SJ BIOMED Inc.

Key Technology Highlights

Technology II: Diagnostic kit/antibody for Acute Myocardial Infarction

NCM test is an immunoassay for the rapid qualitative detection of NCM in human whole blood or serum at a cut off level of 0.5ng/ml. With heart tissue specificity and accuracy with high sensitivity as early as 2 hr after the onset, it may provide an aid in the diagnosis of myocardial infarction in emergency room. Further quantitative and qualitative study in patients will provide more information ensuring that NCM test may enable physician to decide whether patients can be replaced in thrombolytic therapy or not.

- Diagnosis kit : detection sensitivity needs to be increased further
- Antibody: best pairing process for best quantification
- Human data needs to be acquired to confirm our hypothesis and rationale

NCM test is an immunoassay for the rapid qualitative detection of NCM in human whole blood or serum at a cut off level of 0.5ng/ml. With heart tissue specificity, sensitivity, accuracy as early as 2 hr after chest pain and assay read time 15 minutes, it may provide an aid in the diagnosis of myocardial infarction less than 6hr in emergency department. Further quantitative and qualitative study in patients will provide more information ensuring that NCM test may enable physician to decide whether patients can be placed in thrombolytic therapy or not.

Technology Overview

Technology II: Diagnostic kit/antibody for Acute Myocardial Infarction

1. Technology Background:

Myocardial infarction is a complete blockade of the coronary artery, which carries blood to the myocardium. The blockage often is a result of a blood clot, but can also be caused by plaque (cholesterol) or other material. According to the American Heart Association, 12.6 million people alive today have a history of heart attack, chest pain or both. Among chest pain patients, the highest acuity is associated with those suffering a heart attack called myocardial infarction. For these patients, time is a significant factor in the race to save heart muscle and their lives.
40-60 minute occlusion cause serious damage in heart and the damaged heart tissues cannot be regenerated or recovered at all, in the later life if treatment is not made within first 6h of chest pain. If diagnosis of MI within 6hr can be made, patients can be placed in antiplatelet and anti-ischemic agents, thrombolytic therapy, and primary percutaneous transluminal coronary angioplasty (PTCA). Reperfusion therapy (thrombolysis or primary PTCA) works

best when given within 4-6 hours of the onset of signs and symptoms. Thus prompt recognition of signs and symptoms, accurate diagnosis, and timely treatment are essential to limit the size of the infarct, preserve myocardial function, and reduced mortality. Although progress was made, acute chest pain remains a difficult diagnostic problem as autopsy results indicate many myocardial infarctions go undetected. When patients arrive in a emergency room, the evaluation process for chest pain patients under WHO guide line will: 1) clinical history of ischemic chest discomfort more than 30 minutes in duration, 2) evolution of typical electrocardiographic changes, Q-waves, and 3) rise and fall of cardiac specific markers. Surveys suggest that approximately 80 percent of chest pain patients receive electrocardiograms (ECG) as part of the diagnostic process. ECG, however, has been found to be only 50% sensitive for diagnosis of MI. Thus ancillary cardiac markers provide a highly effective means of evaluating chest pain.
Currently available cardiac diagnostic markers include Creatine Kinase MB, Troponin and LDH.
However their lack of heart tissue specificity and low sensitivity and accuracy in less than 6hrs, the bio-marker myoglobin is kept on using in spite of its extremely low specificity to heart injury to diagnosis MI in less than 6hr.
Here, SJ Biomed would like to introduce novel heart tissue specific cardiac maker released as early as 1-2hr after MI to replace myoglobin or confirmatory marker for diagnosis of MI less than 6 hr along with myoglobin.

