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Proceedings from the Third National Institutes of Health International Congress on Advances in Uterine Leiomyoma Research: comprehensive review, conference summary and future recommendations

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BACKGROUND: Uterine fibroids are the most common gynecologic tumors in women of reproductive age yet the etiology and pathogenesis of these lesions remain poorly understood. Age, African ancestry, nulliparity and obesity have been identified as predisposing factors for uterine fibroids. Symptomatic tumors can cause excessive uterine bleeding, bladder dysfunction and pelvic pain, as well as associated reproductive disorders such as infertility, miscarriage and other adverse pregnancy outcomes. Currently, there are limited noninvasive therapies for fibroids and no early intervention or prevention strategies are readily available. This review summarizes the advances in basic, applied and translational uterine fibroid research, in addition to current and proposed approaches to clinical management as presented at the 'Advances in Uterine Leiomyoma Research: 3rd NIH International Congress'. Congress recommendations and a review of the fibroid literature are also reported.

METHODS: This review is a report of meeting proceedings, the resulting recommendations and a literature review of the subject.

RESULTS: The research data presented highlights the complexity of uterine fibroids and the convergence of ethnicity, race, genetics, epigenetics and environmental factors, including lifestyle and possible socioeconomic parameters on disease manifestation. The data presented suggest it is likely that the majority of women with uterine fibroids will have normal pregnancy outcomes; however, additional research is warranted. As an alternative to surgery, an effective long-term medical treatment for uterine fibroids should reduce heavy uterine bleeding and fibroid/uterine volume without excessive side effects. This goal has not been achieved and current treatments reduce symptoms only temporarily; however, a multi-disciplined approach to understanding the molecular origins and pathogenesis of uterine fibroids, as presented in this report, makes our quest for identifying novel targets for noninvasive, possibly nonsystemic and effective long-term treatment very promising.

CONCLUSIONS: The Congress facilitated the exchange of scientific information among members of the uterine leiomyoma research and health-care communities. While advances in research have deepened our knowledge of the pathobiology of fibroids, their etiology still remains incompletely understood. Further needs exist for determination of risk factors and initiation of preventive measures for fibroids, in addition to continued development of new medical and minimally invasive options for treatment.

Key words: NIH conference summary and comprehensive review / uterine leiomyoma / fibroids / clinical treatment/basic research / conference recommendations

Introduction

The National Institutes of Health (NIH) along with other federal partners sponsored a two-day conference titled 'Advances in Uterine Leiomyoma Research: 3rd NIH International Congress' on 22 and 23 November 2010. This conference was the latest in a series of three NIH-wide meetings presenting research strategies to increase our knowledge and understanding of uterine leiomyomas, the biological processes that lead to their development and long-term sequelae, and present a clinical framework to address key issues related to disease burden, prevention and evidence-based treatment options (Newbold *et al.*, 2000; Dixon *et al.*, 2006). The meeting featured scientific presentations that summarized the current state of knowledge, concentrated on promising and innovative research, enhanced our understanding of the basic underpinnings of uterine leiomyoma pathophysiology and known risk factors for disease development, and focused on innovative treatment modalities. Since the first NIH conference in 1999, great strides have been made. Nevertheless, a dearth of information remains about a condition that causes significant reproductive morbidity. This article is a summary of the proceedings of the conference, and presents comprehensive reviews of the current progress in molecular and translational research, and clinical data on efficacy of medical, minimally invasive and surgical treatment modalities.

Overview of uterine fibroids

Molecular etiology and potential therapeutic targets for uterine fibroids

Uterine leiomyomas (i.e. fibroids, myomas, leiomyomata) occur in over 77% of all women and arise from the smooth muscle compartment (myometrium) of the uterus (Cramer and Patel, 1990). Uterine leiomyomas are clonal in origin, appear after menarche, typically grown during reproductive years and then stabilize or regress after menopause. These tumors remain benign, despite the fact that they can be numerous, large and are promoted by hormones. They are diagnosed in African American women three times more frequently than in Caucasian women (Marshall *et al.*, 1997; Baird *et al.*, 2003). Fibroids can induce abnormal uterine bleeding, bladder dysfunction and pelvic pain, in addition

to associated reproductive disorders such as infertility, miscarriage and other adverse pregnancy outcomes (Hart *et al.*, 2001; Surrey *et al.*, 2001; Sheiner *et al.*, 2004). Surgical procedures are of major concern, because uterine leiomyomas remain the primary indication for hysterectomy in women of reproductive age and account for >200 000 hysterectomies a year in the USA (Mauskopf *et al.*, 2005). It has been estimated that uterine fibroids cost the USA $5.9–34.4 billion annually (Cardozo *et al.*, 2012). Despite the fact that uterine leiomyomas represent the most common gynecologic tumor in women and constitute a significant public health concern, the mechanisms that initiate uterine leiomyoma growth and pathogenesis are still not completely understood.

It is compelling to appreciate the benign nature of uterine leiomyomas despite the fact that they are hormone responsive and can become quite large. Studies have shown that the phenotype of parturient myometrial cells and uterine leiomyomas is similar in terms of gene expression (Cesen-Cummings *et al.*, 2000, 2003). These results have led to the suggestion that uterine leiomyomas are more similar to myometrial cells of pregnancy than to nongravid myometrial cells (Busnelli *et al.*, 2010; Andersen and Barbieri, 1995). It has been noted that the increase in uterine weight during pregnancy is due to both hypertrophy and cellular proliferation of smooth muscle cells. The origin of the new smooth muscle cells may be from preexisting smooth muscles or the product of stem cell differentiation. Recently, myometrial stem cells found in a side population (SP) of myometrial cells (myoSP) have been identified in the nonpregnant uterus (Ono *et al.*, 2007). Compared with the main population of myometrial cells, the myoSP reside in quiescence, have an undifferentiated phenotype and can replicate under low oxygen conditions into mature myometrial cells (Ono *et al.*, 2007). Recent studies have confirmed the mesenchymal origin and undifferentiated nature of SP cells and have proposed that they are stem cells with characteristic features of tumor-initiating cells (Mas *et al.*, 2012). It is suggested that they may be responsible for cell proliferation and tumor growth observed in fibroids (Ono *et al.*, 2012).

Another hypothesis of uterine leiomyoma etiology takes into account phenotypic similarities between uterine leiomyomas and keloids, which may occur in response to wounds. A key observation is that both have disorganized collagen fibrils and decreased expression of dermatopontin that is similar to keloids (Catherino *et al.*, 2004; Leppert *et al.*, 2004). Keloids and uterine leiomyomas are more prevalent in African Americans, suggesting a common etiology. The transforming growth factor-beta (TGF-β), mammalian target of rapamycin (mTOR) and progesterone receptor signaling pathways have been considered important in fibroid growth and as potential therapeutic targets (Laping *et al.*, 2007; Levens *et al.*, 2008; Crabtree *et al.*, 2009; Li *et al.*, 2013). Since fibroids are hormone dependent, steroid hormone pathways have long been targets of investigation, in particular, selective estrogen receptor modulators (SERMs) and selective progesterone receptor modulators (SPRMs). Clinical studies have shown that treatment with SPRMs, such as Mifepristone (Engman *et al.*, 2009), CDB-2914 (Ulipristal) (Nieman *et al.*, 2011), CDB-4124 (Luo *et al.*, 2010), Asoprisnil (Yoshida *et al.*, 2010) and CP-8947 (Catherino *et al.*, 2010), generally decrease uterine leiomyoma size, increase primary apoptosis or inhibit cellular proliferation. Based on experimental models and clinical trials, caution is expressed in the use of some of the receptor modulators and inhibitors due to risk of cancer liability in other organs and/or cell types (Laping *et al.*, 2007; Ioffe *et al.*, 2009). Other novel therapeutic agents that are being evaluated for treatment of uterine leiomyoma include the antifibrotic drug halofuginone (Grudzien *et al.*, 2010), gene therapy (Hassan *et al.*, 2009), tyrosine kinase inhibitors (Shushan *et al.*, 2004, 2007; Islam *et al.*, 2013), aromatase inhibitors (Bulun *et al.*, 2005), cyclin-dependent kinase (CDK) inhibitors (Shime *et al.*, 2002), antiproliferative agents (Zhang *et al.*, 2010a, b), vitamin D (Halder *et al.*, 2013a, b), herbals (Li *et al.*, 2012; Liu *et al.*, 2013) and curcumin (Tsuiji *et al.*, 2011). Next generation approaches to treatment of fibroids include the use of new drug delivery systems utilizing smart nanocarrier technology (Taylor and Leppert, 2012) and identification of epigenetic targets, such as DNA methylation sites (Navarro *et al.*, 2012), microRNAs (Luo and Chegini, 2008) and histone modification enzymes (Wei *et al.*, 2011) thought to play a role in uterine leiomyoma initiation and growth.

Genetic studies of uterine leiomyomas from a variety of populations have demonstrated translocations in the high mobility group (HMG) protein genes, specifically HMGA1 and HMGA2. Aberrant expression of HMGA2 may affect the expression of growth factors and growth inhibitors, fibroblast growth factor 2 (FGF2) and p19 alternate reading frame ($p19^{Arf}$), respectively. Moreover, the overexpression of HMGA2 in leiomyomas correlates with increased FGF2 levels and tumor size, and repression of the growth inhibitor factor $p19^{Arf}$ (Markowski *et al.*, 2010a, b). More recently, mutations of the Mediator Complex Subunit 12 gene (MED12) have been reported in uterine leiomyomas from American women of varied ethnicities and racial backgrounds (McGuire *et al.*, 2012), and in South African (Mäkinen *et al.*, 2011a, b) and Finnish (Mäkinen *et al.*, 2011a, b, 2013a, b) women. Mutations in MED12 appear to be common in exon 2, and Je *et al.* (2012) have shown that exon 2 mutations are tissue specific to uterine leiomyomas, rare in other tumors, and may contribute to the development of fibroids. Studies in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) allow examination of uterine leiomyomas that develop in the setting of a hereditary syndrome. These studies suggest that additional factors, such as germline mutations in fumarate hydratase (FH), play a role in the development of hereditary leiomyomatosis and renal cell cancer, observed in a subset of leiomyomas (Toro *et al.*, 2003). The risk of uterine fibroids in FH mutant-positive women with clinical HLRCC is significantly increased compared with women with no identifiable FH germline mutations (Stewart *et al.*, 2008). Environmental factors are known contributors to many disorders and their relationship to fibroid growth and development are now becoming more apparent (Newbold *et al.*, 2002; Cook *et al.*, 2005; Moore *et al.*, 2007; Di *et al.*, 2008; Gao *et al.*, 2010; Laughlin *et al.*, 2010; Yu *et al.*, 2010, 2012; Gao *et al.*, 2012). Endocrine disruptors that may play a role in leiomyoma growth include developmental exposures to phthalates and DES (Baird and Newbold, 2005; Bredfeldt *et al.*, 2010; Huang *et al.*, 2010). Obesity is an established risk factor for fibroid development. Recent studies demonstrated that adiponectin can inhibit leiomyoma growth in culture (Wakabayashi *et al.*, 2011). Thus, it is likely that both genetic and environmental factors differentially contribute to the development of uterine leiomyomas in selected populations of women. Early life exposure during the key developmental periods could reprogram the genome to predispose to leiomyoma (Greathouse *et al.*, 2008; D'Aloisio *et al.*, 2012; Chahine and Catherino, 2013).

