

**DE NOVO CLASSIFICATION REQUEST FOR
BIOXMARK**

REGULATORY INFORMATION

FDA identifies this generic type of device as:

Phase-changing fiducial marker for radiation therapy. A phase-changing fiducial marker for radiation therapy is a single-use, sterile liquid material that changes phase *in situ* when injected in tissue for the purposes of aiding radiation therapy treatment. The device is intended to be visualized using one or more radiologic imaging modalities.

NEW REGULATION NUMBER: 21 CFR 892.5727

CLASSIFICATION: Class II

PRODUCT CODE: QUV

BACKGROUND

DEVICE NAME: BioXmark

SUBMISSION NUMBER: DEN220017

DATE DE NOVO RECEIVED: March 4, 2022

SPONSOR INFORMATION:

Nanovi A/S
Diplomvej 378
C/O DTU Science Park
Kgs. Lyngby
Denmark 2800

INDICATIONS FOR USE

The BioXmark is indicated as follows:

BioXmark is indicated for use to radiographically mark lung, bladder and lymph nodes in adult patients for whom it has been determined that radiographical marking of tissue for radiation treatment is indicated for their cancer treatment.

BioXmark is implanted via image guided injection into tissue relevant for radiotherapy planning at a healthcare facility. BioXmark can be implanted in the tumor, lymph nodes or tissue adjacent to the tumor subject to irradiation or healthy tissue which should not be irradiated.

BioXmark is intended to mark tissue for at least 3 months after implantation.

LIMITATIONS

The sale, distribution, and use of the BioXmark are restricted to prescription use in accordance with 21 CFR 801.109.

The device is sterile, single-use and cannot be reprocessed.

Do not inject the device directly into the bloodstream.

Avoid injections into anatomical structures such as the heart, central nervous system, necrotic tissue, and air-filled cavities.

Do not inject into patients with hypersensitivity to iodine or other components of BioXmark.

Do not exceed maximum injection volume.

PLEASE REFER TO THE LABELING FOR A COMPLETE LIST OF WARNINGS, PRECAUTIONS AND CONTRAINDICATIONS.

DEVICE DESCRIPTION

The device is a single-use, implantable device consisting of a sterile transparent liquid in a one-point-cut (OPC) glass ampoule. Upon injection of the liquid into soft tissue, efflux of alcohol leads to the formation of a radiopaque, sticky, gel-like fiducial marker *in vivo*, which is visible using fluoroscopy, CT, MRI, and ultrasound. The subject device is a mixture of ethanol, sucrose acetate isobutyrate (SAIB) and an iodinated and acylated derivative of sucrose (x-SAIB). Each ampoule contains 1 mL of liquid and is steam sterilized. On injection, a miniscule amount of ethanol diffuses in 1 to 2 hours, causing an increase in marker viscosity and resulting in a hydrophobic semisolid gel-like marker at the injection site.



Figure 1. BioXmark in single ampoule

Table 1. BioXmark composition

Liquid

Component	Description(s)	Nominal Content (w/w%)	Function
SAIB	Sucrose acetate isobutyrate	(b)(4)	Formation of marker <i>in vivo</i>
x-SAIB	Iodinated and acylated derivate of sucrose*		Contrast component providing radiopacity
EtOH	Ethanol		Solvent, reducing the viscosity of the liquid to enable injection through thin needles ($\leq 25G$)

*Sucrose 6,6'-di(2,4,6-triiodophenoxy) isobutyrate.

Primary Packaging

Component	Description(s)	Nominal Content (w/w%)	Function
Type 1 OPC Glass ampoule	Borosilicate	N/A	Sterile barrier

BioXmark is a mixture of ethanol, SAIB and x-SAIB in the ratio (b)(4) (w/w%). Upon injection, the ethanol diffuses in 1 to 2 hours, causing an increase in marker viscosity and resulting in a hydrophobic semisolid gel-like marker at the injection site. Based on performance data provided the rate of ethanol diffusion and formation of the marker is more dependent on the amount injected than the polarity of the tissue it is injected into. Ethanol diffusion rates do not vary significantly in different tissue types.

