SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro diagnostic immunohistochemistry (IHC) test for detection of MAGE-A4 antigen in formalin-fixed, paraffin-embedded (FFPE) human tissue sections
Device Trade Name:	MAGE-A4 IHC 1F9 pharmDx
Device Procode:	SBL
Applicant's Name and Address:	Agilent Technologies, Inc. 6392 Via Real Carpinteria, CA 93013
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P230016
Date of FDA Notice of Approval:	August 1, 2024

II. INDICATIONS FOR USE

For in vitro diagnostic use.

MAGE-A4 IHC IF9 pharmDx is a qualitative immunohistochemical (IHC) assay using monoclonal mouse anti-MAGE-A4, Clone OTI1F9, intended for use in the detection of MAGE-A4 protein in formalin-fixed, paraffin-embedded (FFPE) synovial sarcoma tissue using the EnVision FLEX visualization system on Autostainer Link 48. MAGE-A4 protein expression in synovial sarcoma is determined by using the MAGE-A4 Tumor Intensity Proportion Score (TIPS), which is the overall percentage of viable tumor cells showing MAGE-A4 nuclear and/or cytoplasmic staining at staining intensity ≥ 2+. The specimen should be considered positive if MAGE-A4 TIPS (≥ 2+) is ≥ 75%.

MAGE-A4 IHC 1F9 pharmDx is indicated as an aid in identifying patients with synovial sarcoma for whom TECELRA® (afamitresgene autoleucel), a MAGE-A4-directed genetically modified autologous T-cell immunotherapy, is being considered.

See the TECELRA® product label for specific clinical circumstances guiding MAGE-A4 testing.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the MAGE-A4 IHC 1F9 pharmDx labeling.

V. DEVICE DESCRIPTION

MAGE-A4 IHC 1F9 pharmDx contains a protocol and reagents required to complete an immunohistochemistry (IHC) staining run on formalin-fixed and paraffin-embedded (FFPE) specimens using the PT Link Pre-Treatment Module and Autostainer Link 48. The principal component of the kit is the mouse monoclonal anti-MAGE-A4 clone OTI1F9 antibody that binds MAGE-A4 protein expressed on FFPE tissue. Each kit includes 15 mL of MAGE-A4 primary antibody (approximately 3 µg/mL protein concentration) and the reagents shown in Table 1, necessary to perform 50 tests in up to 10 individual runs. Wash buffer and hematoxylin are required for the assay but are not included in the kit. PT Link Pre-Treatment Module is required for deparaffinization, rehydration, and target retrieval of the tissues. Cover-slipping is required but can be performed by either manual or automated methods. Results are interpreted using a light microscope by a qualified observer. The supplied kit components are shown in Table 1.

Table 1. Device Kit Components

Reagent	Description	Qty x Vol
Peroxidase-Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent, and 0.015 mol/L sodium azide	1 x 34.5 mL
Monoclonal Mouse Anti-MAGE-A4, Clone OTI1F9	Monoclonal Mouse anti-MAGE-A4 in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.	1 x 15 mL
Negative Control Reagent (NCR)	Monoclonal Mouse (IgG2a) antibody in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.	1 x 15 mL
Visualization Reagent-HRP	Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins in a buffered solution containing stabilizing protein and an antimicrobial agent.	1 x 34.5 mL
DAB+ Substrate Buffer	Buffered solution, containing hydrogen peroxide and an antimicrobial agent.	15 x 7.2 mL
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride in organic solvent.	1 x 5 mL
Target Retrieval Solution, pH 9.0, 50x	Buffered solution, pH 9.0, containing detergent and an antimicrobial agent.	6 x 30 mL

Device Instrument and Software
MAGE-A4 IHC 1F9 pharmDx is performed on Autostainer Link 48 automated staining system using the DakoLink software (v4.1.0). The Autostainer system is designed to mimic the staining steps performed manually by the lab technician. The DakoLink software has been designed to recognize and group MAGE-A4 IHC 1F9 pharmDx reagents, mandating that the reagents are run together. The kit components can only be used on the specified instrument with the MAGE-A4 IHC 1F9 pharmDx protocol. Deparaffinization, rehydration, and target retrieval procedures are performed in the PT Link Pre-Treatment module (PT100/PT101/PT200).

