Preclinical Review/additional information
P030004

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Review
Additional information regarding DMSO/coil compatibility, the effect of radiation on the polymer, endotoxin quantities of the catheter and syringe, the amount of silicone oil placed into the syringe and polymer solidification time was requested. As mentioned in the first mail-out, the repeat injection issue related to DMSO potential toxicity is discussed. Finally, additional review information is provided in this email as excerpted from the manufacturing reviewer/chemist's and preclinical reviewer's review of the chemistry and toxicology of the device.

1. DMSO/coil compatibility
In the experiment the sponsor used HPLC to detect any leachable chemical entities from platinum, GDC or Cook fiber coils due to contact with DMSO. The concomitant use of coils and the polymeric embolization agent is likely to occur in the presurgical embolization of AVMs. A minor peak had been identified that was different from control in 6 of 12 coils evaluated. I reviewed the control and suspect chromatograms. The control chromatogram shows a broad peak in the area where the additional peak appears in 6 of 12 samples. In those 6 samples the peak appears to have been split into two. FDA agrees with the sponsor's chemist that the peak identifies a minor chemical entity and that the splitting of the broad peak identified in the control into 2 minor peaks is of minor importance, chemically, and does not necessarily indicate a new degradation by-product or leached chemical. The sponsor has adequately addressed the deficiency.

2. Effect of radiation on polymer
The sponsor had conducted evaluations to determine if radiation could cause degradation of the polymer in vivo. Although the device is intended for use as a presurgical embolication agent, in some cases a patient's AVM may not be resected due to various reasons. In those situations, an alternative means for "resecting" the AVM is to irradiate it. Radiation causes the tissue to undergo fibrosis and thereby, stabilization. The sponsor had not included the IR spectra or GPC chromatograms to support their contention that the material had not degraded after having been irradiated.

The IR spectra provided of material (n = 2) irradiated with 30 Gy were identical, or nearly identical. The control and irradiated material spectra are qualitatively the same. The GPC chromatograms (n = 2) of irradiated material and control EVOH were nearly superimposable.

In addition, the sponsor has provided information that adequately addresses the concerns raised regarding the endotoxin amounts, the silicone oil used in the syringe and the polymer solidification time.

3. Repeat injection issue

In the first mail-out FDA provided the following draft preclinical question for consideration: *Preclinical animal evaluations have shown that the rate and amount of DMSO can cause vasospasm and vascular wall damage. Patients undergoing staged embolization procedures for Cerebral Arteriovenous Malformations will be exposed repeatedly to the potential for DMSO-mediated vessel damage. Do you believe additional animal evaluation should be conducted to more completely assess for repeat-DMSO vessel wall exposure and potential adverse effects? Do you have any recommendations regarding the amount of DMSO a patient should be exposed to over a 24 hour period or the length of time between embolization procedures?*

FDA indicated that this issue would be discussed in more detail in the second mail-out. The following information is a summary of relevant data that you may find helpful in determining how you think this issue is best resolved.

Onyx requires a two-step procedure employing anhydrous DMSO. MTI data shows that the initial catheter-priming step used 0.2 – 0.26 mL DMSO injected intra-arterially at = 0.4 mL/min. The second step involves slow injection (mean rates = 0.115 mL/min) of a mixture of EVOH polymer and tantalum powder dissolved in pure DMSO. Animal studies have shown that if concentrated DMSO is given too quickly, severe vasospasm and angiotoxicity will occur. In early evaluations of the embolic agent, Chaloupka et al studied the device in the swine rete mirabile. The investigators encountered visualization, catheter-compatibility and vascular toxicity complications. The DMSO infusion caused moderate to severe vasospasm immediately; subarachnoid hemorrhage or stroke occurred frequently. Histopathology showed variable endothelial denuding, thrombosis, and internal elastic lamina disruption acutely; an intense mixed inflammatory response with organized thrombus formation and transmural necrosis with extravasation was noted in subacute and chronic specimens. The investigators concluded that undiluted DMSO was angiotoxic.