SUMMARY OF BENCH STUDIES

BIOCOMPATIBILITY

As indicated in Table 1, BioXmark is a mixture of ethanol, SAIB and x-SAIB in the overall ratio of (b)(4) (w/w%). Animal testing showed evidence of limited resorption occurring for 3-9 months followed by stabilization of the marker volume. It is characterized as a permanent (>30 days) implant, tissue/bone contacting. Biocompatibility evaluation of the implant has been completed according to FDA Guidance, *Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process."* Cytotoxicity, sensitization, intracutaneous reactivity, acute systemic toxicity, genotoxicity, implantation, subacute/subchronic toxicity, chronic toxicity, and material-mediated pyrogenicity testing were conducted per the appropriate standards in the ISO-10993 series. Biocompatibility testing was performed on the sterilized device. A toxicological risk assessment was performed to evaluate the carcinogenicity endpoint for the subject device. No risk of carcinogenicity was identified. Based on all testing and evaluations, BioXmark was determined to be biocompatible.

Biocompatibility testing showed evidence of some resorption. Degradation appears to stop at the three-to-nine-month time point. The provided studies are sufficient to demonstrate lack of sub-chronic and chronic toxicity caused by the degradation products.

STERILITY/PACKAGING/SHELF LIFE

Sterility:

The subject device is provided sterile to the end user. The device is sterilized by moist heat in accordance with ISO 17665-1:2006, “Sterilization of health care products – Moist heat – Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices.” The operation cycle selected was 121°C for 20 minutes. The sterilization cycle used for terminal sterilization of the device by moist heat achieved a $F_0 \geq 15$ and an SAL value of 10^{-6} .

Packaging:

The subject device is packaged in a glass ampoule. Five glass ampoules are packaged in a retail box with implant cards and instructions for use.

Shipment integrity was conducted in accordance with ASTM D4169-16, “Standard Practice for Performance Testing of Shipping Containers and Systems.” Packaging validation testing included stacking compression, loose load vibration, low pressure, random vibration, concentrated impact, handling, and visual inspection.

Shelf Life/Stability:

Representative sterilized samples were aged two years to determine the shelf life of the device. Testing was conducted to verify the devices still functioned as expected with two-year real time and accelerated-aged samples. Material and packaging properties did not degrade significantly during real time or accelerated aging. All tests were passed successfully.

Pyrogenicity:

Bacterial endotoxin testing performed in accordance with ANSI/AAMI ST72:2019 “Bacterial endotoxins – Test methods, routine monitoring, and alternatives to batch testing”.

MAGNETIC RESONANCE (MR) COMPATIBILITY

The subject device is composed entirely of non-metallic components; per the FDA guidance document “Testing and Labeling Medical Devices for Safety in the Magnetic (MR) Environment,” the device is MR Safe and poses no safety hazards in the MR environment.

HUMAN FACTORS TESTING

A human factors evaluation was conducted for the subject device in compliance with IEC 62366-1:2015+A1:2020 and FDA guidance document, “Applying Human Factors and Usability Engineering to Medical Device.” Usability testing was conducted to determine if use errors associated with use of the subject device have been reduced to an acceptable level. The usability report described the validation study methods and findings that were used to evaluate the ability

of the subject device to be used as intended by the intended users in the intended use environment. Percutaneous and endoscopic implantation methods were examined, and clinical users included gastroenterologists, interventional radiologists, advanced endoscopists, and radiation oncologists, and clinical support personnel with varying levels of experience.

The validation study consisted of user performance on comprehension questions that evaluated participants' understanding of device-specific knowledge presented in the technical documentation. Simulated-use tasks were also evaluated to promote the evaluation of the BioXmark for its intended use. Usability testing was performed for both percutaneous injections and endoscopic injections.

Across the sample of thirty (30) participants, a total of 241 critical user-device interactions were scored, including 61 simulated-use tasks with discernable outcomes and 180 comprehension questions. The overall success rate on critical tasks was 98.8% across all participants, types, and contexts. In particular, the 241 critical user-device interactions yielded 238 successes (98.8%), 0 difficulties (0.0%), and only 3 use errors (1.2%). The three (3) critical use errors were all committed by the same user group, on the same task, during the simulated endoscopic implantation procedure. Importantly, all 3 of the critical use errors are attributable to study artifacts and were associated with aspects of the procedure more generally, meaning they are not specific to BioXmark. Thus, these findings demonstrate that most critical user-device interactions attempted during device use were successfully completed without any form of difficulty or use error.

