Overcoming the Safety Challenges of Aldose Reductase Inhibition: Development of AT-001 for Diabetic Cardiomyopathy

R. Perfetti[1], G. Yeppuri[2], N. Quadri[2], R. Ramasamy[2], S. Shendelman[1]: NYU Langone Medical Center[2], and Applied Therapeutics[1], New York, NY

APPLIED THERAPEUTICS

Abstract

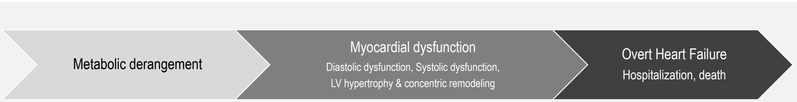
Diabetic Cardiomyopathy (DbCM) leading to overt heart failure is a common sequalae of both Type 1 and Type 2 Diabetes. Previous attempts to develop treatments for DbCM through inhibition of the enzyme Aldose Reductase (AR) were unsuccessful, due to low AR binding affinity and poor specificity. Off-target inhibition of Aldehyde Reductase (AldR), an enzyme critical for detoxification of aldehydes in the liver and normal hepatocyte physiology, led to common liver-related safety and tolerability issues with first generation ARI compounds. We report on the selectivity and specificity of AT-001, a novel small molecule ARI with optimized affinity and specificity for AR and minimal to zero off-target AldR activity.

Methods and Results: AT-001 was evaluated for AR binding affinity as compared to zopolrestat, a first-generation ARI. AT-001 demonstrated a >300-fold greater affinity for AR versus zopolrestat (IC50 of 30 pmol for AT-001 and ~10 nmol for zopolrestat). In contrast to zopolrestat, AT-001 showed no inhibition of AldR at 50x and 100x EC50 levels in assay medium, while zopolrestat inhibited Aldehyde Reductase by 50% and 60% respectively (spec activity of 2.3 and 1.9 mmol NADPH/min/ mg protein). In addition to these analyses, AT-001 was evaluated in a standard off-target receptor binding analysis of 87 substrates (13 enzyme, 74 binding assays) to characterize pharmacological specificity and selectivity. No significant off-target results (defined as ⍰50% inhibitory activity) were observed in this panel.

Conclusion: The unique structure and activity of AT-001 provide selectivity for Aldose Reductase and lack of off-target effects. The in-vitro safety of this agent together with the positive safety data from the phase 1/2 program, supports the ongoing pivotal study in DbCM.

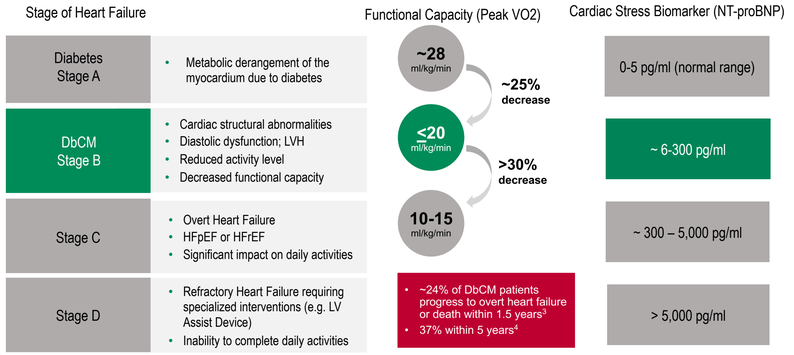
Definition of Diabetic Cardiomyopathy (DbCM)[1]

- Abnormal cardiac structure and/or performance
  - Resulting **from diabetes-associated metabolic alterations**
  - In the absence of coronary artery disease (CAD) as well as hypertensive, valvular or congenital heart disorder
- Progresses to overt heart failure (HF)[2,3]



1. Ryden L Eur Heart J. 2013; 34:3035–3087. 2. Jia G, et al. Circ Res. 2018;122:624-638. 3. Borghetti et al. Frontiers in physiology 2018;9:1514

Diabetic Cardiomyopathy as a Form of Stage B Heart Failure[1-4]



LVH left ventricular hypertrophy, DbCM diabetic cardiomyopathy, HFpEF heart failure with preserved ejection fraction, HErEF heart failure with reduced ejection fraction
1. Kosmala et al, J Am Coll Cardiol 2015;65:257–66.; 2. Swank et al. Circ HF 2012; 3. Wang et al. JACC: Cardiovasc Imaging 2018; 4. From et al. JACC 2010

High Prevalence of DbCM and Unmet Medical Need

Approximately, **17-24%** of patients with diabetes have DbCM in the absence of other forms of heart disease. [1,2]

~77 M patients worldwide have DbCM[3]
- ~ 8.0M in North America
- ~ 10.0M in Europe