Over the years, several animal models have been developed and are now available for investigating the etiology and possible therapies for fibroids, although each model has its own limitations. Therefore, one single animal model may not be applicable for all indications. The Eker rat model has similarities to the human disease and has been used extensively for studying fibroid development and growth (Cesen-Cummings

et al., 2003). Newer innovative models that utilize xenografts of human uterine leiomyoma tumors (Suo et al., 2009a, b; Tsuiji et al., 2010), or human fibroid tumor explants transfected with adenoviral vectors for β-galactosidase, adenoviral-vascular endothelial growth factor-A, adenoviral-cyclooxygenase-2 (Hassan et al., 2008) and implanted into immune-compromised mice are also being used to study the pathogenesis of fibroids. Significant strides have been made with *in vitro* models, such as human leiomyoma cells immortalized with telomerase (Carney et al., 2002; Chang et al., 2009; Halder et al., 2013a, b) that retain many of the hallmarks of the parental cell lines (Carney et al., 2002), in addition to the use of other human leiomyoma cell lines immortalized with human papilloma virus (HPV-16 E6) (Malik et al., 2008). Tumor-derived cell lines from the Eker rat model (Howe et al., 1995) have also been used in many *in vitro* fibroid studies (Tsuiji et al., 2011; Tanfin and Breuiller-Fouche, 2012).

Treatment of uterine fibroids

Surgical tools to treat women with uterine fibroids have expanded dramatically in the last decade. Currently, there are four therapies approved by the US Food and Drug Administration (FDA) for treatment of fibroids: (i) Lupron; (ii) embolic agents for uterine artery embolization; (iii) magnetic resonance imaging-guided focused ultrasound and (iv) robotic assisted surgery. Despite the advancement of minimally invasive surgical procedures, hysterectomy remains the mainstay of leiomyoma therapy.

Comparative data are lacking for surgical procedures such as hysterectomy and myomectomy, causing a serious dilemma for physicians when deciding upon the most effective therapy to recommend to patients. This is a particularly vital issue when reviewing clinical trials. Most of the randomized trials comparing uterine artery embolization with surgery took place outside of the USA. This arrangement excludes the distinctive aspect of diversity that would include racial and ethnic minorities unique to the USA. Assessing outcomes in large-scale observational and randomized trials is the core of determining efficacy. The 2007 Agency for Healthcare Research and Quality report on uterine leiomyomas included 106 studies to determine racial diversity in enrollment. Most studies did not report participation by race or ethnicity. Analyses of 22 of 25 studies where race was reported determined that African American representation was closer to 15% of all women in clinical trials. This percentage is an estimation of the proportion of African American women based on population demographics (Taran et al., 2010a, b). Notwithstanding the prevalence of uterine fibroids in the African American population, this population makes up a low percentage of all women in clinical trials.

Epidemiology, genetics and environment

Finding genes for uterine fibroids

Cytogenetic studies indicate that 60% of women diagnosed with fibroids have a normal karyotype (46, XX) while 40% have chromosomal abnormalities. Chromosomal abnormalities that have been associated with uterine leiomyoma development include trisomy 12, translocation involving chromosomes (t12; 14) (q14–q15; q23–q24), deletions on chromosome 7 (q22q32), 3q and 1p, and rearrangements of 6p21, 10q22 and 13q21–q22 (Hodge et al., 2012). The HMG gene, HGMA2, is often translocated to the der(14) in uterine leiomyomas. Fine mapping in the large Women's Genome Health Study which followed 25 000 women (Ridker et al., 2008), of which a subset were diagnosed with fibroids, discovered a peak signal on chromosome 17 that could be replicated in a cohort of Australian twins. It is now known that a haplogroup in chromosome 17 is likely to be important in predisposing women to uterine fibroid development (Moore et al., 2004).

Uterine fibroids and the exposure paradigm

In utero exposures may be associated with later onset of urologic and gynecologic diseases. In addition to *in utero* exposures, periconception and transgenerational exposures may play a role in disease development (Buck Louis and Sundaram, 2012). Examples of compounds where early or transgenerational exposures in experimental animal models are associated with later disease onset include diethylstilbestrol (DES) and bisphenol A (BPA). A review of the literature has shown that in animal models BPA and DES exposure have been associated with the development of fibroids (Newbold et al., 2002, 2007). Comparatively, human studies have shown that exposures to DES and phthalates can lead to fibroid development (D'Aloisio et al., 2010, 2012; Huang et al., 2010; Weuve et al., 2010). A proposed avenue of study has been defined as the 'exposome', which takes into account the exposures over a lifetime (Buck Louis and Sundaram, 2012). It was recommended that cumulative exposures, those defined as 'inherited' and 'acquired', be assessed and considered when evaluating risk factors associated with development of fibroids and in understanding etiology of the disease.

Epidemiologic insights into ethnic differences in uterine fibroid burden

Several studies have demonstrated that there are ethnic differences in fibroid burden (Kjerulff et al., 1996; Marshall et al., 1997; Baird et al., 2003; Moore et al., 2008; Peddada et al., 2008; Weiss et al., 2009). Previous results from the NIEHS Uterine Fibroid Study and others demonstrated that the cumulative incidence of fibroids is greater for blacks than whites (Baird et al., 2003; Viswanathan et al., 2007). It was noted that while the rate of increase in fibroid incidence was similar for black and white women, the onset of the disease is ~10–15 years earlier in black females compared with white females (Borgfeldt and Andolf, 2000; Baird et al., 2003; Eskenazi et al., 2007; Bower et al., 2009; Laughlin et al., 2009, 2010). The NIEHS Fibroid Growth Study showed that there could be significant variation in fibroid growth rate over a 6-month period in black and white women (Peddada et al., 2008).

The risk factors that account for the ethnic differences in the natural history of fibroids are largely unknown. Vitamin D, which has been shown to be protective for breast cancer, is one proposed risk factor that is being evaluated for fibroids (Halder et al., 2011, 2012). In the NIEHS Uterine Fibroid Study, women were asked about sun exposure, which was then used as a surrogate for Vitamin D levels. When correlating women with defined sufficient Vitamin D levels to development of fibroids, a reduced incidence of ~30% was observed in both black and white women, which suggests that adequate Vitamin D levels may be important in preventing fibroids. In addition to Vitamin D levels, other environmental risk factors that may be associated with fibroid development include diet (Radin et al., 2010; Wise et al., 2011), stress (Vines et al., 2010), reproductive tract infections, endocrine disruptors (Laughlin et al., 2010) and prenatal/early life exposures (Newbold et al., 2002, 2007; Cook et al., 2005; D'Aloisio et al., 2010, 2012).

Risk factors for uterine fibroids in the Black Women's Health Study

The Black Women's Health Study (BWHS) is a US prospective cohort study of almost 60 000 black women aged 21–69 years at baseline in 1995 (Wise *et al.*, 2005a, b). An initial questionnaire was collected in 1995 and has been updated every 2 years since. The cohort retention rate has been >80%. Fibroids were diagnosed by ultrasound or surgery, and the cohort was restricted to premenopausal women at baseline. Published results showed that age, early menarche, years since last birth, being overweight, weight gain, polycystic ovary syndrome, alcohol and perceived racial discrimination were positively associated with fibroid development (Wise *et al.*, 2004, 2005a, b, 2007). An inverse association was found with parity, age at birth of first child, age at first oral contraceptive use, use of progestin-only injectable contraceptives and cigarette smoking.

More recently, evaluations were conducted to test the hypothesis that high levels of exposure to estrogen during fetal or childhood development may affect later responses of the uterus to sex hormones, thereby influencing fibroid development. By linking the data from BWHS with Massachusetts Department of Public Health Registry of Vital Records for participants born in the state, some early life factors could be validated. When birth characteristics were evaluated in women with fibroids in the BWHS, positive associations between soy formula consumption and pre-term birth were not observed (Wise *et al.*, 2010). These results are in contrast to the outcomes noted in the NIEHS Sister Study where low parental education (as a measure of childhood socioeconomic status) was positively associated with fibroid development in women younger than 35 years (D'Aloisio *et al.*, 2010). Small positive associations were also found for young maternal age and first-born status.

As for dietary risk factors and fibroids, in the BWHS a 30% reduced risk of fibroids was observed in women that consumed four or more dairy products per day compared with women that consumed less than one product per day. An inverse association was noted in relation to intake of bioavailable calcium (expressed as a ratio: calcium: phosphorous) (Wise *et al.*, 2010). Results evaluating the association of soy intake with fibroid development were limited due to low consumption rates and large confidence intervals. Fruit and vegetable intake also was associated with decreased risk, with the trend greater for fruits than vegetables. Lycopene and other carotenoids (e.g. beta-carotene) and vitamins (e.g. vitamin C) were not associated with decreased fibroid risk in the BWHS (Wise *et al.*, 2011).

Race and fibroid tumor burden

In a study of racial differences in incidence and growth trends of fibroids, the growth rate of fibroids was assessed in women with and without contraceptive therapy and steroid hormone levels were measured in urine and serum. Criteria for inclusion in the study were leiomyoma ≥10 mm on ultrasound and age between 18 and 45 years. The cohort study start date involved 180 patients, 90 cases and 90 controls, which were monitored by ultrasound (mostly transvaginal) every 6 months for 2 years. However, subject retention for both black and white women in the study was very low, but more so among the black women (Sweet *et al.*, 2008).

Analysis of the parameters evaluated at the initial visit for this study indicated significant differences in patient weight, BMI, uterine volume, number of fibroids, total fibroid volume, median fibroid volume and volume of largest fibroid. Comparison of growth trend values showed that uterine volume, number of fibroids, volume of largest fibroid and total fibroid volume remained consistently greater for African American women throughout the course of the study.

Pathogenesis: growth factors, cytokines, cell signaling and the extracellular matrix

Mediators and integrators of the molecular microenvironment in uterine fibroids

While the factors that initiate uterine fibroid formation are not known, excessive cell growth and phenotypic modifications due to myofibrotic transformation that enhance extracellular matrix deposition result in some of the characteristics of leiomyomas (Luo and Chegini, 2008). It is proposed that myofibrotic transformation to fibroids can occur as a result of mechanical injury (Rogers *et al.*, 2008; Norian *et al.*, 2012), inflammatory mediators or regulatory and signaling proteins including growth factors, cytokines, chemokines, angiogenic factors, hormones and extracellular proteinases [e.g. interleukin (IL)-10, matrix metalloproteinases (MMPs), tissue plasminogen activator inhibitor-1] (Chegini, 2010). Microarray analyses of fibroids have identified no single common gene but increased or decreased expression of several genes including ADAM metallopeptidase domain 17 (ADAM17), E2F1, growth arrest-specific 1 (GAS1), early growth response 3 (EGR3), endothelial cell-specific molecule 1 (ESM1), extracellular matrix protein 2 (ECM2), insulin-like growth factors (IGFs), runt-related transcription factor 3 (RUNX3), tat-binding protein 1[TBP-1]-interacting protein (TBPIP), thrombospondin 1 (THBS1), cystatin E/M (CST6), fibulin 5 (FBLN5) and collagen, type XVIII, alpha 1 (COL18A1) (Tsibris *et al.*, 2002; Ahn *et al.*, 2003; Luo *et al.*, 2007; Pan *et al.*, 2007) have been found. The role these genes play in the development of leiomyoma and their interactions with cellular components and the molecular microenvironment are the basis of ongoing research.