PERFORMANCE TESTING - BENCH

Bench testing was conducted to demonstrate that the BioXmark performs as expected under the anticipated conditions of use. The following bench testing was conducted to demonstrate the device performance characteristics:

Table 2. Performance testing completed for BioXmark

Test	Purpose	Results
Radiation effects on stability	To determine if radiation causes degradation of the device when exposed to appropriate energy and type of beams	No radiation effects shown on stability.
Calculated mass attenuation curves	To determine attenuation of the subject device	Mass attenuation curves showed that attenuation could be calculated based on elemental composition of BioXmark and X-Ray Mass Attenuation Coefficients from individual elements
Effect of volume and shape	To determine if marker volume and shape affects visibility on X-ray imaging	All markers were visible in X-ray imaging
Artifacts	To assess marker visibility and artifact evaluation	Data provided to support CNR and streaking artifacts. Contrast levels, and streaking index were comparable to currently marketed fiducial markers. Artifacts did not impede the ability of the marker to be used for its intended purpose.

Effects with proton beams	To measure relative proton stopping power	Effects appear to be equivalent or smaller than solid markers.
BioXmark visibility and artifacts in MR	To evaluate marker visibility and artifacts	All volumes of markers tested were identified with low degrees of artifacts.

1. Radiation effects on stability

BioXmark markers were stress tested after exposure to a single irradiation dose of 150 Gy and compared to non-irradiated samples. HPLC analysis was performed at time: 0, ~30, ~60 and >90 days. Irradiation of BioXmark markers with a single dose of 150 Gy prior to storage had no effect on the hydrolysis rate. Marker degradation of non-irradiated samples was comparable to irradiated samples.

An additional study was performed, where 2.5-year-old samples were exposed to an irradiation dose of 150 Gy and compared to the non-irradiated samples. Visual inspection of BioXmark and High-performance liquid chromatography (HPLC) analysis were performed. Data showed no difference between BioXmark exposed to radiation versus the control product.

The stability of BioXmark was also studied under normo-fractionated and single-fraction proton beams to determine its stability if subjected to the conditions expected from proton beam therapy. Four markers were irradiated for a period of 51 days with 43 fractions ranging from 1.44-1.86 Gy resulting in an accumulated dose of 67.4 Gy. Four other markers were irradiated with a single dose of 155.4 Gy. BioXmark showed no signs of deterioration when exposed to proton beams.

In animal studies, absorption of the marker is seen and thought to be due to the increased inflammation following radiation therapy and increased vascularization and flow of fluid, which could lead to an increased resorption rate and a delayed formation of encapsulation typically observed in untreated animals, due to continued disturbance of the tissue. This increased resorption was not seen during the clinical testing in patients, though in some tissues, absorption was seen through months 3-9 followed by volume stabilization. Differences in resorption characterization between animal models and humans has been seen with other devices and in literature. It was concluded that this difference is not due to direct effect of radiation on the marker.

2. Calculated mass attenuation curves

Mass attenuation coefficients as a function of photon energy were calculated for BioXmark after complete ethanol efflux, gold and soft tissue (ICRU-44) based on the elemental composition of BioXmark and X-Ray Mass Attenuation Coefficients from individual elements. Mass attenuation curves were provided. The high total iodine content of ~15 w/w% results in a high mass attenuation coefficient of the marker.

3. Effect of volume and shape on visibility under x-ray imaging

The x-ray contrast of BioXmark markers with a volume of 10 μl shaped into an elongated cylinder (rod-like), a flat cylinder (plate-like) and a sphere was investigated in a thorax phantom using CT-imaging. All markers were casted into 10 w/w% gelatin to mimic the soft tissue environment in terms of background contrast level and an anthropomorphic thorax phantom was used to mimic clinical use in patients. All markers, irrespective of 3D marker shape, were clearly visible in x-ray imaging using clinically relevant settings. A mean contrast level for the spherical BioXmark markers, flat cylindrical BioXmark markers and elongated cylindrical BioXmark markers were in the range of 700-1200 HU, 400-800 HU and 500-900 HU depending on the contouring threshold, resulting in a contrast-to-noise ratio (CNR) of > 110 in all cases.

4. Artifacts

The visibility (contrast-to-noise ratio (CNR)) and evaluation of artifacts were investigated in a thorax phantom. BioXmark was filled into hollow polypropylene spheres. CT imaging was conducted using a clinical multi slice CT scanner. Images were acquired from three different gantry angles. Exposure settings were varied. Visibility of BioXmark and the included commercial markers were based on the CNR. CBCT scans were then performed for each marker using standard exposure settings. All scans were also performed with no marker present for a baseline image with no artifact. CBCT and CT scans were used to quantify artifacts introduced by the included markers in both imaging techniques based on the streaking index (SI). The SI of BioXmark in this study was comparable to solid fiducial markers included in the study.