Specimen Preparation
Only FFPE tissues are suitable for use. FFPE tissue specimens should be cut into sections of 4-5 µm. After sectioning, tissues should be mounted on FLEX IHC Microscope Slides or Superfrost Plus slides, and then placed in a 58 ± 2 °C calibrated oven for 1 hour. To preserve antigenicity, tissue sections mounted on slides should be stained within 4 months of sectioning when stored in the dark at 2 to 8 °C (preferred), or at room temperature up to 25 °C. Slide storage and handling conditions should not exceed 25 °C at any point post-mounting to ensure tissue integrity and antigenicity.

Test Controls
Control slides are not supplied as part of the MAGE-A4 IHC 1F9 pharmDx. Instead, positive and negative control tissues (lab-supplied) with known expression should be included in each staining run to ensure the validity of the staining procedure, including reagents, tissue processing, and instrument performance. Synovial sarcoma is the recommended tissue control. In instances where synovial sarcoma is unavailable, squamous non-small cell lung cancer (sqNSCLC) can be utilized. Some sqNSCLC samples were used to supplement some of the analytical studies to support that sqNSCLC can be used as an alternative control tissue. If controls are not fixed, processed, and embedded in the same manner as the patient tissue, the control may only be used as a staining control for reagents and instrument performance.

The kit includes a Negative Control Reagent (NCR) that is used in parallel with the MAGEA4 Clone OTI1F9 primary antibody on patient tissue. The matched negative control reagent aids the reader in differentiating specific staining at the antigen site from tissue-specific background staining that occurs from the reaction with detection chemistry and not the MAGE-A4 primary antibody.

Additional information about the use of controls is available in the product labeling.

Principle of Operation
MAGE-A4 IHC 1F9 pharmDx contains the reagents and protocol required to complete an IHC staining run on FFPE specimens using the PT Link Pre-Treatment Module and Autostainer Link 48. After the PT Link pre-treatment procedure, specimens are first incubated with Peroxidase-Blocking Reagent. Following incubation with the primary monoclonal antibody to MAGE-A4 or the Negative Control Reagent (NCR), specimens are incubated with a visualization reagent consisting of secondary antibody molecules

and horseradish peroxidase (HRP) molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added chromogen results in precipitation of a visible reaction product at the site of antigen. The specimen is then counterstained and cover slipped. Results are interpreted using a light microscope.

Staining Protocol
The staining protocol for MAGE-A4 IHC 1F9 pharmDx on Autostainer Link 48 is as follows:

Rinse in 1x EnVision FLEX Wash Buffer
Peroxidase-Blocking Reagent (2 drop zones x 150 µL): 5 minutes
Rinse in 1x EnVision FLEX Wash Buffer
Mouse monoclonal anti-MAGE-A4 (or NCR) (2 drop zones x 150 µL): 20 minutes
Rinse in 1x EnVision FLEX Wash Buffer
Visualization Reagent-HRP (2 drop zones x 150 µL): 20 minutes
Rinse in 1x EnVision FLEX Wash Buffer
Rinse in 1x EnVision FLEX Wash Buffer: 5 minutes
DAB+ Substrate-Chromogen solution (2 drop zones x 150 µL): 2 x 5 minutes
Rinse in 1x EnVision FLEX Wash Buffer
EnVision FLEX Hematoxylin (2 drop zones x 150 µL): 5 minutes
Rinse in distilled or de-ionized water
Rinse in 1x EnVision FLEX Wash Buffer: 5 minutes
Rinse in distilled or de-ionized water

Interpretation of MAGE-A4 Staining in Synovial Sarcoma
MAGE-A4 IHC 1F9 pharmDx evaluation should be performed by a qualified pathologist using a light microscope; objectives of 4x – 20x magnification are appropriate. The MAGE-A4 stained specimen must contain a minimum of 50 evaluable viable tumor cells to be considered adequate for evaluation.