Growth factor signaling pathways in uterine fibroids

Reactive oxygen species (ROS) can lead to activation of extracellular signal-regulated kinases 1 and 2 (ERK 1/2) and increased leiomyoma cell proliferation. Specifically, the roles of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) in stimulating ROS formation show that stimulation of the tyrosine kinase receptors increases ROS formation through phosphorylation of a subunit of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Mesquita *et al.*, 2010) (see Table I). Treatment of leiomyoma cells with EGF or PDGF resulted in increased intracellular ROS levels that could be blocked by an NADPH oxidase inhibitor and interfered with EGF- and PDGF-induced cell proliferation (Mesquita *et al.*, 2010). It is proposed that NADPH oxidase-derived ROS for growth factor signaling pathways may be a potential target for the treatment of fibroids.

Another pathway thought to be important in the pathogenesis of uterine fibroids is the mammalian target of rapamycin (mTOR) signaling pathway that is activated by steroid hormones, growth factor receptors, other regulatory peptides and genomic and nongenomic signals (Makker *et al.*, 2012). mTOR is a kinase that is downstream of receptor tyrosine

Table I Proposed molecular mechanisms involved in the pathogenesis of uterine fibroids.

Molecular mechanism	Leiomyoma cell growth effect	Extracellular matrix (ECM)	Cell line or model used	Signaling/effectors
Growth factors				
Insulin-like growth factor (IGF)	Proliferation (+) (Yu *et al.*, 2010)		Fibroid tissue (Peng *et al.*, 2009; Yu *et al.*, 2010) Human uterine leiomyoma(UtLM) cells (Yu *et al.*, 2008)	Phospho (p)-AKT[a] activity (Peng *et al.*, 2009) RTK[b] and Shc[c]/Grb2[d]/MAPK[e] (Yu *et al.*, 2008, 2010)
Transforming growth factor- beta (TGF-β)			UtLM cells (Moore *et al.*, 2010; Di *et al.*, 2012)	activin A and Smad3 (Di *et al.*, 2012; Levens *et al.*, 2005) Smad 2 and 3; MAPK (Moore *et al.*, 2010; Ding *et al.*, 2004)
TGF-β3	Proliferation (+) (Lee and Nowak, 2001)	Collagen 1A1 (+), fibronectin 1 (+) and CTGF[f] (+) (Joseph *et al.*, 2010)	Human uterine leiomyoma and myometrial tissues/cells (Norian *et al.*, 2009; Tang *et al.*, 1997; Joseph *et al.*, 2010)	Dual effects of TGF-β3 on the growth of uterine cells (Tang *et al.*, 1997) Glycosaminoglycan (GAG)-rich versican variants (Norian *et al.*, 2009)
Epidermal growth factor (EGF)	Proliferation (+) (Mesquita *et al.* 2010)		Cultured leiomyoma smooth muscle cells (Mesquita *et al.*, 2010)	NADPH oxidase-derived reactive oxygen species (ROS) (Mesquita *et al.*, 2010)
Fibroblast growth factor (FGF)	(+) (Wolanska and Bankowski, 2006; Helmke *et al.*, 2011)	(+) (Wolanska and Bankowski, 2006	Human leiomyoma and myometrial tissues (Wolanska and Bankowski, 2006; Helmke *et al.*, 2011)	HMGA2 locus (Helmke *et al.*, 2011)
Platlet-derived growth factor (PDGF)	Proliferation (+) (Mesquita *et al.*, 2010)		Cultured leimyoma smooth muscle cells (Mesquita *et al.*, 2010)	NADPH oxidase-derived ROS (Mesquita *et al.*, 2010)
PDGF C	Proliferation (+) (Suo *et al.*, 2009a)	(+) (Suo *et al.*, 2009a)	Fibroid-derived uterine smooth muscle cells (Suo *et al.*, 2009a)	PDGF CC/PDGF receptor-alpha (Suo *et al.*, 2009a)
Vascular endothelial growth factor (VEGF)	(+) (Lewicka *et al.*, 2010)	(+) Angiogenesis (Gentry *et al.*, 2001)		
Cytokines				
Tumor necrosis factor-alpha (TNF-α)	(+) PCNA[h], cyclin D1, BCL-2[i] (Nair and Al-Hendy, 2011)		Human leiomyoma cells cocultured with SW872 cells (Nair and Al-Hendy, 2011)	
Interleukin (IL)-1				Polymorphism in IL-1 beta gene (Pietrowski *et al.*, 2009)
IL-8			Myometrial and leiomyoma tissue (Senturk *et al.*, 2001)	Growth of myometrium adjacent to leiomyomas (Senturk *et al.*, 2001)
Genomic/nongenomic				
MicroRNA (miR)-21			Myometrial and leiomyoma tissues from patients (Fitzgerald *et al.*, 2012b)	Cellular apoptosis/proliferation and translation (Marsh *et al.*, 2008; Zavadil *et al.*, 2010; Fitzgerald *et al.*, 2012)
miR-200c		TIMP2[j] and FBLN5[k] (Chuang *et al.*, 2012b)		Ovarian steroids and the VEGFA signaling pathway (Chuang *et al.*, 2012b)
Lethal 7 (Let-7) miR	Proliferation (-) (Peng *et al.*, 2008)			Target gene HMGA2[l] (Wang *et al.*, 2007; Peng *et al.*, 2008)
miR-93/106b			Human leiomyoma tissue and myometrium/cells (Chuang *et al.*, 2012a)	Tissue factor (F3), IL-8, CTGF and PAI-1[m] expression (Chuang *et al.*, 2012)
Trimethylated lysine 27 on histone 3 (H3K27me3)			Eker rats (Greathouse *et al.*, 2012)	Nongenomic PI3K[n]/AKT signaling (Greathouse *et al.*, 2012)
Mediator complex subunit 12 gene (MED12)			Human leiomyomas (Mäkinen *et al.*, 2011a, b, 2013a, b; Je *et al.*, 2012; McGuire *et al.*, 2012)	Mutations of the MED12 (Mäkinen *et al.*, 2011a, b, 2013a, b; Je *et al.*, 2012; McGuire *et al.*, 2012)

Continued

Table I *Continued*

Molecular mechanism	Leiomyoma cell growth effect	Extracellular matrix (ECM)	Cell line or model used	Signaling/effectors
Genome-wide DNA methylation			Human leiomyoma tissue (Navarro *et al.*, 2012)	Tumor suppressors KLF11[o], DLEC1[p] and KRT19[q] and verified promoter hypermethylation (Navarro *et al.*, 2012)
Whole-genome sequencing			Human leiomyoma tissue (Mehine *et al.*, 2013)	Translocations of HMGA2 and RAD51B[r] loci; aberrations at COL4A5[s]-COL4A6[t] locus (Mehine *et al.*, 2013)
Receptor tyrosine kinases (RTKs)			Uterine fibroid and myometrial tissues (Yu *et al.*, 2008, 2010; Jiang *et al.*, 2010)	MAPK (Yu *et al.*, 2010)

+refers to stimulation or increased expression, − refers to inhibition.
[a]Serine/threonine protein kinase Akt.
[b]Receptor tyrosine kinase.
[c]Src homology/collagen.
[d]Growth factor receptor-bound protein 2.
[e]Mitogen-activated protein kinase.
[f]Connective tissue growth factor.
[g]Matrix metalloproteinase.
[h]Proliferating cell nuclear antigen.
[i]B-cell lymphoma 2.
[j]Tissue inhibitors of metalloproteinases 2.
[k]Fibulin 5.
[l]High mobility group AT-hook 2.
[m]Plasminogen activator inhibitor-1.
[n]Phosphatidylinositide 3-kinase.
[o]Krüppel-like factor 11.
[p]Deleted in lung and esophageal cancer 1.
[q]Keratin 19.
[r]RAD51 homolog B.
[s]Collagen, Type IV, Alpha 5.
[t]Collagen, Type IV, Alpha 6.

kinases such as the insulin-like growth factor I (IGF-I) and EGF receptors. Growth factor receptors can also mediate their effect through the phosphatidylinositol-3 kinase (PI3K) pathway and mTor is downstream of PI3K signaling. mTOR is thought to play a role in the development of some cancers (Dhingra *et al.*, 2011) and transcriptional profiling has shown that mTOR is activated with a high frequency in human fibroids (Crabtree *et al.*, 2009). Animal studies showed that treatment with rapalogs, which target mTOR, reduced tumor incidence, multiplicity and size (Crabtree *et al.*, 2009). Recently, investigators have shown that in fibroids loss of the RE1-silencing transcription factor (REST), a silencer or transcriptional repressor, results in the expression of a G protein-coupled receptor, GPR10, which when activated promotes PI3K-AKt-mTOR/rapamycin pathways and cell proliferation (Varghese *et al.*, 2013).

Regulation of growth factor signaling pathways in uterine fibroids by endogenous and environmental factors

Estrogen and progesterone influence leiomyoma growth through regulating growth factors and cytokines and their signaling pathways (Flake *et al.*, 2003). Activation of steroid hormone receptors can have a myriad of effects including the regulation of growth factors and their receptor tyrosine kinases (RTKs) that can result in the activation of downstream effector proteins, such as mitogen-activated protein kinase (MAPK) p44/42 (ERK1/2) (Yu *et al.*, 2008, 2010) (see Tables I and II). Fibroids may also be targeted by environmental chemicals whose biological effects are mediated by hormone receptors (Di *et al.*, 2008). Genomic events and nongenomic signaling in fibroids can result in 'cross talk' between hormone and growth factor receptors with activation of the downstream effectors such as MAPK and phosphorylation of estrogen receptor alpha (ERα) at serine 118 in fibroids (Swartz *et al.*, 2005; Di *et al.*, 2008; Hermon *et al.*, 2008; Yu *et al.*, 2010, 2012).

Environmental estrogens derived from natural plant compounds (phytoestrogens), synthetic and industrial by-products (industrial estrogens) have been found to increase the incidence of uterine leiomyomas in animal models (Newbold *et al.*, 2002, 2007). Both *in vivo* and *in vitro* models have shown that the enhanced sensitivity of uterine leiomyomas to environmental estrogens can be modulated via ERα (Di *et al.*, 2008; Greathouse *et al.*, 2012). Genistein, a soy-derived phytoestrogen, is commonly consumed in the diet, and there is some concern as to the beneficial and/or adverse physiological effects of this compound. It has been found that human UtLM cells proliferate in response to a low dose of genistein treatment, whereas high doses are inhibitory (Moore

Table II Hormonal regulation and hormone receptor interactions in uterine fibroids.