A second study was performed to assess the visibility and artifacts of smaller volumes of BioXmark (10 μl , 25 μl , 50 μl , 100 μl , 200 μl , 300 μl , and 400 μl). Each marker was cast into gelatin in a hollow low-density polyethylene rod container. Imaging was performed with the filled rod container placed inside a CIRS IMRT thorax phantom. CT scans were performed using a standard clinical lung protocol. The SI of BioXmark for volumes $\leq 200 \mu\text{l}$ (range 10.79-20.14) was significantly lower than for commercial solid soft tissue markers (range 16.23-62.36) while still maintaining high contrast level as measured in HU (699-1925).

5. Effects on proton beam therapy

The relative proton stopping power (RSP) of BioXmark was measured using a multi-layer ionization chamber to determine the water equivalent thickness. To determine the range shift of the proton beam due to BioXmark, the relative ionization of the proton beam was measured with clinical energies between 100 and 230 MeV. The water equivalent thickness for each sample was used to determine the measured RSP for BioXmark. The RSP was calculated using the chemical composition and density of BioXmark. There was good agreement between the calculated (1.174) and measured (1.164) RSP.

BioXmark markers (10 μl , 25 μl , 50 μl and 100 μl) were prepared by injecting the desired volume into distilled water to form spherical, semi-solid, gel markers. All markers were fixed in a gelatin phantom. All markers were investigated with five set-ups with different proton energies, designed to investigate the perturbation of the beam with the markers positioned at different depths relative to the Bragg Peak. For the solid markers the parallel orientation resulted in a higher dose perturbation as the protons travel through the full span of the marker material.

Only the largest of the BioXmark marker perturbed the proton beam in the spread-out Bragg Peak delivery and then a maximum perturbation of 4.8% was found in the deepest film position. No influence of the BioXmark directly after the gelatin phantom could be measured.

6. BioXmark visibility and artifacts in MR

The visibility and introduction of possible artifacts on 3T MRI (T1w FFE and T2w) of BioXmark was investigated. The visibility and possible artifacts of BioXmark (10 µl, 25 µl, 50 µl and 100 µl) in MRI was evaluated in a pancreatic equivalent spherical MRI phantom together with two commercially available solid soft tissue markers. All volumes of BioXmark ranging from 10-100 µl were identified in both T1w FFE and T2w MRI as hypo intense spots with low degree of artifacts compared to solid soft tissue markers.

PERFORMANCE TESTING - ANIMAL

Objectives of the following animal studies were to perform a safety and performance evaluation of the BioXmark under simulated clinical use. All objectives of the evaluations were accomplished. The device has been appropriately evaluated in pre-clinical studies. The following tests were performed:

1. *In vivo* kinetics of ethanol efflux

Injection of BioXmark into tissue causes an efflux of ethanol from the marker into the adjacent tissue based on the mechanism of non-solvent induced phase separation (NIPS). The ethanol efflux kinetics from BioXmark markers *in vivo* were unaffected by the local tissue composition at the target injection site but dependent on the marker injection volume. The required time for complete ethanol efflux was determined to be 15-120 min post-implant. This is considered to be within the timeframe between marker placement and subsequent planning CT scan acquisition used in clinical practice, therefore the kinetics of ethanol efflux does not affect the clinical use of BioXmark.

2. *In vivo* resorption of BioXmark

With ethanol efflux the material forms a highly viscous non-crystalline liquid. It was observed that resorption of the device *in vivo* is via chemical hydrolysis which gradually degrades the surface of the implant.

3. Visualization of BioXmark markers from chronic toxicity study

The objective of this study was to visualize BioXmark markers following necropsy at the day of sacrifice. The rats used were from the chronic toxicity study. Subcutaneous injections at 5 injection sites in the dorsal part of the neck each administered on Day 1 of the study. BioXmark markers were visible in rats after an observation period of one year.