MAGE-A4 expression in synovial sarcoma stained with MAGE-A4 IHC 1F9 pharmDx is determined by using MAGE-A4 Tumor Intensity Proportion Score (TIPS). To determine the MAGE-A4 TIPS (≥ 2+), the entire viable tumor area must be evaluated.

$$\text{MAGE-A4 TIPS } (\geq 2+) \quad \frac{\#\text{ of MAGE-A4 positive viable tumor cells staining at } \geq 2+\text{intensity}}{\text{Total \# of viable tumor cells}} \times 100$$

When executing the MAGE-A4 TIPS (≥ 2+), levels of MAGE-A4 staining intensity of tumor nucleus and cytoplasm should be evaluated using a 0 – 3+ scale. Staining intensities tend to follow the magnification levels listed in Table 2.

Table 2. Evaluation of MAGE-A4 nuclear and/or cytoplasmic staining intensity

Staining Intensity	Magnification Guidance for MAGE-A4 Score: Nuclear or Cytoplasmic Staining
No Staining (0)	No staining observed at any magnification.
Weak (1+)	Light brown staining is generally identified with the 10x or 20x objective lens. Staining is weak but is perceptible and convincing.
Moderate (2+)	Medium brown staining is generally identified with the 4x or 10x objective lens.
Strong (3+)	Dark brown to black staining is generally identified with the 4x objective lens.

Tables 3 and 4 describe the inclusion and exclusion criteria for the numerator and denominator, respectively. Synovial sarcoma tissue specimens that are tested for MAGE-A4 diagnostic status are scored and considered MAGE-A4 diagnostic negative or positive based on the MAGE-A4 TIPS (≥ 2+) for the whole tissue: The specimen should be considered MAGE-A4 positive if MAGE-A4 TIPS (≥ 2+) is ≥ 75%.

Table 3. MAGE-A4 TIPS (≥ 2+) numerator inclusion/exclusion criteria

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	Viable tumor cells with convincing nuclear and/or cytoplasmic staining (at 2+ and 3+ staining intensity)	• Non-staining viable tumor cells • Viable tumor cells with convincing nuclear and/or cytoplasmic staining at 1+ staining intensity • Non-viable/necrotic tumor cells and/or cellular debris • Apoptotic nuclei/nuclear debris • Tumor cells in areas with obscuring artifacts (i.e., poorly preserved areas and processing artifacts)
Other Cells	Not Included	• Stromal cells • Immune cells • Benign cells • Red blood cells

Table 4. MAGE-A4 TIPS (≥ 2+) denominator inclusion/exclusion criteria

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable tumor cells	• Non-viable/necrotic tumor cells • Apoptotic nuclei/nuclear debris • Tumor cells in areas with obscuring artifacts (i.e., poorly preserved areas and processing artifacts)

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Other Cells	Not Included	• Stromal cells • Immune cells • Benign cells • Red blood cells

For each staining run, tissue slides should be examined in the order recommended in the labeling to determine the validity of the staining run and enable assessment of the staining of the tissue. Patient specimens stained with MAGE-A4 primary antibody and the NCR from MAGE-A4 IHC 1F9 pharmDx should be examined when evaluating MAGE-A4 expression. Specimens stained with NCR must have 0 specific staining and ≤ 1+ nonspecific staining.

The MAGE-A4 IHC 1F9 pharmDx Interpretation Manual for synovial sarcoma is available to users to assist in interpretation of assay results.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is no other FDA-cleared or approved alternative class III IHC assays available for detection of MAGE-A4 in FFPE synovial sarcoma specimens to aid in identifying patients with synovial sarcoma for whom TECELRA, a MAGE-A4 directed genetically modified autologous T- cell immunotherapy, is being considered.

VII. MARKETING HISTORY

MAGE-A4 IHC 1F9 pharmDx has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect MAGE-A4 test results and subsequently improper patient management decisions.