Hormonal regulator	Leiomyoma cell growth	Extracellular matrix (ECM)	Cell line or model	Effector /signaling pathway
Estrogens				
17-beta estradiol	Cell cycle progression genes (+) (Yu *et al.*, 2012), proliferation genes (+) (Yu *et al.*, 2012)	COL1A1 (+) (Yu *et al.*, 2012)	Human UtLM cells (Yu *et al.*, 2012)	ERα[a]/IGF-IR[b]/MAPK[c]p44/42 pathways (Yu *et al.*, 2012)
Diethylstilbestrol (DES)	PCNA[d] (+) (Newbold *et al.*, 2002)		CD-1 mice (Newbold *et al.*, 2002)	TGF-α[e] and EGF-R[f] (Newbold *et al.*, 2002) Prenatal estrogen exposures (Newbold *et al.*, 2002)
Genistein	Proliferation (+) (Hunter *et al.*, 1999; Moore *et al.*, 2007; Di *et al.*, 2008) PCNA (+) (Moore *et al.*, 2007), apoptosis (+) (Moore *et al.*, 2007)		ELT[g]3 and ELT6 rat uterine leiomyoma cell lines (Hunter *et al.*, 1999); Human uterine leiomyoma (UtLM) cells (Moore *et al.*, 2007; Di *et al.* 2008)	Mimics endogenous estrogens (Hunter *et al.*, 1999) Lower concentrations elicit proliferation, higher concentrations inhibit proliferation (Moore *et al.*, 2007) ERK[h]/MAPK pathway (Di *et al.*, 2008)
Estrogen Receptors				
Estrogen receptor alpha (ERα)	Proliferation (+) (Hunter *et al.*, 1999; Glace *et al.*, 2009; Di *et al.*, 2008)		ELT3 cells (Hunter *et al.*, 1999; Glace *et al.*, 2009) UtLM cells (Di *et al.*, 2008)	Stromal cell-derived factor-1 (SDF-1/Cxcl12) (Glace *et al.*, 2009) MAPK (Di *et al.*, 2008)
ERα phospho-serine118	PCNA (+) (Hermon *et al.*, 2008)		Human leiomyoma and myometrial tissues (Hermon *et al.*, 2008)	MAPK activation (Hermon *et al.*, 2008)
ER-beta (ERβ)			Leiomyoma and myometrial tissue samples (Grings *et al.*, 2012; Bakas *et al.*, 2008)	Overexpression of ERα, ERβ are not the cause of tumor growth (Grings *et al.*, 2012) ERα/ERβ expression ratio (Bakas *et al.*, 2008)
G protein-coupled receptor 10 (GPR10)	Proliferation (+) (Varghese *et al.*, 2013)		Human leiomyoma cells and tissue/mice (Varghese *et al.*, 2013)	GPR10 when activated promotes PI3K[i]-Akt[j]-mTOR[k] pathways (Varghese *et al.*, 2013)
Progesterone	Proliferation (+) (Ishikawa *et al.*, 2010)		Leiomyoma xenografts (Ishikawa *et al.*, 2010) ELT3 (Glace *et al.*, 2009)	L-type amino acid transporter 2 and 4F2hc (Luo *et al.*, 2009)
	Cell numbers (+) (Hoekstra *et al.*, 2009)		Progestin/cultured uterine leiomyoma cells (Hoekstra *et al.*, 2009)	Phospho(Ser 256)-FOXO1, phosphoglycogen synthase kinase-3b, and AKT pathway (Hoekstra *et al.*, 2009)
	PCNA (+) (Maruo *et al.*, 2000)			EGF and Bcl-2[l] (Maruo *et al.*, 2000)
Progesterone receptor			Human uterine leiomyoma smooth muscle cells (Yin *et al.*, 2010; Yin *et al.*, 2012)	KLF11[m] (Kim and Sefton, 2012 Yin *et al.*, 2010)
Selective estrogen receptor modulaors (SERMs)	Tamoxifen (−), raloxifene (−) (Walker *et al.*, 2000; Walker, 2002)	Reduce tumor size (Walker *et al.*, 2000)	Eker rats (Walker *et al.*, 2000) Leiomyoma-derived ELT cell lines (Fuchs-Young *et al.*, 1996)	Reduce tumor incidence (Walker, 2002) High affinity to ERα and β (Hummel *et al.*, 2005) Tissue-specific estrogen agonist or antagonist effects (Cook and Walker, 2004)
Selective progesterone receptor modulators (SPRMs)	CP8947 (−) (Catherino *et al.*, 2010) CDB-2914 (−) (Yoshida *et al.*, 2010) CDB4124 (−) (Luo *et al.*, 2010)		Immortalized human leiomyoma and myometrial cells (Catherino *et al.*, 2010)	Apoptosis (−) (Roeder *et al.*, 2011) KLF11 (Luo *et al.*, 2010)

Continued

Table II *Continued*

Hormonal regulator	Leiomyoma cell growth	Extracellular matrix (ECM)	Cell line or model	Effector /signaling pathway
Glucocorticoid	Proliferation (−) (Whirledge *et al.*, 2012)		Immortalized human uterine leiomyoma cells (Whirledge *et al.*, 2012)	Reduces S-phase cells (Whirledge *et al.*, 2012)
Gonadotropin-releasing hormone (GnRH)			Leiomyoma and myometrial tissues/cells (Parker, 2007; McCarthy-Keith *et al.*, 2011)	GnRH agonist decreases NFAT5[n] expression (McCarthy-Keith *et al.*, 2011)
Vitamin D	Proliferation (−) (Sharan *et al.*, 2011)	(−) Collagen type 1, and fibronectin (Halder *et al.*, 2013b)	Immortalized human uterine leiomyoma cells (Halder *et al.*, 2011) Eker rats (Halder *et al.*, 2012)	Catechol-*O*-methyltransferase (COMT) protein (Sharan *et al.*, 2011)
Retinoic acid (RA)	Proliferation (−) (Gilden *et al.*, 2012)	(−) (Gilden *et al.*, 2012)	Immortalized leiomyoma cells (Gilden *et al.*, 2012)	RA and PI3K/Akt pathways (Ben-Sasson *et al.*, 2011)

+ refers to stimulation or increased expression, − refers to inhibition or decreased expression.
[a]Estrogen receptor alpha.
[b]Insulin-like growth factor I receptor.
[c]Mitogen-activated protein kinase.
[d]Proliferating cell nuclear antigen.
[e]Transforming growth factor-alpha.
[f]Epidermal growth factor receptor.
[g]Eker rat leiomyomas.
[h]Extracellular signal-regulated kinase.
[i]Phosphatidylinositide 3-kinase.
[j]Serine/threonine protein kinase Akt.
[k]*Mammalian target of rapamycin (mTOR).*
[l]B-cell lymphoma 2.
[m]Krüppel-like factor 11.
[n]Nuclear factors of activated T cells.

et *al.*, 2007; Di *et al.*, 2008, 2012). Growth factor receptor pathways, such as the RTK, IGF-I receptor, are important in uterine leiomyoma cell regulation and growth and could represent possible targets for fibroid treatment (Yu *et al.*, 2008, 2012). By identifying environmental risk factors and delineating molecular signaling mechanisms and novel proteins activated in fibroids during their growth, it is possible to develop preventive measures and nonsurgical treatment strategies that target unique molecules important in fibroids.

Hormonal regulation and hormone receptor interactions

Studies suggest that estrogen and progesterone act in combination to stimulate myoma growth (Shimomura *et al.*, 1998; Maruo *et al.*, 2004) (see Table II). Specifically, progesterone stimulates EGF production while estrogen stimulates EGF receptor production. Since myoma growth is a balance between cell proliferation and apoptosis, the effects of progesterone on apoptosis have been shown to increase the expression of the anti-apoptotic protein Bcl-2 (B-cell lymphoma 2) in cultured leiomyoma cells (Matsuo *et al.*, 1997; Luo *et al.*, 2005); however, estradiol has no effect. Additionally, progesterone has been found to inhibit the expression of tumor necrosis factor-alpha (TNF-α), an apoptosis-inducing factor and IGF-I expression in cultured leiomyoma cells (Kurachi *et al.*, 2001).

In vitro studies have shown that progesterone increases cellular proliferation of uterine leiomyoma cells (Shimomura *et al.*, 1998; Ishikawa *et al.*, 2010). Progesterone receptors are proposed to produce effects through interaction with progesterone elements that can modulate transcription of genes or through interaction with membrane signaling components to modulate second messenger systems (Yin *et al.*, 2007; Kim *et al.*, 2009; Kim and Sefton, 2012) (see Table II).

While SERMs have questionable efficacy (Deng *et al.*, 2012), aromatase and selective progesterone receptor modulators (SPRMs) have been more encouraging. Two SPRMs, CP8863 and CP8947, both have been found to increase alkaline phosphatase activity in leiomyoma cells and inhibit cell growth (Catherino *et al.*, 2010) (see Table II). SPRMs can induce leiomyoma inhibition in a cell-specific manner through decreased cellular proliferation and increased apoptosis in *in vitro* studies (Yoshida *et al.*, 2010). Some SPRMs have been shown to decrease the expression of vascular endothelial growth factor (VEGF)-A, VEGF receptor (VEGFR)-1, VEGFR-2, adrenomedullin (ADM) and ADM receptor (ADMR) selectively in uterine leiomyoma cells when compared with myometrial cells (Xu *et al.*, 2006), and suggests that progesterone receptor modulators may selectively suppress angiogensis in leiomyomas. Also, SPRMS have been reported to cause enhanced expression of extracellular matrix (ECM) degradation factors (extracellular matrix metalloproteinase inducer [EMMPRIN] and matrix metalloproteinase [MMP]-1 and MMP-2) in a cell-type specific manner (Xu *et al.*, 2008). It is proposed that the use of a progesterone receptor modulator IUD could be utilized

in the management of fibroid growth and menorrhagia associated with fibroids (Maruo et al., 2001).

Pilot trials have found that administration of aromatase inhibitors decreases fibroid size (Shozu et al., 2003, 2004; Varelas et al., 2007; Gurates et al., 2008; Parsanezhad et al., 2010). Aromatase inhibitors have also been reported to interfere with estrogen synthesis *in situ* in fibroids, thereby reducing estrogen levels within a fibroid tumor (Shozu et al., 2004). Recent studies have shown that aromatase levels are significantly higher in leiomyomas from African American, Caucasian American and Japanese women when compared with myometrial tissues, and aromatase levels were much higher in leiomyomas from African American women when compared with the other two racial groups of women (Ishikawa et al., 2009). These results suggest that biological differences can be identified for better targeting of medications and that differences do exist between populations.