4. Implantation and visibility in a porcine model

The studies were conducted in order to evaluate; i) the usability of BioXmark using the identical equipment (endobronchial ultrasound (EBUS) and endoscopic

ultrasound (EUS)) currently used in the clinic operated by professional pulmonologists; ii) the visibility of BioXmark (Ultrasound-, fluoroscopy- and CT-imaging) following implantation in various tissue types including lymph nodes, liver, lung, and thymus tissue. EBUS, EUS, and percutaneous injection techniques can be successfully used with the test device. Ultrasound allowed real-time monitoring of injection and the test device could be visualized using fluoroscopy after injection throughout the 6 week study. BioXmark markers did not migrate and retained their 3D shape over the course of the study.

5. Use of BioXmark for high-precision radiotherapy in a pancreatic tumor mouse model

A study conducted by Dobiasch et al., 2017 established an orthotopic pancreatic tumor mouse model for high-precision IGRT using BioXmark fiducial marker. BioXmark could be detected by CBCT (SARRP and Skyscan). The position of BioXmark was monitored at least weekly by CBCT and was stable over 4 months. BioXmark was shown to be well tolerated; no changes in physical condition or toxic side effects were observed in comparison to control mice.

6. Evaluation of BioXmark for canine oral tumors for image-guided radiation therapy planning

A study conducted by Clerc-Renaud et al., 2019 evaluated BioXmark to improve identification of the superficial component of oral tumors in dogs with computed tomography imaging. This study demonstrated potential utility for combining liquid fiducial markers with post-contrast computed tomography images for improved oral tumor localization and gross tumor volumes contouring for radiation therapy planning.

7. Evaluation of BioXmark for small animal image-guided radiotherapy applications

A study conducted by Brown et al., 2020 followed the positional stability of the marker at 1 hour, 24 hours, 1 month and 5 months in mice (subcutaneous implantation) without reporting any adverse events or withdrawal of animals from the study. The cumulative data supports that the subject device is safe for use with no toxicological concerns in animal subjects.

SUMMARY OF CLINICAL INFORMATION

Effect of BioXmark on dose distribution *in vivo*

The effect on the dose distribution from BioXmark was investigated for 6 patients treated with radiotherapy (photon therapy) for locally advanced non-small lung cancer. Two algorithms were used to evaluate the effect of dose perturbation from BioXmark. The relative dose difference in Gross Tumor Volume were calculated using both algorithms. The effect on the dose distribution from BioXmark was shown to be negligible.

The following clinical studies were provided to support the Indications for Use of the BioXmark:

A. 310-01 Lung Cancer

The Sponsor collected data from patients having BioXmark implanted prior to radiotherapy. The investigation had a Part A investigating the device in the intended use period and a Part B, a follow up period. Part A of the study was started in February 2014 and Part B was completed in October 2018.

Part A primary endpoint: Stability of the geometrical configuration and visibility of the injected gel marker in 2D kV x-rays and CBCT acquired throughout the treatment course and quantified by vectors and contrast to noise ratio (CNR). BioXmark should be visible using CT or CBCT during radiotherapy with a contrast-to-noise ratio > 2 (160 HU or more). BioXmark does not migrate during radiotherapy using a predefined procedure for capturing and measuring BioXmark marker volume, contrast and positional stability.

Part B primary endpoint: Quantification of risk of pneumonitis and cardiotoxicity estimated by V20 lung volume, mean lung dose and V40 heart volume and late lung tissue damage for DIBH versus free breathing technique (NTCP). These endpoints are not related to BioXmark performance.

Subjects: Twenty (20) subjects were screened, eighteen (18) subjects were found suitable for inclusion and fifteen (15) subjects gave their consent. Thirty-five (35) markers were implanted in the fifteen (15) subjects, 1-3 markers (100 -300 μ l) in each subject. Eleven (11) markers were placed into or near the primary tumor and twenty-four (24) into the hilar or mediastinal lymph nodes. Imaging data were not recorded for two (2) subjects due to progression of their disease (3 markers), hence for these two subjects there are no medical imaging and data are available only for assessment of procedural safety. No subjects were withdrawn or discontinued from the investigation. Two (2) markers were lost between implantation and planning CT, and one marker was not suitable as a marker for radiotherapy (placed in the wall of a tumor cavity and was dispersed). Therefore, a total of twenty-nine (29) markers were followed from implantation through radiotherapy and follow up.