There was no device related serious adverse events (SAEs) or unanticipated adverse device effects (UADEs) reported in the retrospective testing of the phase 2 SPEARHEAD- 1 clinical study using MAGE-A4 IHC 1F9 pharmDx.

IX. SUMMARY OF NON-CLINICAL STUDIES

The nonclinical studies conducted to support the safety and effectiveness of MAGE-A4 IHC 1F9 pharmDx in synovial sarcoma patients include analytical sensitivity, specificity, precision, external reproducibility, robustness, and stability. Where applicable, the cutoff of ≥ 75% tumor cell staining [MAGE-A4 TIPS (≥ 2+)] was applied.

A. Laboratory Studies

1. Analytical Sensitivity

Analytical sensitivity of MAGE-A4 IHC 1F9 pharmDx was tested on 99 unique cases of commercially procured FFPE synovial sarcoma specimens, stages I-IV, using a manufactured production lot. Assessment of MAGE-A4 expression demonstrated staining across a range of 0-100% positive tumor cells and 0-3 staining intensity. The MAGE-A4 positivity rate in commercially procured synovial sarcoma specimens based on MAGE-A4 TIPS (≥ 2+) ≥75% was 30.3%.

2. Analytical Specificity

Specificity testing was conducted to demonstrate that MAGE-A4 IHC 1F9 pharmDx will detect the target antigen in the appropriate tissue elements and cellular compartment (Table 5 & Table 6). In addition, analytical specificity of the anti-MAGE-A4 antibody was tested with western blotting, peptide inhibition (Table 7) and ELISA. Prevalence estimates for MAGE-A4, MAGE-A8, and MAGE-A10 proteins are provided based on literature (Table 8).

a. Immunoreactivity in normal tissues
Table 5 summarizes MAGE-A4 immunoreactivity on the recommended panel of normal tissues. All tissues were FFPE and stained with MAGE-A4 IHC 1F9 pharmDx according to the instructions in the package insert. Nuclear and cytoplasm staining was observed in spermatogonia and spermatocytes in the testis tissue, but not other tissue types tested.

Table 5. Summary of MAGE-A4 Normal Tissue Reactivity

Tissue Type (# tested)	Nuclear Staining: Tissue Elements	Cytoplasmic Staining: Tissue Elements
Adrenal (3)	0/3	0/3
Bladder/Urinary (2)	0/2	0/2
Bone marrow (2)	0/2	0/2
Breast (3)	0/3	0/3
Cerebellum (3)	0/3	0/3
Cerebrum (2)	0/2	0/2
Cervix (3)	0/3	0/3
Colon (3)	0/3	0/3
Esophagus (2)	0/2	0/2
Kidney (3)	0/3	0/3
Liver (3)	0/3	0/3
Lung (3)	0/3	0/3
Mesothelial cells (2)	0/2	0/2
Heart (3)	0/3	0/3
Muscle, skeletal (3)	0/3	0/3

Tissue Type (# tested)	Nuclear Staining: Tissue Elements	Cytoplasmic Staining: Tissue Elements
Nerve, peripheral (3)	0/3	0/3
Ovary (3)	0/3	0/3
Pancreas (2)	0/2	0/2
Parathyroid (3)	0/3	0/3
Pituitary (3)	0/3	0/3
Prostate (3)	0/3	0/3
Salivary gland (1)	0/1	0/1
Skin (3)	0/3	0/3
Small intestine (2)	0/2	0/2
Spleen (3)	0/3	0/3
Stomach (3)	0/3	0/3
Testis (3)	3/3 Spermatogonia 3/3 Spermatocytes	3/3 Spermatogonia 3/3 Spermatocytes
Thymus (3)	0/3	0/3
Thyroid (3)	0/3	0/3
Tonsil (3)	0/3	0/3
Uterus (3)	0/3	0/3

b. Immunoreactivity in neoplastic tissues
Table 6 summarizes MAGE-A4 immunoreactivity on a panel of neoplastic tissues. Nuclear and/or cytoplasm staining was observed in neoplastic tissues. Nonspecific staining ≤ 1+ was observed in bronchioalveolar, clear renal cell carcinoma, malignant melanoma, and laryngeal squamous cell carcinoma. All tissues were FFPE and stained with MAGE-A4 IHC 1F9 pharmDx according to the instructions in the package insert. This nonspecific staining was not recorded as positive staining. There were no unexpected results observed in the tumor specimens tested. The MAGE-A4 staining that was observed in some neoplastic tissues is consistent with report literature.