GnRH agonists or antagonists act at the pituitary level resulting in a decrease in gonadotrophin production, which produces a hypoestrogenic state. While these compounds ultimately decrease leiomyoma size, they do produce numerous side effects. Microarray analyses have shown that specific collagen isoforms and veriscan, which contains high levels of proteoglycans which absorb water, are overexpressed in leiomyoma cells and addition of GnRH analogs can reduce their expression (Malik and Catherino, 2007; Parker, 2007; Britten et al., 2012) (see Table II). Conversely, while MMPs are not highly overexpressed in leiomyoma cells, addition of GnRH analogs increases their expression. It has been proposed that the therapeutic effects of GnRH analogs are through direct effects on leiomyomas (e.g. decreased veriscan production leads to decreased water retention in the fibroid tumors which decreases the size). Therefore, studies are progressing towards examining whether compounds, such as GnRH analogs, which appear to have direct effects on fibroid tumors could be applied locally (e.g. IUD) rather than through systemic administration, thus providing safer, targeted treatment options without affecting the hypothalamic–pituitary–ovarian axis.

Developing new model systems

Green tea extract for the treatment of uterine fibroids

Catechol-*O*-methyltranferase (COMT) is involved in metabolizing estrogens, by methylation of the catechol-estrogens 2- and 4-hydroxyestradiol. The catechol estrogens are anti-estrogenic, but conversion to the methoxy counterparts increases the estrogenic milieu. Evaluation of leiomyoma tissues showed that COMT RNA and protein levels are increased in leiomyomas when compared with myometrial tissues (Al-Hendy and Salama, 2006). Genetic evaluation shows the presence of a single nucleotide polymorphism at site 158, a Val/Met site, in the COMT gene. In a Val/Val genotype, high enzyme activity is observed. When compared with the Met/Met activity, the Val/Val genotype is approximately four times greater. When the distribution of these two genotypes was evaluated in different ethnic groups, it was consistently shown that the Val/Val genotype was associated with increased risk of fibroids. In general, the Val/Val genotype was highly prevalent in the African American women. Therefore, one potential treatment avenue for fibroids is inhibition of COMT (Hassan et al., 2011) (see Table III). To date, some COMT drugs are available for use in the treatment of Parkinson's disease.

Epigallocatechin gallate (EGCG), an extract of the green tea, is a COMT inhibitor. *In vitro*, EGCG dose dependently decreases PCNA labeling and increases apoptosis factors, such as bcl-2 (Zhang et al., 2010a, b). Cell cycle studies show that EGCG increases the percentage of cells in G2/M phase. *In vivo* studies in nude mice injected s.c. with Eker rat tumor-derived uterine leiomyoma (ELT)-3 cells show that EGCG treatment decreased tumor size when compared with water. EGCG also arrested growth and decreased uterine leiomyoma size in Eker rats as early as 2 weeks after treatment initiation. Clinical trials have been initiated to evaluate the effects of EGCG in women with symptomatic uterine leiomyomas (Roshdy et al., 2013).

Uterine β-catenin mouse model for uterine fibroids

β-catenin plays two roles in the cell, one role is at the adherens junctions, the other is as a downstream effector of Wnt signaling (Tanwar et al., 2009). Mice with a mesenchymal deletion of β-catenin at 6 weeks of age develop an increase in adipocytes on the surface of the uterus, and after 10 weeks post-natal, there is little myometrium remaining. It appears that β-catenin levels are diminished in the uterus and the muscle cells convert to adipocytes.

In an *in vivo* mouse model that accumulates β-catenin in the cell nucleus there is an increase in the myometrium when compared with controls (Tanwar et al., 2009) (see Table III). Examination of these mice after a few weeks showed large uterine protrusions that upon histologic examination are similar (e.g. progesterone receptor expression) to leiomyomas present in women. The lesions also have high levels of mTOR, and a downstream target of mTOR, phospho-S6-kinase, indicating increased activity of mTOR is present in these mutant mice. The control of mTOR is by the tuberous sclerosis complex, Tsc1 and Tsc2. In the Eker rat model, Tsc2 is mutated. It is proposed that the constitutive action of β-catenin, which induces mTOR expression and activity, mimics mutations in Tsc1 and Tsc2. It also has been shown that in rats and humans, the downstream targets of mTOR are up-regulated in leiomyomas (Crabtree et al., 2009). Therefore, the constitutive activation of β-catenin mimics the end-points observed in humans.

Mouse xenograft model for human uterine fibroids

A xenograft model has been developed to evaluate the cellular mechanisms underlying fibroid growth regulation by estrogen and progesterone (Ishikawa et al., 2010) (see Table III). In this model, human leiomyoma tumors are grafted subrenally. The xenografts retain histological characteristics of the original tumor and grow in response to estrogen and progesterone. Removal of the steroid hormones leads to decreased tumor size, which is associated with reduced cell size. Studies have shown that progesterone receptor expression in human leiomyoma is dependent on the presence of estrogen (Englund et al., 1998), and based on this observation it was proposed that progesterone's action requires estrogen.

Advances in clinical and translational research

Opportunities and challenges in identification of new treatment modalities for uterine fibroid therapy

Disease and pharmacodynamic models that are required to validate targets and screen profile novel drug candidates should be clinically relevant and feasible. To assess treatment options, a toolbox of *in vitro* models is needed to assess efficacy. However, there are a limited number of *in vitro* models that can be used for this purpose. The limited number of models available reflects their complexity, as well as the underlying pathophysiological mechanisms that remains incompletely understood. There are currently two treatment paradigms: hormonal and nonhormonal. Most treatments have favored the hormonal paradigm; however, recently anti-fibrotic, anti-proliferation, anti-angiogenesis, anti-hypertrophy and anti-inflammatory small drug compounds are being evaluated as potential therapeutics. Therefore, additional efforts are needed to develop *in vitro* models to assess therapeutic options for uterine fibroids.

Target identification is the first step in developing an appropriate therapeutic agent for treatment of uterine fibroids. Sources used for target identification include results from microarray analysis, protein expression studies, the literature and personal communications. *In vitro* and *in vivo* models are used to validate the target, for pharmacodynamic studies and safety assessment. Prior to development of a lead compound, specific properties must be developed or evaluated, and this includes patentability, safety, selectivity, production costs and formulation.

Pregnancy loss and uterine fibroids

To address the challenges of studying early pregnancy loss, the Right from the Start (RFTS) study (Promislow *et al.*, 2004) was developed to investigate the association of uterine fibroids with adverse pregnancy events in a nonclinical, prospective cohort with uterine fibroids. The RFTS study evaluated women with uterine fibroids to determine whether they were at higher risk of spontaneous abortion or preterm birth; whether size or location of fibroid was associated with the risk and whether there was a delayed time to conception as a potential surrogate for impaired fertility. Within the study population, the risk of spontaneous abortion increased with age and BMI. Small uterine fibroids (<3 cm) and those located submucosally increased the risk of spontaneous abortions slightly. In comparison, larger fibroids and those located subserously or intramurally did not increase the risk of pregnancy loss. The presence of uterine fibroids did not increase the risk of preterm birth in the study population. Additionally, subserous and submucosal fibroids slightly increased the risk of pre-term birth. The mean time to conception was similar between women with and without uterine fibroids. African American women were more likely to have a uterine fibroid, to have more than one fibroid, and to have a larger uterine fibroid than their Caucasian counterparts.

Based on this population-based sample, it was concluded that the presence of uterine fibroids is not independently associated with delays in conception or increased risk of miscarriage or preterm birth. While there was no evidence that larger or subserous uterine fibroids have a profound influence on risk, smaller intramural and submucous uterine fibroids may require additional evaluation. Based on these findings, it is likely that the majority of women with uterine fibroids will have normal pregnancy outcomes. To confirm these results and given the limited research on the effects of uterine fibroids on pregnancy outcomes, additional research is warranted (Laughlin *et al.*, 2009).

Stem cell origin of fibroids and effects of resveratrol

Collagen is the predominant extracellular matrix component expressed in fibroids, and its expression is influenced by cytokines, estradiol and growth factors (Flake *et al.*, 2003; Walker and Stewart, 2005; Ciarmela *et al.*, 2011). Studies have shown that within the uterus there is a SP of stem cells that is multipotent (Flake *et al.*, 2003; Walker and Stewart, 2005; Ono *et al.*, 2007, 2012; Ciarmela *et al.*, 2011; Mas *et al.*, 2012). These stem cells can spontaneously differentiate into muscle cells *in vitro* and can differentiate into other tissues, including reconstituting into myometrial cells *in vivo*. A study was undertaken to determine whether there were stem cells present in uterine leiomyomas and myometrium. Samples were obtained from hysterectomies for routine indications. Results showed that myometrium from uterine fibroids contain more stem cells than myometrium from a normal uterus suggesting that an increased density of stem cells may be associated with uterine fibroids. Mas *et al.* (2012) have further characterized leiomyoma stem cells or SP cell lines that have a normal karyotype and express genes of undifferentiation, such as OCT-4 (octamer-binding transcription factor 4), NANOG, DNMT3B [DNA (cytosine-5)-methyltransferase 3 beta] and GDF3 (growth differentiation factor-3). These cells also have markers of mesenchymal differentiation and establish tissue populations in ~8 weeks that histologically resemble human leiomyomas when grown in NOD-SCID mice in the presence of E_2 and P_4 (Mas *et al.*, 2012). Further research is needed to investigate the role of stem cells in the pathophysiology of uterine leiomyomas.

Resveratrol, a dietary phytoalexin and a component of red wine, has been shown in studies of uterine leiomyoma cells to increase formation of apoptotic cells, decrease cell viability and number, and increase the percentage of cells arrested in the G1 phase in a dose-dependent manner by preventing cell cycle progression from the G1 to S phase (Catherino *et al.*, 2011). Studies also revealed that resveratrol possesses a potent antifibrogenic effect by reducing the mRNA production and protein expression of collagen types III and I in a dose-dependent manner *in vitro* (Catherino *et al.*, 2011). Resveratrol also altered the TGF-β/Smad pathway, and this impairment contributed to a reduction in collagen production. This research suggests that resveratrol might prove to be an effective and novel preventive agent due to its ability to reduce collagen production, induce apoptosis and reduce cellular proliferation in uterine leiomyoma cells.

Clinical advances in the treatment of uterine fibroids

Women wishing to preserve their fertility are opting for uterine preservation and minimally invasive approaches. Myomectomy has been the mainstay for women who wish to preserve their fertility. To find a better way to manage uterine fibroids, several new minimally invasive surgical techniques are available or in development. Robotic methods have been used in recent years in an attempt to provide a less invasive

surgical option for women. Generally, limitations of robotic surgery include lack of haptic feedback, larger port sizes and increased operating time during the steep learning curve. There are limited studies on the comparison of robotic procedures with laparoscopic procedures for hysterectomy for benign disease. In one study of surgical outcomes in a community practice, a comparison of 100 laparoscopic hysterectomies and 100 robotic hysterectomies (Payne and Dauterive, 2008) showed that operating times, estimated blood loss, length of hospital stay and conversion to open surgery were lower for robotic hysterectomy than for laparoscopic surgery. However, the cost of robotic hysterectomy remains a significant limiting factor (Pasic *et al*., 2010; Sarlos *et al*., 2010).

