Addendum to FDA Briefing Document

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Nonclinical Effects of Testosterone on the Cardiovascular System

The following is a brief literature review of several cardiovascular effects of subphysiological (castrate levels) and supraphysiological levels (many times normal) of testosterone in male animals. This is not a comprehensive review of all the relevant literature. Recent review articles discussing nonclinical cardiovascular effects of androgens and primary literature based on key word searches in PubMed were reviewed. Key search words included but were not limited to names of numerous animal species, androgen, testosterone, dihydrotestosterone, anabolic, androgen receptor, heart, cardiovascular, cardiac, atherosclerosis, inflammation, edema, polycythemia, vasodilation, and myocardial infarction.

Cardiac Hypertrophy: One of the principle cardiac pathologies in response to sustained and large supraphysiological androgen exposure is myocyte hypertrophy in animals (Papamitsou *et al.* 2011). Hypertrophy of cardiac myocytes by testosterone and DHT was determined to be androgen receptor dependent by an in vitro assessment (Marsh *et al.* 1998). Findings in animals are consistent with signs of cardiac hypertrophy reported in athletes abusing anabolic androgenic steroids which has been associated with sudden cardiac death with ventricular hypertrophy, myocytolysis, myocardial fibrosis, and/or myocyte necrosis (Darke *et al.* 2014; Higgins *et al.* 2012; Montisci *et al.* 2012).

Hypertension and Peripheral Edema: It is well known that androgens may promote retention of sodium and water which may be a serious complication in patients with or without congestive heart failure with preexisting cardiac, renal, or hepatic disease. In normal rats or in rodent models of hypertension, studies suggest that androgens promote sodium and water resorption and consequently elevate blood pressure (Reckelhoff *et al.* 2005). Studies in normal male rats suggest that the increased systolic blood pressure caused by supplemental DHT exposure was due to up regulation of the renin-angiotensin system in the proximal tubules leading to retention of water and sodium (Quan *et al.* 2004; Quigley 2008; Reckelhoff *et al.* 2005).

Polycythemia: It is well known that androgens promote erythropoiesis in animals and humans and that polycythemia is a known risk of testosterone replacement therapy (Shahani *et al.* 2009). Although androgens are known to elevate erythropoietin levels, increase bone marrow activity, and increase iron incorporation into the red cells, the molecular mechanism for this is not entirely clear (Bachman *et al.* 2014; Shahani *et al.* 2009).

Vasodilation: Although there are conflicting data, the majority of the nonclinical literature surveyed suggests that testosterone can stimulate vasodilation at either physiological or supraphysiological exposures (Herring *et al.* 2013; Kelly and Jones 2013; Wu and von 2003). The mechanism for this response is not fully understood but it is believed to be due to rapid inactivation of L-type voltage gated calcium channels and/or activation of potassium channels in vascular smooth muscle and increased nitric oxide production in vascular endothelial cells (Kelly and Jones 2013).

Potential Effects on Cardiac Rhythm: The clinical significance of the effects of androgens on cardiac rhythm in animals is unclear. In tissue cultures and castrated animal models, subphysiological levels of testosterone appear to suppress contractile performance and prolong QT interval which can be restored with androgen supplementation to physiological levels (Bai *et al.* 2005; Herring *et al.* 2013; Liu *et al.* 2003; Scheuer *et al.* 1987). Evidence suggests that the functional effects on the heart may be mediated by a non-transcriptional mechanism by enhancing I_{KS} currents, suppressing L-type calcium currents ($I_{Ca,L}$), and potentially by regulating nitric oxide release.

Diet and Cardiovascular Risk: In a model of metabolic syndrome induced by a high fat diet in normal male rabbits, testicular weight and serum testosterone were decreased (Filippi *et al.* 2009). Maintaining testosterone at near normal physiological exposures for 12 weeks in rabbits on a high fat diet reduced visceral fat accumulation while improving fasting glucose levels, glucose tolerance, and mean arterial pressure without altering the fat induced elevation of cholesterol and triglycerides.

Atherosclerosis: Although the effect of androgens on vascular inflammation is not clear because of conflicting and contradictory findings in nonclinical models, physiological levels of androgens appear to have beneficial effects on preventing plaque formation in animals on a high fat diet while plaque formation may be promoted by subphysiological testosterone levels (Alexandersen *et al.* 1999; Bruck *et al.* 1997; Kelly and Jones 2013; Nathan *et al.* 2001; Nettleship *et al.* 2007).

Effects on Recovery from Myocardial Ischemia: The potential effect of testosterone on recovery from myocardial infarction (MI) is unclear because of conflicting findings in animal models. Castration improved survival and recovery from surgically induced regional myocardial infarction in mice but castration impaired recovery in rats. Conflicting findings were also reported in the hearts of rats that were isolated and recovering from acute ischemic conditions ex-vivo where castration caused beneficial effects in some reports and detrimental effects in other reports.

In mice with surgically induced myocardial infarction, suppression of endogenous testosterone by castration reduced infarct size, cardiac rupture, and death and also improved cardiac function which suggests that physiological testosterone exposures may impair healing of the infarction site and decrease survival (Cavasin *et al.* 2006). However, in rats the opposite findings were observed, where castration increased the infarct size, decreased angiogenesis at the infarct, increased the number of apoptotic

cardiomyocytes, and worsened cardiac function after MI (Chen *et al.* 2012). Additionally restoring testosterone to physiological exposures in castrated rats with surgically induced MI reversed these findings. Supraphysiological exposure to testosterone in intact rats with surgically induced MI did not adversely affect infarct size, survival, cardiac output, or blood pressure although it did increase cardiac hypertrophy and left ventricular weight (Nahrendorf *et al.* 2003).

The effects of altering testosterone exposure in vivo on the response to acute ischemia in isolated perfused hearts of rats were mixed. Some ischemia reperfusion models indicated that castration enhanced recovery of contractile function (Wang *et al.* 2005) and decreased infarct size (Le *et al.* 2014). Others found the opposite where castration impaired contractile function and increased infarct size (Callies *et al.* 2003; Liu *et al.* 2006), and testosterone or DHT supplementation helped to reverse these adverse findings in castrated rats (Callies *et al.* 2003; Liu *et al.* 2006).

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