Table 6. Summary of MAGE-A4 IHC 1F9 pharmDx neoplastic tissue reactivity

Tumor Type	Location/Organ	MAGE-A4 positive /total (n=211)
Adenocarcinoma	Breast, lobular, invasive	0/4
	Breast, mixed ductal and lobular, invasive	0/1
	Cervix	0/6
	Cervix, papillary	0/2
	Colon	1/6
	Colon, mucinous	0/2
	Esophagus	1/3
	Lung, alveolar	0/2
	Lung, papillary	0/2

Tumor Type	Location/Organ	MAGE-A4 positive /total (n=211)
	Pancreas, ductal	0/8
	Prostate, acinar	0/7
	Prostate, ductal	0/1
	Stomach	3/7
	Uterus, endometrial	0/8
	Ovary, serous	1/5
	Ovary, papillary serous	0/3
Adenoid cystic carcinoma	Oral cavity	2/2
Astrocytoma	Brain, interstitial	0/4
Bronchioalveolar carcinoma	Lung	0/3
	Lymph node	0/1
Cholangiocarcinoma	Liver	1/8
Ductal carcinoma		
Ductal carcinoma, Invasive	Breast, nonspecific	0/8
Glioblastoma	Brain	0/4
Hepatocellular carcinoma	Liver	0/8
Large cell carcinoma	Lung, endocrine	0/4
Leukemia	Lymph node	0/3
	Ovary	0/1
Lymphoma		
Diffuse large B-cell	Bone Marrow	0/1
	Testis	1/3
Hodgkin lymphoma	Lymph nodes	0/4
Medullary carcinoma	Breast	0/3
Melanoma	Skin, malignant	0/3
Mesothelioma	Lung	0/1
	Unknown	0/1
Neuroblastoma	Retroperitoneum	1/8
Papillary carcinoma	Thyroid	0/4
Renal cell carcinoma		
Clear cell	Kidney	0/8
Sarcoma		
Leiomyosarcoma	Soft tissue	1/6
Liposarcoma	Small Intestine	0/1
	Soft Tissue	0/1
Malignant fibrous histiocytoma	Soft tissue	0/2
Osteosarcoma	Bone	2/8
Seminoma	Testis	3/8
Signet ring cell carcinoma	Stomach	1/1
Small cell carcinoma	Lung, complex	0/1
	Lung	1/3

Tumor Type	Location/Organ	MAGE-A4 positive /total (n=211)
Squamous cell carcinoma	Cervix	1/8
	Esophagus	4/5
	Larynx	1/4
	Lung	1/4
	Oral cavity	1/2
Urothelial carcinoma	Bladder, invasive papillary	0/1
	Bladder, invasive	2/3
	Bladder, invasive to gallbladder	0/4

c. Western Blot

Western blot (WB) analysis was performed on cell lysates from five cancer cell lines with varying expression levels of MAGE-A4: A431 (epidermoid carcinoma), MDA-MB-436 (breast adenocarcinoma), NCI-H929 (multiple myeloma), NCI-H1299 (large cell lung carcinoma), and NCI-H1581 (non-small cell lung carcinoma). MDA-MB-436 and NCI-H1299 are known to also express MAGE-A10. MAGE-A4 protein was detected as a band slightly above 37 kDa with the anti-MAGE-A4 (OTI1F9) primary antibody and a goat anti-mouse immunoglobulin secondary antibody in the five cell lysates with known relative mRNA expression levels for MAGE-A4. An additional faint high molecular weight band at ~50 kDa, corresponding to MAGE-A10 protein, was observed in two cell lines MDA-MB-436 (breast adenocarcinoma) and NCIH1299 (large cell lung carcinoma). No bands of these sizes were observed in the MAGE-A4 negative cell line: HPAFII (pancreatic ductal adenocarcinoma).