One retrospective study compared 15 robotic myomectomies with 35 laparoscopic myomectomies (Nezhat *et al*., 2009). The robotic technique removed fibroids that were fewer, smaller and lighter when compared with those removed using a laparoscopic technique, similar to what had been reported in other studies (Advincula *et al*., 2007; Bedient *et al*., 2009), although the difference was not significant. When compared with abdominal myomectomy, robotic myomectomy had less blood loss and shorter hospital stays, but longer operative time (Advincula *et al*., 2007; Sarlos *et al*., 2010). Robotic surgery and laparoscopic surgery continue to advance rapidly with trends toward single port access surgery, image guidance and networking with other consoles during surgery. More data are needed on long-term clinical outcomes, especially as it pertains to fertility and pregnancy outcomes, particularly for robotic myomectomy.

When laparoendoscopic single site surgery was compared with traditional laparoscopic procedures, a retrospective comparative analysis showed that recovery time was better and immediate post-operative pain was lower with single site surgery (Yim *et al*., 2010). Laparoscopic uterine artery ligation/occlusion under Doppler guidance is a surgical procedure that results in infarction and necrosis of uterine fibroids. The procedure appears to have no impact on ovarian reserve, although there are no data on fertility or pregnancy post-operatively (Brill, 2009; Qu *et al*., 2010). A randomized controlled trial (RCT) comparing laparoscopic uterine artery ligation/occlusion with uterine artery embolization demonstrated a greater decrease in the uterine size, more complete devascularization of uterine fibroids and lower symptom recurrence rate with uterine artery embolization (Hald *et al*., 2009). On the farthest frontier is natural orifice translumenal endoscopic surgery (NOTES). The procedure is performed on pelvic organs via a single-puncture transgastric approach, thereby eliminating all incisions and making the pelvic organs accessible from the gastric approach. Animal studies have shown some promise (Mintz *et al*., 2007).

Clinical management and therapeutic options

Medical treatment options for women with symptomatic uterine fibroids

RCTs of medical treatments were few, and many do not emphasize symptoms (Makarainen and Ylikorkala, 1986; Coutinho and Goncalves, 1989; Friedman *et al*., 1989). Current options are high-dose progestins, oral contraceptive pills (OCPS), nonsteroidal anti-inflammatory drugs (NSAIDs), tranexamic acid and GnRH agonists (GnRHa) (see Table IV). For short-term management of symptoms, such as heavy uterine bleeding, progestins, NSAIDs and OCPs have been used off-label; however, they do not reduce fibroid volume (Stewart, 2001). GnRHa successfully reduce fibroid volume and induce amenorrhea, but may only be used preoperatively due to side effects of estrogen deprivation. No add-back therapy for GnRHa has been effective. For the treatment of women with cyclic heavy menstrual bleeding with or without uterine fibroids, tranexamic acid, a plasminogen inhibitor, has been recently approved by the FDA (Lukes *et al*., 2010).

Progesterone receptor agonists, SPRMs and aromatase inhibitors have been investigated, and targeting of progesterone receptors appears to be an attractive approach for symptomatic fibroid treatment (Chwalisz *et al*., 2005). Both SPRMS (e.g. J867; asoprisnil) and progesterone receptor agonists (e.g. mifepristone and ulipristal acetate) induce amenorrhea and reduced fibroid volume; however, they are associated with cystic changes of the endometrium of unknown clinical significance (Mutter *et al*., 2008; Spitz, 2009; Williams *et al*., 2012). Recently, letrozole and other aromatase inhibitors have been evaluated for their use as a possible monotherapy for uterine fibroids (Parsanezhad *et al*., 2010). Although they decrease fibroid volume, they have minimal effect on uterine bleeding, and may be associated with ovarian cyst formation.

As an alternative to surgery, an effective long-term medical treatment for uterine fibroids should reduce heavy uterine bleeding as well as fibroid/uterine volume without excessive side effects. This goal has not been achieved, and unfortunately, current treatments only reduce symptoms temporarily.

Uterine fibroid surgical therapy

RCTs and reviews from 2005 to 2010 were evaluated on the indications, options and complications of uterine fibroid surgical therapy. For women who want to maintain fertility, myomectomy remains the gold standard (see Tables IV–IX). Data on myomectomies should include preoperative evaluation of the uterine cavity. The data suggest that surgical options should be individualized based on desires for future fertility, location and size of fibroids, and surgical risks. Further research on prevention of infection and post-operative adhesions is needed.

With technical advances, there has concurrently been an increase in minimally invasive procedures. Magnetic resonance guided focused ultrasound using the *ExAblate 2000*® is the only device currently FDA approved to integrate real-time advanced imaging feedback into a treatment for leiomyoma, targeting and treating each fibroid individually (Tempany *et al*., 2003). Normal tissue injury is minimized, but some fibroids may not be treated. Volume reductions similar to that seen after uterine artery embolization (UAE) and GnRHa treatment were observed (see Tables IV and V). No RCTs on focused ultrasound surgery (FUS) have been published. However, the FIRSTT (Fibroid Interventions: Reducing Symptoms Today and Tomorrow) Study, a randomized trial comparing UAE and FUS in a racially diverse cohort, is underway. The FIRSTT Study will elucidate clinical outcomes and will include an assessment of economics and ovarian reserve (Bouwsma *et al*., 2011).

Another minimally invasive alternative to traditional surgery for fibroids is UAE, also referred to as uterine fibroid embolization (UFE). Current knowledge on UAE was summarized by describing the results of the Embolization versus Hysterectomy (EMMY) and the Randomized Study of Embolization and Surgical Treatment for Uterine Fibroids

Table III Model system/concepts for studying uterine fibroids.

Model system	Tumor/cell characteristics	Time to tumor development	Model characteristics	Application
***In vitro* models**				
Human uterine leiomyoma cells	ERα[a] (+), ERβ[b] (+) (Di *et al.*, 2008) Receptor tyrosine kinases (+) (Yu *et al.*, 2010)		Human cell lines	Molecular mechanisms of environmental estrogens (Di *et al.*, 2008; Yu *et al.*, 2008, 2010; Gao *et al.*, 2010; Gao *et al.*, 2012)
Htert-human uterine leiomyoma cells	ERα and PR[c] (+) (Carney *et al.*, 2002) Glucocoticoid receptor (+) (Whirledge *et al.*, 2012)		Human telomerase immortalized leiomyoma and myometrial cell lines (Carney *et al.*, 2002)	Molecular mechanisms of fibroid cell growth and inhibition (Whirledge *et al.*, 2012)
3D *in vitro* model			Immortalized cells of patient-matched myometrium and leiomyoma (Malik and Catherino, 2012)	Assessing the mechanism of aberrant ECM formation, effectiveness of potential therapies (Malik and Catherino, 2012)
Eker leiomyoma tumor-derived (ELT) 3 cells		4–8 weeks in nude mice (Zhang *et al.*, 2010b); approximately 4 weeks in nude mice (Salama *et al.*, 2007)	Eker rat leiomyoma tumor cells defect in Tsc2[e] tumor suppressor gene (Howe *et al.*, 1995)	Green Tea Therapy (Zhang *et al.*, 2010b) Cdk[d]4 (+) (Zhang *et al.*, 2010a, b)
***In vivo* models**				
Eker rat	High frequency (~65% or >) in females (Everitt *et al.*, 1995)	12 months or > (Everitt *et al.*, 1995)	Defect in the Tsc2 tumor suppressor gene (Crabtree *et al.* 2009)	Assessing COMT[f] inhibitor (Hassan *et al.*, 2011) Rapamycin pathway (Crabtree *et al.*, 2009)
Guinea pig	8.4% incidence (Field *et al.*, 1989)	47.6 months (Field *et al.*, 1989)		Spontaneous reproductive tract tumor in aged female guinea pigs (Field *et al.*, 1989)
Miniature pet pigs	<1 cm in diameter to as large as 35 × 30 × 40 cm (Ilha *et al.*, 2010)	4 months to 19 years (Ilha *et al.*, 2010)	ER and PR positive (Ilha *et al.*, 2010)	Aging was associated with the development of uterine lesions (Ilha *et al.*, 2010)
Pot bellied pig	Tumors ranged from microscopic to 45 kg, multiple (Mozzachio *et al.*, 2004)	Possibly 5 years and > (Mozzachio *et al.*, 2004)	Smooth muscle actin (+) and has collagenous component (Mozzachio *et al.*, 2004)	Valuable animal model for studying human fibroids (Mozzachio *et al.*, 2004)
Japanese quail		350 days (Sahin *et al.*, 2009b) 315 days (Sahin *et al.*, 2009a)		Dietary supplementation of genistein (Sahin *et al.*, 2009a) Dietary zinc picolinate supplementation (Sahin *et al.*, 2009b)
CD-1 mice	PCNA[g] (+), TGF-α[h](+) EGF-R[i] (+) (Newbold *et al.*, 2002)	13 or 17 months (Newbold *et al.*, 2002)		Pre- and perinatal exposures to diethylstibesterol (DES) (Newbold *et al.*, 2002)
Calcium-binding protein (CaBP)9 K/Tag[j] transgenic mice		2.5–3 (shortest) months or >6 months (longest) (Romagnolo *et al.*, 1996)	9 K/-117-Tag and 9 K/-1011-Tag (Romagnolo *et al.*, 1996)	Therapeutic approaches to fibroids (Romagnolo *et al.*, 1996)
Transplanted fibroid cells in mice (xenografts)	Bioluminescence (BL)-based whole animal imaging (Suo *et al.*, 2009b); ERα (+) (Ishikawa *et al.*, 2010)	BL signal peaked at 28 days (Suo *et al.*, 2009b) 10 weeks (Ishikawa *et al.*, 2010)	Implantation of 17β-estradiol-releasing pellets in the recipient mice, fibroid tissues have higher engraftment potential (Suo *et al.*, 2009b)	Freshly dissociated fibroid cells can generate stable xenografts in subcutaneous Matrigel implants (Suo *et al.*, 2009b) Progesterone dependent (Ishikawa *et al.*, 2010)

Continued

Table III *Continued*

Model system	Tumor/cell characteristics	Time to tumor development	Model characteristics	Application
Immunodeficient (NOD/SCID/gammac-null: NOG) mice	Ki-67 proliferation marker (+), (Tsuiji *et al.*, 2010)	4 or 8 weeks (Tsuiji *et al.*, 2010)		Development of novel therapeutic strategies (Tsuiji *et al.*, 2010)
Adenovirus-enhanced human fibroid explants	Ki-67 proliferation marker (+), ER (+), PR (+) (Hassan *et al.*, 2008)	30 days post-implantation (Hassan *et al.*, 2008)	Adenoviral- cyclooxygenase-2 and adenoviral-VEGF-A[k] transfection in immunodeficient mice (Hassan *et al.*, 2008)	A novel model for human uterine leiomyoma (Hassan *et al.*, 2008)
Beta-catenin mice	TGFβ3[l] (+) (Tanwar *et al.*, 2009)	4 weeks (Tanwar *et al.*, 2009)	Activated beta-catenin in uterine mesenchyme by Cre recombinase knocked into the Müllerian-inhibiting substance type II receptor promoter (Tanwar *et al.*, 2009)	WNT[m]/beta-catenin signaling (Tanwar *et al.*, 2009)
Stem cells				
Human uterine leiomyomas	CD[n]90+/− (Chang *et al.*, 2010)		Stem/reservoir cell characteristics (Chang *et al.*, 2010)	Separate origins and/or divergent transformation pathways for ULM[o] and ULMS[p] (Danielson *et al.*, 2010)
Leiomyoma-derived side population (LMSP) stem/reservoir cells	Comprise ~1% of all leiomyoma- and 2% of myometrium-derived cells (Ono *et al.*, 2012)		mRNA levels for ERα and PR are minimally detectable in LMSP cells (Ono *et al.*, 2012)	Stem/reservoir cell characteristics, are necessary for *in vivo* growth of leiomyoma xenograft tumors (Ono *et al.*, 2012)

[a]Estrogen receptor alpha.
[b]Estrogen receptor beta.
[c]Progesterone receptor.
[d]Cyclin-dependent kinases.
eTuberous sclerosis complex 2.
[f]Catechol-*O*-methyltransferase.
[g]Proliferating cell nuclear antigen.
[h]Transfroming growth factor-alpha.
[i]Epidermal growth factor receptor.
[j]Simian virus 40 large T Antigen.
[k]Vascular endothelial growth factor-A.
[l]Transfroming growth factor beta 3.
[m]Wingless-type MMTV integration site family.
[n]Cluster of Differentiation.
[o]Leiomyoma.
[p]Leiomyosarcoma.