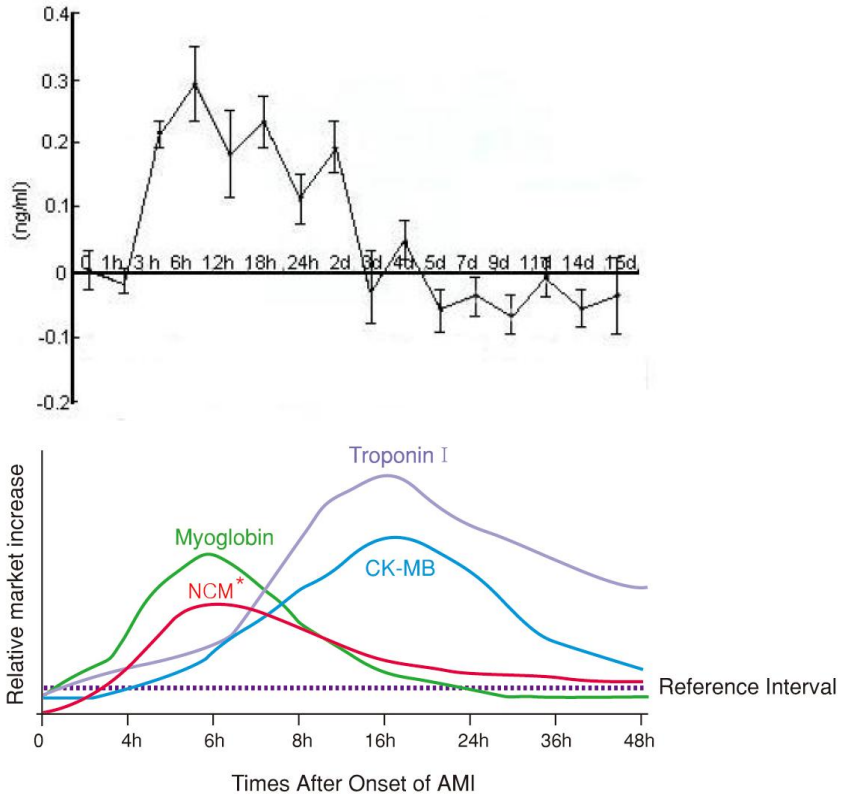
☐ **Heart tissue specific expression:**

As a mitochondrial matrix enzyme involved in the biological energy metabolism, NCM is expressed restrictively at heart, liver, kidney, but not skeletal muscles in human body.
No detection is made at the basal level, but detected from 0.1ng/ml to 10ng/ml after the MI onset.

☐ **Early release within 2 h after MI (in rat model)**

- NCM is released upon MI.
Though the NCM localized in the mitochondrial matrix, it can be released as early as myoglobin
which locates in the cytosol. As the hypoxic stress triggers the apoptosis of myocytes, mitochondria begin to disrupt and the contents are released into the cytosol before the necrosis of the myocytes. (CK-MB or troponins are still captured in the myofilaments right after the myocyte necrosis and accordingly, are released slowly into the blood stream and detected after 6 hours from onset symptom).
- The molecular size is small enough to be released into the circulation easily (better than CK-MB, the same with troponins, worse than myoglobin)
- Availablility of sandwich immunoassay (better than myoglobin or troponins, worse than CKMB). Therefore, it is quite reliable to detect the NCM with the same hour window as myoglobin which is 2-4h earlier than CK-MB or cTns.

Time dependent NCM releasing pattern from rat acute myocardial infarction.



***** The NCM expression pattern was assumed based on rat releasing pattern data provided above.

Human pattern was not examined yet but to be planned.

☐ Accuracy

No mitochondrial enzymes are currently being investigated as markers for myocardial injury. The accuracy of the diagnostic test was as excellent as no false negative was detected from 70 patient serum samples. False positive was detected from ca. 10% of liver diseased patients and very rarely from patients with brain infarct. The results are summarized in Table II. Here, the likelihood ratios guided by AHA are shown in the Table I. Take into consideration of borderline of likelihood ratios recommended by AHA (10 for positive, and 0.1 for negative), NCM showed an excellent marks at the pre-clinical tests.

Table 1. Clinical features of 70 patients with MI and 70 non-MI patients in relation to NCM detection in serum.

	NCM Positive	NCM Negative
AMI (cases)	70	0
Non-AMI (cases)	3	67

- Likelihood Ratio (+) ; [70/(70+0)]/[3/(67+3)] = 23.3 > 10
- Likelihood Ratio (-) ; [0/(70+0)]/[70/(67+3)] =0 < 0.1

These characteristics enables the NCM can substitute for the combinatorial usage of Myoglobin and CK-MB or cTns or replacement of myoglobin.

Existing Biochemical Markers

The advantages of NCM over existing markers are shown in Table 1.

Table .1 Comparison of NCM with the existing biochemical markers

	NCM	Myoglobin	Cardiac Troponin		CK-MB	MB Isoform
			cTnI	cTnT		
MW	30	17	23	33	86	86
Sensitivity 100%(h)	3-5	4-8	8-12		8-12	6-10
Peak (h)	ND	4-8	10-24		10-24	6-12
Duration(h)	ND	0-5-1	5-10	5-14	2-4	0-5-1.0

cTnI cardiac specific troponin I: cTnT, cardiac specific troponin T, Adapted from Adams J.Abendschein D, Jaffe A Biochemical markers of myocardial injury : Is MB creatine kinase the choice for the 1990s? Circulation, 1993;88;750-76317 ND: not determined

CM-MB

- Standard clinical laboratory test used in diagnosing AMI
- Elevation in CK-MB in the absence of surgery and trauma is highly correlated with myocardial ischemia.
- Traditional CK-MB assay requires serial sampling on admission and about 12 hours later
- Low sensitivity of single CK-MB pose a diagnostic dilemma for emergency physicians because thrombolytic therapy must be initiated within four to 6 hous after infarction.
- Lack of absolute cardiac specificity of CK-MB occuring in renal failure has let to the development of aasays of more specific proteins, particularly troponins.
- Since the frst 4-6 hours are crucial for any reperfusion strategy, a bimarker that reaches elevated levels early is attractive. But much of the data indicate a low sensitivity for CM-MB in the first 6 hours

LDH

Although LDH and its isoenzymes are less accurate than total CK amd MB-CK, LDH determinations can be helpful in patients who delay their arrival at the hospital for more than 48-72hours and in whom CK levels may have already returned to baseline.