WB analysis was also performed on cell lysates from cancer cell lines with known MAGE-A Family mRNA expression. MAGE-A10 protein was detected as a faint ~50 kDa band in cell lines that express MAGE-A10 without MAGEA4: NCI-H1792 (lung adenocarcinoma), NCI-H510A (small cell lung carcinoma), and Hs-695T (melanoma). No additional bands were detected in cancer cell lines tested with known MAGE-A (A1, A3, A6, A8, A11, A12) mRNA expression pattern. MAGE-A9/A9B was detected as a ~50 kDa band in HEK293T overexpression cell lines expressing high levels of MAGE-A9/9B protein. No bands corresponding to MAGE-A2 and MAGE-A8 were detected in HEK293T overexpression cell lines expressing MAGE-A2 and MAGE-A8, respectively.

d. Peptide Inhibition

Anti-MAGE-A4 antibody (OTI1F9), in addition to reacting with MAGE-A4, cross-reacts with exclusive MAGE-A10 expression in human cancer cell lines and to high levels of MAGE-A8 and MAGE-A9/A9B, individually overexpressed in cell lines. The specificity of the anti-MAGE-A4 antibody (OTI1F9) was evaluated and the epitope was confirmed with peptide inhibition using the MAGE-A4 IHC assay. Table 7 summarizes the results of peptide inhibition with MAGE-A4, MAGE-A8, MAGE-A9/A9B, and MAGE-A10 derived peptides corresponding to

the antibody binding region (epitope) of the anti-MAGE-A4 antibody. Molar excess of different concentrations of MAGE-A4, MAGE-A8, MAGE-A9/A9B and MAGE-A10 derived peptides corresponding to the epitope sequence were incubated with the anti-MAGE-A4 antibody, which was at ready-to-use concentration. FFPE sections of MAGE-A4 positive tissue samples were stained with antibody peptide mixes at the varying peptide concentrations. The staining was compared to a positive control consisting of the anti-MAGE-A4 antibody alone. The anti-MAGE-A4 staining showed a decrease in MAGE-A4 TIPS (≥ 2 +) with increased MAGE-A4, MAGE-A8, MAGE-A9/A9B and MAGE-A10 peptide concentration (Table 7). MAGE-A4 derived peptide showed the highest reduction of IHC signal compared to MAGE-A10 and MAGE-A8 derived peptide at every concentration tested between 2×10^{-7} – 2×10^{-6} M. The IHC signal was completely abrogated in most of the specimens at the lowest concentration of MAGE-A4 peptide of 2×10^{-7} M. MAGE-A10 peptide showed higher reduction in IHC signal compared to MAGE-A8 peptide, but less than MAGE-A4. Compared to MAGE-A4 peptide and MAGE-A10 peptide, MAGE-A8 peptide showed the smallest reduction in IHC signal and did not completely abrogate the IHC signal even at the highest peptide concentration of 2×10^{-6} M for most of the specimens tested. MAGE-A9/9B derived peptide did not decrease IHC signal even at the highest peptide concentration of 2×10^{-6} M for all specimens tested. No decrease in IHC signal was detected with the negative control peptide, which is a scrambled version of MAGE-A4 derived peptide with no sequence homology to the epitope sequence. These results indicate that there are differences in antibody affinity to different MAGE-A proteins with the antibody affinity being highest for MAGE-A4, followed by MAGE-A10, and MAGE-A8. These differences in antibody affinity to different MAGE-A proteins was confirmed with competitive ELISA, in which the antibody concentration was held constant and MAGE-A (A8, A9/A9B and A10) peptides at varying concentrations were allowed to compete with the MAGE-A4 peptide. MAGE-A9/A9B did not compete with MAGE-A4 peptide. MAGE-A8 and MAGE-A10 peptides competed with MAGE-A4 peptide for antibody binding but bind at least 50-fold lesser than MAGE-A4 peptide.