Thirteen (13) subjects completed the 2-month course of radiotherapy and the injected BioXmark markers were visible on all defined imaging modalities (ultrasound, CT, CBCT, 4D CT, 2D kV and fluoroscopy). The markers were visible during the entire treatment period with no change in marker contrast level over time, irrespective of placement (healthy lung tissue, primary tumor, or within PET positive lymph nodes). CT and CBCT scans were acquired during radiotherapy and each marker was automatically contoured in the EclipseTM contouring software by using a Hounsfield Unit thresholding function with a lower limit of 500 HU. The CT and CBCT scans and the contoured markers were subsequently analyzed for marker radiopacity, marker volume and positional stability. Visibility was evaluated by the naked eye by scoring yes/no by the medical physicist and investigator. Constant marker visibility was evident from all scans.

Marker visibility using x-ray imaging was quantitatively assessed by measuring the contrast to noise ratio (CNR). All markers had a $CNR > 2$, the cutoff that determines whether the marker is visible. The CNR of the markers remained constant during the entire treatment period. The recorded clinical images were reviewed for potential artifacts by the investigator and the medical physicist independently. No relevant artifacts caused by the markers were observed.

The majority of BioXmark markers showed minimal change in the 3D vector length from the Center of Volume (CoV) of the tumor/lymph node and the CoV of the marker during the entire treatment period within the pre-defined criteria for migration (<5 mm). Two markers, placed in the tumors of two (2) patients, had a maximal 3D vector length decrease of -8.4 mm and -7.8 mm at the end of treatment indicating possible marker migration. Independent manual assessment by both the medical physicist and the investigator resulted in their judgement that the markers had in fact not migrated. For one (1) patient the large decrease in 3D vector length was attributed to movement of the tumor due to significant shrinkage. The marker remained at the anatomically right spot. For the other, the surrounding tissue changed significantly due to atelectasis, the marker followed the tissue. Migration, therefore, was not observed for any of the twenty-nine (29) markers. Marker migration in lung cancer patients of $\leq 25\%$ during the course of radiotherapy can be considered as a performance that is at least as good as for other soft tissue markers based on clinical experience reported in the literature. Based on no observed migration with the twenty-nine (29) markers one can calculate with 95% certainty that migration of BioXmark in this use case is less than 15%.

Serious adverse events: There were fifteen (15) serious adverse events (SAEs) and twenty-five (25) adverse events reported in part A of the clinical trial, none were reported as device related. There was one adverse event that was not attributed to the device since it was the result of a protocol violation, but there is high probability that the adverse event was marker related. In violation of the protocol, the marker was placed without the use of image guidance. There is a possibility that the marker was placed in a small vessel or highly vascularized area in the tumor, and subsequently “washed out”. An x-ray dense area was observed in the lateral periphery of the lung in close proximity to the pleura. The subject experienced mild pain in relation to respiration lasting less than 5 days. Two markers had been placed in the patient, therefore the patient continued in the study with the one remaining marker in situ.

There were fifty-nine (59) SAE's reported during Part B of the study. No Adverse Device Effects or Serious Adverse Device Effects or Unanticipated Serious Adverse Device Effects, were reported in Part B of Clinical Investigation #310-01 at the 36-month timepoint.

Six (6) out of the originally thirty-five (35) implanted markers were not available for analysis: three (3) because patients did not proceed to radiotherapy, two (2) because they were lost and one (1) because the marker fragmented and was not considered suitable to guide radiotherapy.

Results: BioXmark markers were suitable for image guided radiotherapy for locally advanced lung cancer patients. The marker volume and density were stable through the treatment period. The injected markers stayed within the tumor and/or lymph node position during the course of radiotherapy and were most stable for lymph node injections. Positional shifts were observed for patients with large anatomical changes. The volume of BioXmark markers was slowly reduced over the first 9 months with approximately 35% due to resorption of the marker. Further degradation could not be detected for the 9-26 month follow up period. This observation is in line with the observation from the animal studies that a fibrous capsule is created around the marker. The contrast level remained constant for the 24-month period confirming that the degradation takes part from the surface towards the interior. Migration was

assessed by visually comparing CT scans over time. Eight (8) subjects completed the 36 month follow up and fifteen (15) markers out of the thirty-five (35) originally implanted markers remained during the 36 month follow up period. There was no evidence of migration of any of the markers during the follow up period.

B. Investigator initiated study – Bladder cancer

Between July 2018 and July 2019, twenty (20) patients with non-metastasized unifocal muscle invasive bladder cancer, suitable for chemoradiation, were accrued to this prospective Phase I– II trial. The trial was conducted at a tertiary university medical center. Patients were excluded when there was any contraindication for undergoing an outpatient cystoscopy procedure.