Additional studies have shown that if a low dose of DMSO is administered using a very slow injection rate, vasospasm and angiotoxicity is not observed. Murayama et al., evaluated the embolic agent for acute and chronic effects after intra-arterial delivery. The study looked at the importance of slow infusion in reducing arterial damage caused by concentrated DMSO. Injections of 0.5 mL DMSO were given over 5, 15, 30, 60, and 120 seconds; the EVOH mixture used a priming dose of 0.3 mL DMSO administered over 40 seconds followed by 0.3-0.5 mL of the EVOH/DMSO mixture given over 20-40 seconds. Special attention was directed to findings of focal or diffuse angionecrosis, arterial revascularization, and perivascular inflammation. When 0.5 mL DMSO alone was given over 5-15 seconds, vasospasm and endothelial necrosis developed. The same dose infused over 15 seconds yielded focal vasospasm, but no laminal disruption or angionecrosis. No toxicity of any kind was noted if 0.5 mL anhydrous DMSO was given slowly over 30, 60 or 120 seconds. The authors concluded that the two most important elements in controlling vascular toxicity precipitated by intravenous injection of concentrated DMSO were:

- ?? Contact time with the arterial wall
- ?? DMSO volume

Murayama et al. concluded that "slow, controlled intra-arterial delivery of DMSO shows minimal endothelial inflammatory response and no histological evidence of necrotizing arteritis". The preclinical information provided in the PMA and found in the scientific research literature clearly indicates that DMSO can cause vascular toxicities if the rate of its infusion is not carefully controlled. In addition, the preclinical information in the PMA also shows that if the rate of injection is controlled, vasospasm and vascular toxicities are avoided in single infusion experience. Training physicians with regard to the use of the product and how to avoid causing DMSO-mediated vascular toxicity is important to the safe use of the product. The sponsor has an established training program for physicians learning how to use the product that includes the following elements:

- ?? Theoretical presentation: includes discussion of Onyx formulations (Onyx 18 (6%) and Onyx 34 (8%) and rationale of when to use each formulation; overview of preclinical testing; use of DMSO (research papers, animal studies, clinical experience to date), and complete review of Onyx LES (liquid embolic system) tips and techniques, i.e., material preparation, rate of injection of DMSO, compatible micro-catheters, injection technique.
- ?? In vitro bench workshop: bench model that replicates AVM flow characteristics used to provided physicians experience with injecting Onyx 18 and Onyx 34 at various flow rates
- ?? In vivo animal injections or clinical observation: physician is offered opportunity to perform embolizations in the swine rete mirabile, renal arteries or external carotid arteries, or to observe a clinical case performed by the Onyx proctor.
- ?? Case review: training physician shares case films to provided reference regarding clinical use of Onyx – overall clinical experience from Europe, selected case videos, and films are reviewed
- ?? Clinical representative attends the physician's first case

And the product label contains the following information regarding the use of DMSO:

- ?? A DMSO compatible delivery micro catheter that is indicated for use in the neuro vasculature (e.g. Rebar™ or UltraFlow™ HPC catheters) is used to access the embolization site.
- ?? Direction and Warning: Based on clinical practice, it is recommended that Onyx be injected at a slow, steady rate of 0.16 mL/min (0.25 mL/90 sec). Do not exceed 0.3 mL/min. Do not exceed 0.3 mL/min injection rate. Animal studies have shown that rapid injection of DMSO into the vasculature may lead to vasospasm and /or angionecrosis.

The training program and the label instructions/warnings appear to adequately inform the user about the dangers of rapid DMSO vascular infusion. However, little research information or clinically meaningful information is available regarding the safety of repeat infusions of DMSO as might occur during staged embolizations of the product. In an Onyx-unrelated animal assessment of the repeat intravenous administration of DMSO, Willson et al found that undiluted DMSO given to dogs at 0.3, 0.6, 1.2 and 2.4 g/kg/day six times per week for 4 weeks caused injection-site vein occlusion.

Limited information to address repeat DMSO injection as related to the Onyx device and its potential resultant toxicity was provided by the sponsor. In tissue surgically excised from 7 patients of the Mexico City Embolyx Pilot Study and the International Brain Arteriovenous Malformation Clinical study the investigators assessed Onyx for its potential chronic, histotoxic effects. Seven BAVMs embolized with Onyx were surgically excised and submitted for evaluation to a board certified histopathologist. Prior to surgery, 1 of 7 patients received a single embolization treatment, 2 of 7 received 2 treatments, 1 of 7 received 3 treatments and 3 of 7 received 4 treatments. The timing between embolization and surgery ranged from 1 week to 19 months while all patients had pre-surgical embolization periods of at least 3 months, i.e., no one had one embolization procedure and then within 1 week went to surgery whereas some patients may have had multiple embolization procedures with the last one being 1 week prior to surgery. There were no indications of vascular necrosis, rupture or extravasation of the Onyx material. Numerous vessels were observed with disruption of the internal elastic lamina, but there did not appear to be any serious adverse effect on the vessel wall. The information is obviously very limited.

