to the procedure.

3. Potential risks: Work in our laboratory has shown prolonged transesophageal echo study to be safe for the esophagus,[66] and no complications have been encountered. We have only had minimal local bleeding with this procedure, and no perforation. Transthoracic scanning has no associated risk.

5. Risks will be minimized by excluding patients with dysphagia and upper GI disease from TEE.

6. Risks are negligible and the test is clinically indicated to guide treatment, providing benefit to the patient currently in addition to the long-term benefits of research.

f. VERTEBRATE ANIMALS
Protocol # 97-4022

1. As detailed by Llaneras et al.,[23] anesthesia will be induced in Dorsett hybrid sheep (Ovine Biotechnologies, Inc., NJ) with sodium thiopental (12.5 mg/kg IV), and the trachea intubated and ventilated at 15 ml/kg with a mixture of 2% isoflurane and oxygen. All animals will receive glycopyrrolate (0.4 mg IV) and chloramphenicol (1 gm IV) one hour before incision, and one dose of chloramphenicol postoperatively. They will be loaded with lidocaine (3 mg/kg IV) and procainamide (15 mg/kg IV) 30 minutes before infarction and maintained on lidocaine (2 mg/min) during the operation. A micro- manometer-tipped catheter (Millar Instruments, Houston TX) will be introduced into the LV via a carotid artery and LV pressure recorded along with an ECG lead on a multi-channel physiologic recorder. After a sterile left thoracotomy to expose the heart, the second and third obtuse marginal branches of the left circumflex coronary artery will be ligated at their origin; another model involves PDA ligation as well. 2D and 3D echo imaging will be performed as described below through a sterile water bath before infarction, and 30 to 60 minutes following infarction. The thoracotomy will be closed and the animals cared for, with weekly transthoracic monitoring of LV function and MR. Those sur-viving two months later will undergo repeat 3D echo imaging, which for optimal quality and quanti-tation requires repeat thoracotomy. Those undergoing interventions to alleviate ischemic MR (external patch or chordal cutting to relieve tethering) will be tested for efficacy and safety of these interventions by being cared for an additional 8 weeks prior to sacrifice, in order to provide informa-tion needed to justify patient applications. After that, euthanasia will be provided by 100 mg/kg IV pentobarbital overdose and post-mortem studies will be performed to confirm infarct location and size and to measure mitral leaflet area. Postoperative care: Just before the animals wake up, they will be given butorphanol tartrate, 0.1 mg/kg IM, for analgesia, with bupivacaine 0.5% and epinephrine (Sensorcaine) injected into the incision site. They will awaken in the laboratory and be returned to the recovery room (Edwards 6) where our laboratory and Office of Laboratory Animal Research (OLAR) staff will monitor them for discomfort for the first 24 hours 2-3 times a day, admin-istering analgesics as necessary and requested by a veterinarian. Antibiotics (Cephapirin, 0.5 gm IV) will be adminis-tered for the next 5 days. Following complete recovery the animal will be placed in a pen designated by the MGH OLAR veterinary staff. Sheep models are also planned, as described above, to generate global LV dysfunction in an established model of microsphere embolization of the coronary arteries.[60,61] The initial models will use 24 sheep of either sex for each surgical intervention model (6 each for acute segmental, acute global, chronic segmental, and chronic global MI).

2. These animals are selected because the models are reproducible, well-established, and of suitable size to be studied by 3D echo. Rationale for selecting the sheep models is detailed above under Preliminary Studies and Results, the section on Experimental models and 3D analysis (paragraph 1).

3. The Animal Resources Program and centralized facilities at the _____ are under the direction of the Director, Office of Laboratory Animal Resources (OLAR), Research Affairs, a veterinarian certified by the American College of Laboratory Animal Medicine (ACLAM). The_____ has an active and functioning subcommittee on Research Animal Care (SRAC) which serves as the Institutional Animal Care and Use Committee (IACUC) as required by the PHS Policy on Humane Care and Use of Laboratory Animals.

   Within the hospital complex, 55,101 sq.ft. of space are devoted to the housing and servicing of various species used in biomedical research. There are 5 fully-equipped survival surgical suites within the _____ research facilities. These areas are designed to support a variety of surgical procedures. The majority of this space (85%) is located in newly-constructed and/or recently renovated buildings. Two of these areas have been designated Viral-Antibody-Free (VAF) areas for rodents, and containment procedures have been established. The Edwards-6 area contains 3,800 sq.ft. of animal space for the housing of dogs, cats, primates, sheep, swine and other large animals. Each area is equipped with cage/rack washin capability. The remaining 4,450 sq.ft. represent housing facilities for specialized gnotobiotic and conventional rodent breeding colonies. The average daily census is as follows: 21,000 mice, 2,600 rats, 335 rabbits, 60 dogs, 30 cats, 30 primates, 12 sheep, 8 pigs and a variable number of

guinea pigs, hamsters and gerbils. This census increases as additional animals are received in the newly-opened facility.

The OLAR is responsible for administering the microbiological Monitoring Program which is designed to include: the Chairman, Deputy Director of General Affairs of the Cutaneous Biology Research Center; 15 investigators who use animals in their research; 2 members of the MGH research admin-istration; 2 facility managers; and 2 members of the Boston community not otherwise associated with the _____. The _____ also monitors the status of microbial definition in each facility. Commercial suppliers providing animals for use at _____ are required to submit results of their monitoring program on a regular basis. Veterinary medical care is available on a 24-hour basis. The _____employs three veterinarians including the Director, OLAR. All serve as members of the SRAC. The Subcommittee employs more than 18 full-time animal technicians to provide daily health monitoring and animal care. The _____ is registered with the U.S. Department of Agriculture (Reg. # 14-R-014) as an approved research facility and the animal care areas are inspected at least three times yearly by the USDA. Agents of the Massachusetts Society for the Prevention of Cruelty to Animals also inspect the facility frequently. The Hospital has on file with the Office for Protection from Research Risks of NIH an Assurance of Compliance with Public Health Service regulations and requirements and provisions of the Animal Welfare Act (Assurance #A3596-01). The _____ is AALAC certified as of July 1993.

4. Sheep will be anesthetized and given analgesics as described above.

5. Euthanasia will be by barbiturate overdose (100 mg/kg IV pentobarbital), consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

g. Literature Cited

h. Consortium/Contractual Arrangements

i. Consultants

7. Appendix