Primary endpoints: Safety of the marker implantation procedure, marker visibility, and positional stability of the fiducial markers over time.

Predefined acceptance criteria for performance and clinical applicability were that 75% of the markers had to remain visible and positionally stable from the CT acquisition for RT planning to the last CBCT, without causing grade three toxicity (CTCAE v4.0). Formally this corresponds to test the hypothesis $H_0: p \leq 0.75$ against the alternative $H_A: p > 0.75$. H_0 is rejected if the lower limit of the 95% CI for the observed performance success in the trial is lower than 0.75.

Patients: Approximately, 3-5 BioXmark liquid markers of ~0.1 ml (0.07-0.15 ml) each were injected in proximity to the bladder tumor, using flexible cystoscopy. Fluoroscopy after injection as visual quality control measurement was used only in the first five (5) patients (19 markers) but because the implantation with the endoscopic technique was successful, further use of fluoroscopy visualization was not deemed valuable. Visibility was evaluated on radiotherapy planning CT acquisition and weekly CBCT during treatment. Visibility was scored dichotomously (visible/non-visible). Serious adverse events (SAE) associated with BioXmark marker were recorded from the moment of injection until the end of chemoradiation treatment or at least 30 days after the marker implantation. Patients were assessed weekly during treatment and 4 weeks following treatment.

In total, seventy-six (76) markers were implanted in twenty (20) patients. One patient died after CT acquisition but before start of treatment from an intercurrent disease cause. There were twenty (20) evaluable patients for marker visibility on CT scan and nineteen (19) evaluable patients for marker visibility and stability on CBCT. All nineteen (19) patients finished treatment as planned.

Of the seventy-six (76) markers implanted, sixty (60) (79% (95% CI 70–88%)) were visible on treatment planning CT scan. All separate spots were continuously classified as clearly visible without artifacts. The preset threshold of 75% was overlapped by the CIs, thereby this Phase I study failed to show a statistical difference with the prospectively defined test. However, the study showed a clear learning curve as visibility scored 58% for the first 5 patients and 86% for the next 15. The investigator concluded that the majority of the lost markers were the result

of the implantation technique learning curve.

In patients where BioXmark was injected successfully, the fiducial marker was determined to be an easy and clinically applicable tool for IGRT in bladder-preserving chemoradiotherapy. Blurring, migration, and fading did not occur during treatment. Furthermore, this study resulted in continuous visibility of the fiducial markers. Positional stability was scored on CT scan and latest CBCT (with comparable bladder filling). Of those visible markers (60) on CT scan, all (100%) remained detectable without displacement until the end of the treatment, defined as visible on the last CBCT at week four of follow-up.

Results: BioXmark was safe to use for patients with non- metastasized unifocal muscle invasive bladder cancer. Two patients experienced grade 2 toxicity, but one of those events may be related to the implantation procedure itself and not the marker. One patient presented with urinary tract infection and the other with hematuria. The hematuria was present before the implantation and stopped during the radiation course.

Pediatric Extrapolation

In this De Novo request, existing clinical data were not leveraged to support the use of the device in a pediatric patient population.

LABELING

The labeling consists of Instructions for Use, device label, outer box label, and implant card.

The instructions for use include device description, storage instructions, indications for use, injection volumes and corresponding radiologic imaging modalities, warning and precaution statements, MR compatibility, shelf life, listing of adverse events, instructions for use (i.e., endoscopic assisted injections, percutaneous injection), disposal, treatment planning information and information related to patient follow-up.

The labeling meets the requirements of 21 CFR 801.109 for prescription devices.