(REST) trials (see Tables V–IX). The EMMY trial compared UAE to hysterectomy; symptoms significantly improved by similar magnitudes after both, but reinterventions were more common after UAE due to recurrent symptoms. The REST trial included patients who had undergone myomectomy, hysterectomy and embolization. It found similar symptomatic improvements after 5 years for all procedures, but re-intervention was again more frequent after UAE. Adverse events, such as uterine ischemic injury, were found to be infrequent. Fibroid expulsion after UAE is particularly concerning since it can require gynecological intervention, but has been found to be rare.

To date, no studies have established a rate of successful pregnancy after UAE. Recently, Homer and Saridogan (2010) found that rates of miscarriage, Cesarean section and post-partum hemorrhage were increased after UAE when compared with controls.

It was noted that gaps exist in our knowledge as it relates to certain aspects of the UAE technique not being adequately defined, such as missing an appropriate end-point, or determining whether the current technique used actually represents 'over embolization' with increased risk of potential injury to the endometrium, myometrium or ovary. Precipitating factors for complications are not well understood, and there is a need for more data on reproductive outcomes after UAE and myomectomy, such as whether one treatment might be preferable for certain subgroups.

Conclusions

While advances in research have expanded our knowledge of the pathobiology of fibroids, their etiology still remains incompletely understood. With technical advances, there has concurrently been an increase in minimally invasive surgical procedures; however, an effective long-term non-surgical treatment for uterine fibroids to reduce heavy uterine bleeding as well as fibroid/uterine volume without excessive side effects has not

Table IV Effect of drugs on symptoms for women with symptomatic uterine fibroids.

Drug	Decrease in fibroid size[a]	Decrease in bleeding[a]	Amenorrhea[a]	Adverse events
GnRHa	30–65% (Stewart, 2001; Olive *et al.*, 2004; Parker, 2007a, b; Somigliana *et al.*, 2007; Ezzati *et al.*, 2009; Cook *et al.*, 2010; Donnez *et al.*, 2012a, b; Nodler and Segars, 2013) Improved symptoms 64% (Parker *et al.*, 2007a, b)	89% (Donnez *et al.*, 2012a, b)	Up to 97% (Nodler and Segars, 2013; Parker, 2007a, b)	Bone loss, hot flashes, vaginal dryness, headache (Parker, 2007a, b)
Mifepristone Levonorgestrel IUD (LNG-IUS)	30–57% (Cook *et al.*, 2010; Esteve *et al.*, 2012; Chwalisz and Winkel, 2013; Nodler and Segars, 2013) No decrease (Nodler and Segars, 2013; Magalhaes *et al.*, 2007)	41–93% (Cook *et al.*, 2010; Esteve *et al.*, 2012) 85% (Parker, 2007a, b)	60–65% (Olive *et al.*, 2004; Nodler and Segars, 2013) 10–40% (Grigorieva *et al.*, 2003; Parker, 2007a, b; Nodler and Segars, 2013)	Simple endometrial hyperplasia, endometrial changes, nausea, hot flashes, vomiting, fatigue
Asoprisnil	0.4–36% (Chwalisz *et al.*, 2007; Wilkens *et al.*, 2008) Improved symptoms 67–89% (Chwalisz *et al.*, 2007)	28–91% (Chwalisz *et al.*, 2007; Wilkens *et al.*, 2008)	16–70% (Chwalisz *et al.*, 2007)	Endometrial changes
Ulipristal acetate (CDB-2914)	12–42% (Donnez *et al.*, 2012a, b) 21–36% (Levens *et al.*, 2008)	90–98% (Donnez *et al.*, 2012a, b) 81–90% (Levens *et al.*, 2008)	73–82% (Donnez *et al.*, 2012a, b)	Possible endometrial Hyperplasia
Tranexamic acid	No decrease	up to 50% (Chwalisz and Winkel, 2013)		
NSAIDs	No decrease (Parker, 2007a, b)			
Aromatase inhibitors Letrozole	45.6–59.7% (Cook *et al.*, 2010) 45.6% (Parsanezhad *et al.*, 2010; Nodler and Segars, 2013)			

[a]Depends on the dosage, time since treatment and number of fibroids.

been achieved. Further needs exist for determination of risk factors and initiation of preventive measures for fibroids, in addition to continued development of new medical and minimally invasive options for long-term treatment.

Future recommendations

During the Congress participants attended breakout sessions grouped under the broad topics of the meeting. The attendees were asked to address the future needs of their area of interest and to summarize the needs and future recommendations to present at the end of the meeting. What follows is a brief summary of each group based on the breakout session topics. The *Epidemiology, genetics and the environment* breakout group emphasized a need for increased public awareness of fibroids. This would assist in stimulating increased patient interest and possible participation of more patients in health-care activities and clinical studies, particularly in racial and ethnic communities. It was stressed that there is also a need to diversify study populations by increasing enrollment of Hispanic and Asian women, along with African American women. It was also recommended that in order to share information among the fibroid research and medical communities, development of a listserv of experts in the field would facilitate the rapid exchange of information. Creation of a standard tool kit for research that contains a list of resources for investigators that outlines relevant end-points and important data to collect when initiating uterine fibroid research would also be helpful. Due to the increase role of the environment as a trigger for many disease processes, environmental exposures and life-exposure research that incorporates collecting data on potential endocrine disruptors and behavioral factors (diet, physical activity, etc.), and takes into account total (life) exposures, and their association with development of uterine fibroids should be considered. This group also suggested more genetic studies that can integrate full depth genome sequencing of uterine fibroids and involve epigenetic approaches to begin classifying mutations and nongenomic changes, respectively, in fibroids. The *Pathogenesis: growth factor, cytokine, extracellular matrix and cell signaling research* breakout group suggested systematic protein profiling studies of uterine leiomyomas at the tissue and cell culture levels that would increase our knowledge of protein expression as it relates to different tumor phenotypes that might be regulated by post-translational modifications, in addition to the exploration of the proteomics of fibroids. There was a consensus among attendees that it is necessary to optimize cell culture systems and animal models for measuring growth factors and cytokines, including cultures of primary and immortalized uterine leiomyoma and uterine smooth muscle cells. Unification of reporting conditions of samples or patients as it relates to age, tumor size, hormone status, tumor location and ethnicity was encouraged. There should be additional research on the extracellular matrix that takes into account organization and regulation, cell- matrix and cell–cell interactions. Emphasis was placed on the need for further studies on regulation and identification of key transcription factors and their role in uterine leiomyoma growth. Research focusing on the microenvironment of leiomyomas as it relates to the interactions of cell

Table V Effect of procedure on symptoms for women with symptomatic uterine fibroids.

Procedure	Decrease in fibroid size[a]	Decrease in bleeding[a]	Decrease in pain/ dysmenorrhea[a]	Improved or symptom free[a]
Hysterectomy	NA	NA	NA	89–99% (Parker, 2007a, b; Hirst et al., 2008; Heitmann et al., 2013)
Myomectomy		Up to 90% (Heitmann et al., 2013)	67% (Heitmann et al., 2013)	75–87.9% (Parker, 2007a, b; Hirst et al., 2008; Mara et al., 2008; Heitmann et al., 2013)
UAE/UFE	33–75% (Spies et al., 2001; Pron et al., 2003; Olive et al., 2004; Goldberg and Pereira, 2006; Parker, 2007a, b; Cook et al., 2010; Fenlon and Spies, 2013; Nodler and Segars, 2013)	82.7–96% (Spies et al., 2001; Pron et al., 2003; Goldberg and Pereira, 2006; Parker, 2007a, b; Volkers et al., 2007; van der Kooij et al., 2010; Nodler and Segars, 2013) Amenorrhea 5–60% (Stovall, 2001; Pron et al., 2003; Goodwin et al., 2008; Fenlon and Spies, 2013; Nodler and Segars, 2013)	77% (Pron et al., 2003; Goldberg and Pereira, 2006; Parker et al., 2007a, b)	74–91% (Cook and Walker, 2004; Olive et al., 2004; Parker, 2007a, b; Goodwin et al., 2008; Hirst et al., 2008; Mara et al., 2008; Ezzati et al., 2009; van der Kooij et al., 2010; Fenlon and Spies, 2013)
MRgFUS	4–32% (Stewart et al., 2006; Parker, 2007a, b; Al Hilli and Stewart, 2010)			50–71% (Stewart et al., 2006; Parker, 2007a, b)

MRgFUS, magnetic resonance-guided focused ultrasound; UAE, uterine artery embolization; UFE; uterine fibroid embolization.
[a]Depends on the number of years since the procedure.

Table VI Complications by procedure for women with symptomatic uterine fibroids.