Troponin

- The most often used markers are the creatine kinase MB(CK-MB) fraction and the troponin I (TnI) or troponin T (TnT) levels.
- It rise 3.5hr after the onset of chest pain and remain so for at least 5 days. Thus, troponin T determinations are particularly useful in patients with acute infaraction who do not seek medical attention within the 48-72hr window
- Serum level of cardiac troponins are usually low (below the level of detection) but increase quickly when acute myocardial infarction occurs.
- Rise within 4-6hr after injury. Levels remains up to 8-10 days.
- But in some cases of patients with unstable angina or non-Q wave myocardial infarction, duration of elevation is shorter thus need to replace with other marker measurement.

Myoglobin

- Myoglobin released within 2 hours of coronary occlusion and peaks in 6-7 hours.
- Although it has early release, due to its low sensitivity 37% at 2 hrs and 86% at 6 hours, can't be used for the reperfusion injury therapeutic marker
- Serial measurement didn't give much competitiveness to others.
- In addition, it is not cardiac specific

2. Advantages: Summary of Competitiveness compared to others

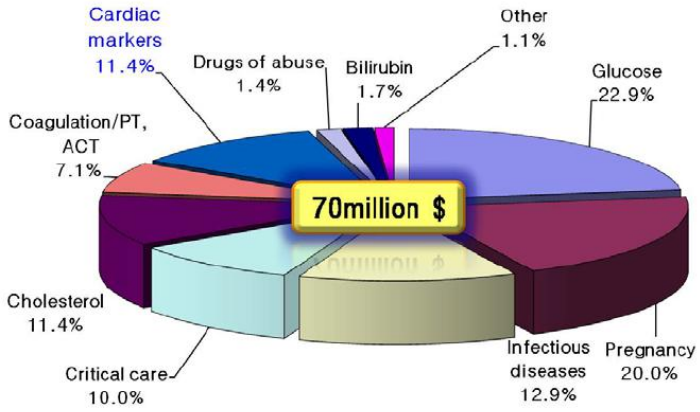
		Opportunity	**T**hreat
		- Growth of Diagnostic market - Early accuracy diagnostic marker is not exist	- Market occupation of Established marker
Strength	- Early release Marker - Heart tissue specific expression - High accuracy - Various application form is possible (ex. ELISA, POCT etc.)	SO strategy - New marker for diagnosis of MI less than 6hr - NCM can substitute for the combinatorial usage of Myoglobin and CK-MB or cTns or replacement of myoglobin - High sensitivity diagnostic kit develoments	ST strategy - Acquirement of Satisfactory clinical research data
Weakness	- New marker - Insufficiency of Clinical case	WO strategy - CE mark wii be gained - Progess of KFDA permission	WT strategy - Acquirement of Satisfactory clinical research data - Biochemical Assay methods establishment

3. Current developmental status, Developmental Plan and Cost estimate

1. Monoclonal antibody for IVD kit ; monoclonal antibody pair for IVD kit were established with the detection limit of 0.5 ng/ml NCM in blood. However, it preferably be improved up to minimal detection level of 0.1 ng/ml
2. ELISA quantification method monoclonal pair for Sandwich ELISA were established with the detection limit of 0.5 ng/ml NCM in serum. However, it preferably be improved up to minimal detection level of 0.1 ng/ml.

Year 2011 : ELISA Kit sensitivity improvement and manufacturing (70,000 USD)
Year 2012: Kit POC test to prove proof of concept and to set quantification range.(150,000 USD)

4. Global Diagnostic market size (2010' estimate)



Worldwide professional-use point-of-care test sales by test category
Source: Kalorama Information.

Patent Information

PATENT (related to Key technology)				
Human NCM Antibody, Immunological Formulation and Diagnostic Kit for Cardiac Disease	414637 2262939 3736798 784153 11/581,146 1165133 NK1049121 00805979.9	2003.12.26. 2005.04.05. 2005.11.04. 2006.05.25. 2006.10.16. 2010.12.29 2011.01.14 2010.11.03	Korea Russia Japan Australia U.S.A Europe Hong Kong China	Registration
	2,366,468 PI 0010712-3	2000.08.10. 2001.10.08.	Canada Brazil	Pending
Monoclonal antibody specific to NCM	0456790 KR2003/001979	2004.11.02. 2003.09.27.	Korea PCT	Registration