A review of CT, MRI and flat film skull x-rays obtained from patients whose BAVMs were treated with Onyx or n-BCA was performed for MTI by a central reader to determine if any direct neurotoxicity due to Onyx can be detected in the brain post-embolization. A total of 54 patients were studied in the Onyx group and 19 in the n-BCA group (total = 73). The central reader was blinded as to treatment. All MRI and CT studies were evaluated for the presence or absence of gliosis, encephalomalacia, edema, leptomeningeal or parenchymal enhancement and hemorrhage. These parameters were pre-defined based on specific imaging characteristics. The average time post-embolization for all imaging studies was 23 months, with a range of 9 to 50 months. Forty-one patients of the 73 had imaging findings that required an assessment as to whether the finding was due to the device. Twelve of the 19 n-BCA patients had imaging changes that were due to: concurrent neurosurgical resection of the AVM, changes in the brain related to neurosurgery, or due to the natural history of the AVM. Twenty-nine of 54 patients in the Onyx groups demonstrated imaging findings post-embolization that were not present pre-embolization. The reader (Director, Clinical Image Processing Service for UCLA Department of Radiological Sciences) asserts that "in all cases the etiology of the post-embolization findings was found to be due to events unrelated to the presence of Onyx." The findings were believed to be due to radiosurgery, surgical resection of the AVM and the natural history of the AVM. An FDA radiologist reviewed the images and found no reasons to disagree with the central reader's interpretation that the image post-embolization observations were due to events unrelated to the presence of Onyx.

The following parameters of the investigational study should be taken into account:

Number of	n-BCA (n = 54)		Onyx (n = 46)	
embolization procedures	#	%	#	%
1	34	63	26	56.5
2	9	16.7	11	23.9
3	7	13	6	13
4	2	3.7	1	2.2
5	2	3.7	1	2.2

6	0	0	0	0
7	0	0	1	2.2

From the sponsor's clinical experience outside of the United States:

Number of	Onyx (n = 161)	
embolization procedures	#	%
1	113	70.2
2	32	19.9
3	12	7.4
>3	4	2.5

So, the information gathered in the sponsor's U.S. clinical study and their experience outside the U.S. clearly indicates that although the majority of AVM patients will undergo one embolization procedure, there is a subpopulation of individuals that will undergo two or more infusions of the embolic agent. The mean volume of Onyx injected in this [U.S.] investigational study was 0.5 mL and the mean volume of DMSO injected was 0.27 mL, whereas clinical experience outside the U.S. found that the mean volume of DMSO per treatment was 1.57 mL and the maximum dose of DMSO ever delivered was 8.36 mL.

It could be argued that since the embolic agent is targeted to a vascular abnormality that the physician intends to remove from the patient, long term evaluation regarding repeat DMSO injections is of little interest. However, not all patients in the study went on to have their AVM surgically excised. Of the 100 patients in the ITT population, 86 had total resection and 89 had total or partial resection. The patients enrolled in the study were identified as surgical candidates but because surgical resection was not in the patients' best interest, surgical resolution did not occur in every case. With respect to vasculature that has been embolized with DMSO more than once we have very limited information in terms of numbers of patients and in length of time of follow-up.

Please consider this information in preparation for discussion of the repeat-injection DMSO toxicity panel question.

4. Chemistry of Device

Ethylene vinyl alcohol copolymer (EVOH) is synthesized by polymerizing a mixture of ethylene gas and vinyl acetate. The resulting ethyl vinyl acetate is treated in a basic pH environment with sodium hydroxide and methanol to hydrolyze the acetate from the polymeric chain resulting in ethyl vinyl alcohol. The EVOH polymer is washed with methanol to remove the acetate and other low molecular weight oligomers.

The EVOH co-polymer requires the use of anhydrous DMSO (Onyx = EVOH in DMSO plus tantalum for radiopacification) as a solvent for delivery through the micro-catheter to the AVM site. If Onyx comes into contact with saline, it will immediately precipitate and block the catheter. A small amount of anhydrous DMSO (0.2-0.26 mL) is used to prime the micro-catheter. After Onyx reaches the aqueous environment of the embolization site, DMSO from the

EVOH/DMSO mixture will be diluted by water in the blood and surrounding tissues. Water contact will cause the EVOH polymer to precipitate and produce an embolus that will conform to the tissues of the embolization site. Formation of the embolic plug begins at its outer surfaces and proceeds inward. Complete embolus formation requires a prolonged period of time, from 3-20 minutes, depending on blood flow and the amount of material injected. Micronized tantalum is added to the EVOH/DMSO solution to provide for fluoroscopic visualization. It is important to note that the catheter priming amount of DMSO represents the free, non-solvent DMSO device component in that it is used simply to prime the catheter. The priming volume of DMSO will be readily transported from the embolization site intravascularly and/or into the interstitial space of the site. DMSO solvating the EVOH will diffuse more slowly from the site, than the free DMSO priming amount, as it is gradually released from the precipitating embolus.