RISKS TO HEALTH

The table below identifies the risks to health that may be associated with the use of Phase-changing fiducial markers for radiation therapy and the measures necessary to mitigate these risks:

Risks to Health	Mitigation Measures
Adverse tissue reaction	Biocompatibility evaluation Animal performance testing

Interference with image-guided radiation therapy or radiotherapy response assessment	Clinical performance testing Non-clinical performance testing Labeling
Treatment delays due to device malfunction, marker migration, or inability to locate marker on imaging	Clinical performance testing Non-clinical performance testing Labeling
Infection	Sterilization validation Shelf life testing Labeling
Inaccurate radiation dose delivery due to incorrect marker positioning, marker migration, or implantation	Clinical performance testing Non-clinical performance testing Usability testing Labeling
Complications due to implantation of marker or marker migration	Clinical performance testing Animal performance testing Labeling

SPECIAL CONTROLS

In combination with the general controls of the FD&C Act, the Phase-changing fiducial marker for radiation therapy is subject to the following special controls:

- (1) Clinical performance data under anticipated conditions of use must evaluate:
 - (i) Risk of marker migration in tissue during the course of radiation therapy through post-treatment follow-up;
 - (ii) The ability to visualize the marker to allow for adequate localization during the course of radiation therapy through post-treatment follow-up;
 - (iii) Risk of device interference with tumor response assessment post-treatment; and
 - (iv) All adverse events.
- (2) Animal performance data under anticipated conditions of use must evaluate device toxicity and the risk of marker migration.
- (3) Non-clinical performance data under anticipated conditions of use must evaluate:
 - (i) Maintenance of physical form throughout the course of therapy and post-treatment follow-up;
 - (ii) Device visibility on one or more radiologic imaging modalities; and
 - (iii) Device interference with radiation dose delivery.
- (4) Performance testing must demonstrate the patient-contacting components of the device are biocompatible.
- (5) Performance testing must support the shelf life of the device by demonstrating continued sterility, package integrity, and device functionality over the labeled shelf life.
- (6) Performance testing must demonstrate device sterility and non-pyrogenicity.
- (7) Usability testing must demonstrate that the device can be positioned as indicated based solely on reading the directions for use.
- (8) The labeling must include:

- (i) A detailed description of the device including materials and composition, chemical and physical properties, a description of the mechanism of the change of phase, and timeframe for achieving final state;
- (ii) Summary of all reported device-related adverse events from clinical testing;
- (iii) Information describing the injection procedure, including any use of image guidance, and the range of compatible injection needle gauges; and
- (iv) A shelf life.

BENEFIT-RISK DETERMINATION

The BioXmark is restricted to prescription use in accordance with 21 CFR 801.109 for the radiographic marking of the lung, bladder and lymph nodes.

Device risks and mitigations:

The risks include: 1) adverse tissue reaction, 2) interference with image-guided radiation therapy or radiotherapy response assessment, 3) treatment delays due to device malfunction, marker migration or inability to locate marker on imaging, 4) infection, 5) inaccurate radiation dose delivery due to incorrect marker positioning, marker migration, or implantation, 6) complications due to implantation of marker or marker migration.

Probable benefits:

In radiation therapy, it is crucial for the physician to accurately locate the intended target volumes in order to focus radiation on the target volumes and spare healthy tissue as much as possible. Accurate target volume localization is made more challenging by organ movement and lack of exact anatomic reference points. The implantation of a fiducial marker near the tumor, or target volume, can facilitate the ease, accuracy, and precision of the localization process. However, in some tissues, implantation can be difficult, and dislocation of implanted markers is observed. As a liquid fiducial marker, BioXmark has the probability to mitigate some of the challenges observed with traditional metal fiducial markers such as imaging artifacts, technical difficulties with implantation, and marker migration or dislodgement. BioXmark is visible on several imaging modalities and creates less interference than the metal fiducial markers. Lastly, evidence has shown that in some tissue types, the marker does not migrate out of the tissue.

Evidence of benefit has only been shown in lung, bladder, and lymph nodes.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

Benefit/Risk Conclusion

In conclusion, given the available information above, for the following indication statement:

BioXmark is indicated for use to radiographically mark lung, bladder and lymph nodes in adult patients for whom it has been determined that radiographical marking of tissue for radiation treatment is indicated for their cancer treatment.

BioXmark is implanted via image guided injection into tissue relevant for radiotherapy planning at a healthcare facility. BioXmark can be implanted in the tumor, lymph nodes

or tissue adjacent to the tumor subject to irradiation or healthy tissue which should not be irradiated.

BioXmark is intended to mark tissue for at least 3 months after implantation.

The probable benefits outweigh the probable risks for BioXmark. The device provides benefits, and the risks can be mitigated by the use of general controls and the identified special controls.

CONCLUSION

The De Novo request for the BioXmark is granted and the device is classified as follows:

Product Code: QUV

Device Type: Phase-changing fiducial marker for radiation therapy

Regulation Number: 21 CFR 892.5727

Class: II