Procedure	Febrile morbidity[a]	Blood loss[b]	Rate of transfusion	Post-operative adhesions[c]
Hysterectomy	14% (Taran et al., 2010a, b)	265 ± 329 ml (Dickersin et al., 2007)	7–13% (Parker, 2007a, b; Taran et al., 2010a, b)	NA
Abdominal hysterectomy	1–11% (LaMorte et al., 1993; Heitmann et al., 2013)	300–400 ml (LaMorte et al., 1993; Heitmann et al., 2013)	20% (LaMorte et al., 1993)	NA
Myomectomy	12–33% (LaMorte et al., 1993; Iverson et al., 1999; Heitmann et al., 2013)	200–800 ml (Heitmann et al., 2013)	2–28% (Heitmann et al., 2013)	Up to 90%[d] (Heitmann et al., 2013)
Abdominal myomectomy	2–5% (Heitmann et al., 2013)	296 ± 204 ml (Vercellini et al., 2003)	7–8% (Vercellini et al., 2003)	75–90% (Heitmann et al., 2013)
UAE/UFE	1% (Parker et al., 2007a, b)		0% (Parker, 2007a, b)	14% (Homer and Saridogan, 2010)
MRgFUS	0.03% (Taran et al., 2010a, b)		3% (Stewart et al., 2006; Al Hilli and Stewart, 2010; Taran et al., 2010a, b)	

MRgFUS, magnetic resonance-guided focused ultrasound; UAE, uterine artery embolization; UFE; uterine fibroid embolization.
[a]Defined as fever and/or infection.
[b]Rate drops if GnRHa is used preoperatively.
[c]Depends on number of fibroids, or if interceed or sepafilm is used.
[d]More common after posterior uterine incisions than after fundal or anterior uterine incisions.

populations (tumor cells, SP stem cells, fibroblasts, vasculature) and how uterine fibroid cells survive and perhaps thrive in their surroundings was suggested. This group also recommended the expansion of the NICHD uterine fibroid tissue/cell bank to increase diversity, histological types and tissue microarrays. The *Hormonal regulation and hormone receptor interactions research* breakout group proposed a further need to explore the complex relationship of estradiol and progesterone in uterine fibroid growth and pregnancy. Additional studies to assess the effects of pregnancy on uterine fibroid growth and biological behavior were recommended. Further evaluation of the side effects of SPRMs to characterize how different regimens modify the histologic response of the endometrium, and the efficacy of IUD use with a progestin as treatment for uterine fibroids was discussed. Research on new molecular targets as therapeutic agents for uterine fibroids and examination of new retinoid compounds as therapy for uterine fibroids, focusing on potential side effects were recommended. In the *Developing new model systems for studying fibroids* breakout group, attendees proposed that reliable uterine fibroid-based animal models with desired features that replicate human disease be developed and used independently to study uterine fibroid biology and to test new therapeutic agents. They

Table VII Recurrence and reoperation rates by procedure for women with symptomatic uterine fibroids.

Procedure	Recurrence rate[a]	Retreatment rate[a]
Hysterectomy	NA	10.7–28.6% (Freed and Spies, 2010; van der Kooij *et al.*, 2010)
Myomectomy	5–67% (Bulletti *et al.*, 1999; Vercellini *et al.*, 2003; Bulletti *et al.*, 2004; Reed *et al.*, 2006; Parker, 2007a, b; Mara *et al.*, 2008; Al Hilli and Stewart, 2010; Fenlon and Spies, 2013; Heitmann *et al.*, 2013; Johnson *et al.*, 2013)	3.2–23.5% (Heitmann *et al.*, 2013; Mara *et al.*, 2008; Reed *et al.*, 2006; Freed and Spies, 2010; Moss *et al.*, 2011)
Hysteroscopic myomectomy	Up to 27% (Reed *et al.*, 2006)	9.5–26.7% (Parker, 2007a, b)
Abdominal myomectomy	15–51% (Reed *et al.*, 2006; Parker, 2007a, b)	11.1–30% (Reed *et al.*, 2006; Parker, 2007a, b; Freed and Spies, 2010; van der Kooij *et al.*, 2010)
Laparoscopic myomectomy	Up to 27% (Reed *et al.*, 2006; Parker, 2007a, b)	
UAE/UFE	10.3–25% (Mara *et al.*, 2008; Freed and Spies, 2010; van der Kooij *et al.*, 2010; Fenlon and Spies, 2013)	10–32.8% (Parker, 2007a, b; Goodwin *et al.*, 2008; Hirst *et al.*, 2008; Mara *et al.*, 2008; Freed and Spies, 2010; van der Kooij *et al.*, 2010; Moss *et al.*, 2011)
MRgFUS	High due to only 10% of fibroids being targeted	8–48% (Stewart *et al.*, 2006; Parker, 2007a, b; Al Hilli and Stewart, 2010)

MRgFUS, magnetic resonance-guided focused ultrasound; UAE, uterine artery embolization; UFE; uterine fibroid embolization.
[a]Depends on the number of years since the procedure, age at procedure, whether childbearing was completed, number of fibroids and if GnRHa was used.

Table VIII Fertility after procedure for women with symptomatic uterine fibroids.

Procedure	Pregnancy rate	Live birth rate
Myomectomy	33–78% (Bajekal and Li, 2000; Vercellini *et al.*, 2003; Bulletti *et al.*, 2004; Casini *et al.*, 2006; Goldberg and Pereira, 2006; Mara *et al.*, 2008; Cook *et al.*, 2010; Heitmann *et al.*, 2013)	25–48% (Bajekal and Li, 2000; Bulletti *et al.*, 2004; Mara *et al.*, 2008)
Hysteroscopic myomectomy	55% (Bajekal and Li, 2000)	80% (Bajekal and Li, 2000)
Abdominal myomectomy	50–60% (Bajekal and Li, 2000; Vercellini *et al.*, 2003; Goldberg and Pereira, 2006)	79% (Bajekal and Li, 2000)
Laparoscopic myomectomy	11–64% (Landi *et al.*, 2003; Malzoni *et al.*, 2003; Goldberg and Pereira, 2006; Chahine and Catherino, 2013)	76% (Bajekal and Li, 2000)
UAE/UFE	33–50% (Carpenter and Walker, 2005; Pron *et al.*, 2005; Hirst *et al.*, 2008; Mara *et al.*, 2008)	19–75% (Pron *et al.*, 2005; Hirst *et al.*, 2008; Mara *et al.*, 2008)
MRgFUS	54 pregnancies in 51 women (Al Hilli and Stewart, 2010; Rabinovici *et al.*, 2010)	41% (Al Hilli and Stewart, 2010; Rabinovici *et al.*, 2010)

MRgFUS, magnetic resonance-guided focused ultrasound; UAE, uterine artery embolization; UFE; uterine fibroid embolization.

suggested that desired features of an animal model for fibroids should include: initiate spontaneous growth of uterine fibroids, express estrogen and progesterone receptors, create extra cellular matrix, have a normal karyotype, be immune competent, develop tumors in the appropriate anatomical location, and retain the benign status of the tumor. This group also recommended organizing a network or registry to share animal models among investigators and placing greater emphasis on projects that involve *in vivo* studies for evaluating new therapeutic agents with a focus on exploring potential side effects on fertility, liver function, normal myometrium and other biological and physiological parameters. The *Advances in clinical and translational research* breakout group suggested the development of a National Uterine Fibroid Research Network that would be similar to other large-scale research networks to facilitate multisite collaborations, conduct clinical trials that reflect community practice and incorporate uterine fibroid translational research. Phenotype standardization was also suggested, as part of a large-scale national phenotyping effort, that would enhance the ability to distinguish differences based on well-delineated patients, focusing on consensus and standardized measures. The need to develop a nationwide data registry that could be linked to electronic medical records and web-based methods that would allow for tracking of large-national cohorts, in addition to tissue and specimen banks to support the inclusion of well-characterized patients was echoed by this group. The *Clinical management and therapeutics options* workgroup proposed the creation of a database with uniform data-reporting instruments to facilitate decision-making pertaining to current and new surgical and medical technologies. Development of basic tools for uterine fibroid classification and standardizing outcome measures was also suggested. Additionally, evaluating existing databases (e.g. HMOs) to obtain comparative information on current treatment outcomes would move the field forward. Prospective comparative trials should be conducted to evaluate outcomes and determine the best treatment options for patients, and patient education tools that are interactive to assist with decision-making and serve as a resource should be made readily available to patients. The *Proposed new fibroid classification system* breakout group proposed the inclusion of a drawing of the uterus and fibroids to indicate clinical severity, location or stage of the disease in the current classification scheme. To ensure clinical relevance of the classification scheme they suggested that it should incorporate the burden of uterine fibroid disease by including bleeding, blood loss and pain. The new classification system should be compared with earlier versions and tested for reliability and reproducibility.

Table IX Obstetric outcomes after procedure for women with symptomatic uterine fibroids.

Procedure	Miscarriage	Preterm delivery	Caesarian section	Placenta previa	Uterine rupture	Post-partum hemorrhage
Myomectomy	7–23% (Bajekal and Li, 2000; Bulletti *et al.*, 2004; Mara *et al.*, 2008)	26.3% (Mara *et al.*, 2008)	68.4% (Bajekal and Li, 2000; Mara *et al.*, 2008)		0.4–1.7% (Landon and Lynch, 2011; Heitmann *et al.*, 2013)	0% (Mara *et al.*, 2008)
Laparoscopic myomectomy	15–26% (Goldberg and Pereira, 2006; Chahine and Catherino, 2013)	3% (Chwalisz *et al.*, 2007)	46%-57% (Bajekal and Li, 2000; Landi *et al.*, 2003; Malzoni *et al.*, 2003; Goldberg and Pereira, 2006)		1% (Chahine and Catherino, 2013)	1% (Chwalisz *et al.*, 2007)
UAE/UFE	15–64% (Ravina *et al.*, 1995; Carpenter and Walker, 2005; Pron *et al.*, 2005; Goldberg and Pereira, 2006; Hirst *et al.*, 2008; Mara *et al.*, 2008; Homer and Saridogan, 2010)	14–28.5% (Ravina *et al.*, 1995; Carpenter and Walker, 2005; Pron *et al.*, 2005; Goldberg and Pereira, 2006; Parker, 2007a, b; Mara *et al.*, 2008; Homer and Saridogan, 2010)	50–88% (Carpenter and Walker, 2005; Pron *et al.*, 2005; Goldberg and Pereira, 2006; Hirst *et al.*, 2008; Homer and Saridogan, 2010)	11% (Pron *et al.*, 2005)		6–20% (Goldberg and Pereira, 2006; Parker, 2007a, b; Mara *et al.*, 2008; Homer and Saridogan, 2010)
MRgFUS	26–28% (Rabinovici *et al.*, 2010)	6.7% (Al Hilli and Stewart, 2010; Rabinovici *et al.*, 2010)	36% (Rabinovici *et al.*, 2010)	9% (Al Hilli and Stewart, 2010; Rabinovici *et al.*, 2010)		

MRgFUS, magnetic resonance-guided focused ultrasound; UAE, uterine artery embolization; UFE; uterine fibroid embolization.

Charting the course: summary of breakout workgroup recommendations

In summary, the meeting met the overarching goals of the conference to bring together multidisciplinary aspects of uterine fibroid research with an eye toward identifying pivotal questions and formulating new collaborations with a focus on exploring the development of research innovations important to prevention of the disease and to optimizing clinical management. The conference provided a forum for experts in the field to concentrate on promising and innovative research that continues to build upon and enhance our understanding of the basic underpinnings of uterine leiomyoma pathophysiology and innovative targets for treatment modalities. Also, the importance of environmental exposures in early life and later expression of disease were addressed in addition to the roles of genetic, environmental and epigenetic events in influencing disease manifestation. New research should shape and expand our basic understanding of uterine leiomyoma pathophysiology and innovative treatment modalities. Although the existing data are impressive, many challenges remain and further research is absolutely warranted. The final session served as a catalyst for discussing future research recommendations, directions and opportunities formalized by speakers and meeting participates in seven breakout workgroups.

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Conflict of interest

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