Master file and release specifications for each device component have been reviewed. From the manufacturing/polymer chemist's perspective, the "three major components of the subject device, Ethylene Vinyl Alcohol Copolymer (EVOH), Dimethyl Sulfoxide (DMSO) and Tantalum do not contain significant amounts of impurities and are spectrascopically pure materials."

There are 2 formulations used in the Onyx LES for AVM treatment: Onyx 18 and Onyx 34. The maximum concentration of DMSO is approximately 90% by volume. In MTI's studies to date the average amount of DMSO used per treatment was 1.57 mL and the maximum volume of DMSO used in any one treatment was 8.36 mL (data collected from 222 procedures). For the calculation of the potential maximum DMSO dose observed in AVM treatments, 8.36 mL x 1.10 (specific gravity of DMSO) equals 9.2 g DMSO, thus yielding (9.2 g/70 kg) a maximal DMSO dose of **131 mg/kg**. If this maximum dose is calculated to be used as the first of a series of staged embolizations, it is reasonable to assume that subsequent embolization treatments would require smaller, more average, quantities of Onyx. Using the average amount of DMSO (1.57 mL) used for 3 additional treatments (1.57 mL x 3 treatments x 1.10 [spec. gravity of DMSO]), plus 9.2 g DMSO per the first treatment, the total maximal dose of DMSO a patient would be likely to be exposed to is 14.37 g DMSO/70 kg, or **205 mg/kg**. [Please note that these values are numerically larger than what was used in the U.S. clinical study, i.e., 0.27 mL free DMSO plus 0.5 mL Onyx.] To calculate a lower range value, using the mean, average dose for the first treatment instead of the maximal dose ever observed, the lower potential DMSO exposure would be, 1.57 mL x 1.10 = 1.73/70 kg = 0.025 g/kg x 4 treatments = **100 mg/kg**. Therefore the range of DMSO concentration that a patient could be exposed to is **100-205 mg/kg**. It is important to note that the embolization procedures would be done over a period of time and the patient would not be exposed to the 100-205 mg/kg total all at once.

Metabolic studies in man and lower animals indicate that the primary metabolites of DMSO are dimethyl sulfone ($DMSO_2$) and dimethyl sulfide (DMS).

5. DMSO Biocompatibility: Toxicities of DMSO and DMSO metabolites

MTI provided a white paper on DMSO toxicity which identified research information regarding the absorption, distribution, metabolism, and excretion of DMSO. The following information is a very brief summary of the most pertinent information of that white paper. DMSO, when used in the treatment of interstitial cystitis in humans is considered a drug. For a point of comparison,

Rimso-50® consists of a 50% solution of DMSO, and as instilled in the bladder as a 50 mL dose, equals a 393 mg/kg dose for a 70 kg person.

DMSO has chemical properties which facilitate its absorption into and distribution throughout biological systems by all routes of administration. DMSO can also carry other substances along with it due to its solvating power. DMSO is a polar nucleophile which has free electron pairs at its sulfur and oxygen terminals. It is considered an aprotic solvent since it does not normally donate hydrogen atoms in chemical reactions. Hydrogen bonding of DMSO with water is 1? times stronger than the hydrogen boding between water molecules themselves, thus yielding DMSO's hygroscopic character. Intravenous administration of DMSO appears to be well-tolerated at concentrations lower than 50%. Higher concentrations, given repeatedly, injure the injected vessels causing fibrosis proportional to the concentration and number of injections. Persistent damage to the blood vessel causes a narrowing of the lumen. As noted above, the Willson study observed injection site vein occlusion in dogs given daily injections of DMSO for 4 weeks. The injection rate of DMSO has been observed to determine toxicity. As noted in a 1971 text, *Toxicology of DMSO in Animals* (Mason), a 5 mL rapid intravenous infusion of DMSO caused death in a dog whereas a dose of 100 mL infused over 4 hours did not cause death.