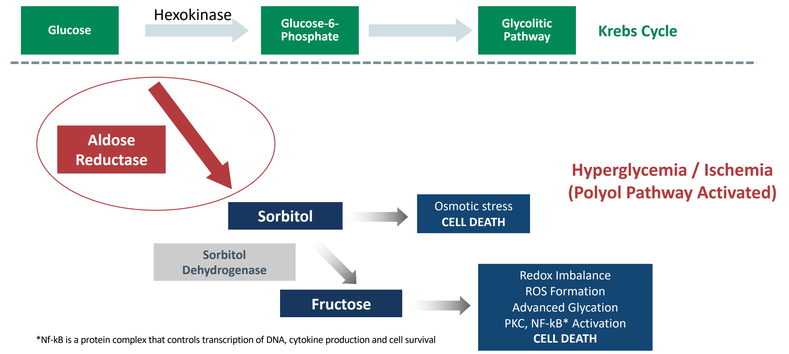
- ~24% of DbCM patients progress to overt heart failure or death within **1.5 years**[4]
- 37% within 5 years[5]

- Patients with diabetes are counseled on HF risk reduction:
  - Lifestyle modification
  - Hypertension
  - Dyslipidemia
  - Hyperglycemia
  - Albuminuria

No Treatment for DbCM
- No therapies target the metabolic derangement responsible for DbCM and subsequent worsening to overt HF
- Heart Failure treatment is only initiated upon onset of clinical symptomatology (stage C heart failure)

1. Dandamudi et al. J Card Fail. 2014;20(5):304-309. 2. Pham et al. Intl J Endocrinology 3. International Diabetes Foundation, 2017,4. Wang et al. JACC: Cardiovasc Imaging 2018; 5. From et al. JACC 2010

Pathogenesis of DbCM & Hyperactivation of Polyol Pathway1,2

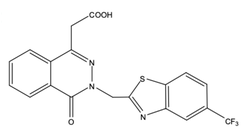


*Nf-kB is a protein complex that controls transcription of DNA, cytokine production and cell survival

1. Brownlee M. Diabetes Care. 2005;54(6):1615-1625. 2. Miki T, et al. Heart Fail Rev. 2013;18(2):149-166.

AT-001 Pre-clinical and Clinical Profile Summary

zopolrestat



Inhibition of Aldose Reductase → **Clinical Efficacy**

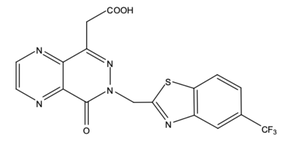
Competitive Inhibition of Aldehyde Reductase → **(Off-Target) Hepatotoxicity**

- First generation Aldose Reductase Inhibitor (zopolrestat) demonstrated clinical efficacy in Diabetic Cardiomyopathy[1]
- Hepatotoxicity was observed in the development program (presumably due to off target competitive binding with Aldehyde Reductase in liver)
- Clinical development was discontinued

[1]Johnson, et al. Diabetes Care, 2004 pp 448-454

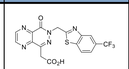
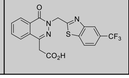
AT-001: A Next Generation Highly Selective Aldose Reductase Inhibitor for Treatment of Diabetic Cardiomyopathy

AT-001



- AT-001 was developed through rational drug design, using the geometric parameters of the active site of the Aldose Reductase enzyme determined via X-ray crystallography.
- Optimal target selectivity for Aldose Reductase and minimization of potential off-target activity with Aldehyde Reductase was achieved.
- Aldehyde Reductase plays an important role in detoxification mechanisms in the liver. Minimization of off-target activity is critical to ensure safety.

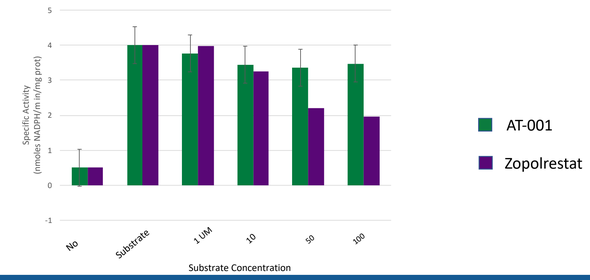
AT-001 Increased Affinity for Aldose Reductase vs. Zopolrestat

Compound	Structure	IC_{50}	MTD in animals	Tissue Penetration (in rats)			
				Systemic/ Heart	Nerve	Retina	CNS
AT-001		30pM	>2,000mg/kg	✓	✓	✓	X
zopolrestat		10nM	100mg/kg	✓	✓	X	X

No AT-001 Off-Target Binding

- Eurofins Panlabs Safety Screen Panel (consisting of 87 primary molecular targets including 13 enzyme and 74 binding assays) was used to evaluate potential off target binding activity of AT-001
- No off-target binding activity (defined as ≥50% inhibition or stimulation for biochemical assays) was observed

Zopolrestat (But Not AT-001) Inhibits Aldehyde Reductase



Conclusions

- AT-001 is logarithmically more potent than zopolrestat in inhibiting Aldose Reductase
- The unique structure and activity of AT-001 provide selectivity for Aldose Reductase and avoid off-target inhibition of Aldehyde Reductase
- The in vitro safety of this agent together with the positive safety data from the phase 1/2 program, support the ongoing pivotal study in DbCM

[1]NYU Langone Medical Center, New York, NY, [2]Applied Therapeutics Inc, New York, NY