Distribution studies regarding DMSO reveal that the molecule is rapidly distributed in a wide-spread manner. Denko et al. found that DMSO accumulated more in soft tissues and it was found in tissues with low and high lipid content. Tissue vascularity or permeability appeared to offer no preferred mode of action. In a study by Nishimura et al. looking specifically at distribution of DMSO in brain and vascular tissue in the rat, calculated tissue (muscle, liver and gray matter) to plasma ratios were observed to be 1:1 two hours after infusion (of a 1g/kg/hour) was initiated. White matter approached this ratio after one hour and had declined some by 2 hours. In the mouse, peak plasma concentration was reached one minute after a bolus injection into the tail vein and it diminished in a biexponential fashion; its rapid distributive phase showed a t½ of 1.5 minutes, while the longer terminal half-life was 90 minutes. In summary, MTI believes that the DMSO in the Onyx solution will be distributed rapidly by the vascular system, a portion likely bound to serum or plasma proteins, or dispersed into interstitial spaces of surrounding tissues. The priming dose (of the catheter) will likely be distributed via the vascular system very quickly whereas the DMSO that slowly elutes from the embolus will likely gain entrance to the endothelial lining cells of the blood vessel and to the interstitial space.

Many metabolic studies in man and animals show that the primary metabolites of DMSO are dimethyl sulfone and dimethyl sulfide. The extent to which DMSO is converted to dimethyl sulfone by non-primates seems to vary somewhat by route and species. All studies reviewed in the white paper indicated that DMSO is not excreted unchanged to some extent, most of it will be either oxidized to dimethyl sulfone and excreted in the urine or reduced to dimethyl sulfide and exhaled. The garlic-breath of individuals treated with DMSO is thought to result from the conversion to dimethyl sulfide. Although unsupported by data, MTI believes, based on animal studies including human and non-human primates, that approximately half of the DMSO in Onyx will not be metabolized with 20-25% being converted to dimethyl sulfone and a small fraction being converted to dimethyl sulfide. Dimethyl sulfoxide is a constituent of plant materials. Dimethyl sulfone is found in milk and dimethyl sulfide has been found in prepared foods. The

manufacturer of the DMSO provided limited LD_{50} information regarding the acute toxicity of dimethyl sulfide in the rat. The oral, inhalation and dermal values were 3.7 g/kg, 40,250 ppm, and 10.2 g/kg, respectively. The small amounts of dimethyl sulfone and dimethyl sulfide should not, based on available toxicology information, cause risk to the patient.

LD_{50}s after intravenous dosing reveals that anhydrous DMSO caused acute toxicity in cats, dogs, monkeys, rabbits and rats in the range of **2.5 g/kg-11g/kg**. Some investigators found that repeated intravenous injections of undiluted DMSO were damaging to the veins of dogs and rats. However other investigators found no negative effects of injecting 40% DMSO into dogs for 33 days. The sponsor cites a 1963 reference that determined the $LD_{0.1}$, or that dose likely to kill 1/1000 animals dosed, for anhydrous DMSO after intravenous administration to be 400 mg/kg in the mouse. As the sponsor notes, this dose is approximately 3 times that of the largest single DMSO dosage used in Onyx clinical experience to date (i.e., 131 mg/kg) and 14-16 times the average dosages for Onyx clinical treatments.

There is sufficient evidence provided in the research literature that demonstrates DMSO to be hemolytic. A number of animal studies noted hematuria and hemoglobinuria – specifically after intravenous administration. Emmerling et al in a 1991 study report to NCI found that male rats given 70% DMSO via a rate of 2 mL/kg/hr/120 hrs showed marked decreases in hematocrit and hemoglobin by the second day. In a study by Bennet et al concentrations of DMSO of 10, 20 and 40% infused at 1g/kg caused decreases in hematocrit of 1.5, 4.9 and 5% in humans. In the study by Willson, anemia, hemoglobinuria, bilirubinuria, increased SGOT levels and slight liver pathology was observed at a dose of 0.3 g/kg/day/6 days/week/4 weeks.

In conclusion, Onyx administration is likely to produce some hemolysis, primarily after the priming dose is given. In addition, endothelial cell damage is possible but should be minimized by adherence to the slow rate of administration determined in the sponsor's animal model investigations evaluating for angiotoxicity. The severity of vasospasm and the occurrence of angionecrosis in swine were reduced when the volume of DMSO was reduced from 0.8 to 0.5 mL. As a result of the recommendation by Murayama et al, that 0.3 mL delivered over 40 seconds was a safe dose, the recommended priming doses now used for Onyx is 0.26 over 40 seconds. The amount of DMSO a patient has been maximally exposed to falls 3-4 times below levels noted in animal toxicology studies to cause adverse effects. As evidenced by the sponsor's U.S. clinical study, the amounts of DMSO most commonly used in the presurgical embolization of AVMs is much lower than the maximal amount reported. The extensive biocompatibility assessments and animal performance evaluations indicate that the product was biocompatible and did not cause adverse tissue responses different than, or greater than what is seen with approved embolic agents.