Table 7. Results of peptide inhibition (IHC) with MAGE-A derived peptides

MAGE-A Peptide	Specimen		MAGE-A4 TIPS (≥ 2+) in SS (N =3) Specimens at Different Peptide Molar (M) Concentrations			
			0 M	2×10^{-7} M	1×10^{-6} M	2×10^{-6} M
MAGE-A4	SS	A	100	0	0	0
		B	99	0	0	0
		C	95	0	0	0
MAGE-A8	SS	A	100	100	100	99
		B	99	99	94	60
		C	95	90	75	10
MAGE-A9/A9B	SS	A	98	98	98	98
		B	100	100	100	100
		C	93	93	90	90

MAGE-A Peptide	Specimen		MAGE-A4 TIPS (≥ 2+) in SS (N =3) Specimens at Different Peptide Molar (M) Concentrations			
			0 M	2×10^{-7} M	1×10^{-6} M	2×10^{-6} M
MAGE-A10	SS	A	100	90	0	0
		B	99	65	0	0
		C	95	70	0	0

e. MAGE-A4, MAGE-A8, and MAGE-A10 prevalence estimates in synovial sarcoma
Prevalence was estimated for MAGE-A4, MAGE-A8 and MAGE-A10, as peptides derived from these proteins showed reduction in IHC signal compared to the no peptide reference. The prevalence of MAGE-A4, MAGE-A8, and MAGE-10 was estimated from gene expression data from three independent RNA sequencing datasets. A specimen was considered positive for gene expression if the corresponding gene Transcripts per Million (TPM) value was greater than or equal to 1. The prevalence estimates are presented in Table 8.

Lower prevalence and lower expression levels of MAGE-A8 and MAGE-A10 compared to MAGE-A4 combined with the co-expression pattern of MAGE-A10 with MAGE-A4 indicates that potential antibody cross-reactivity to MAGE-A8 and MAGE-A10 is unlikely to cause a false positive result with the MAGE-A4 IHC 1F9 pharmDx.

Table 8. MAGE-A4, MAGE-A8, and MAGE-A10 prevalence estimates in synovial sarcoma based on gene expression data from three independent RNA sequencing datasets

RNA-Seq Dataset (source)	Sample Type	Sample Size (n)	Percentage of Specimens Expressing MAGE-A (TPM ≥ 1					
			MAGE-A4	MAGE-A8	MAGE-A10	MAGE-A4 & MAGE-A8	MAGE-A4 & MAGE-A10	MAGE-A4. MAGE-A8, & MAGE-A10
CLB/ADAP (high quality WERS)	FFPE	105	73% (77/105)	0% (0/105)	15% (16/105)	0% (0/105)	15% (16/105)	0% (0/105)
TCGA	Fresh frozen	10	80% (8/10)	0% (0/10)	20% (2/10)	0% (0/10)	20% (2/10)	0% (0/10)
Sun et al., 2022	Fresh frozen	10	70% (7/10)	0% (0/10)	20% (2/10)	0% (0/10)	20% (2/10)	0% (0/10)

WERS: Whole Exome RNA-seq

3. Precision

The precision of MAGE-A4 IHC 1F9 pharmDx was evaluated. The confidence intervals for Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and Overall Percent Agreement (OA) were calculated for each sub-study using the percentile bootstrap method. If 100% agreement was observed, confidence intervals (CI) for PPA, NPA and OA were calculated using 95% Wilson Score limits. The results for each sub-study based on synovial sarcoma only is provided below. Lower-bound CI on the NPA point estimate for inter-instrument, inter-operator, inter-lot, and

inter-observer precision was lower than 85% due to low sample size in this rare cancer type and some discordance in the near-cutoff samples. Actual NPA point estimates were > 90% (Table 9).

Table 9. Precision of MAGE-A4 IHC 1F9 pharmDx in synovial sarcoma (SS) tested at one site, MAGE-A4 TIPS (≥ 2+) ≥ 75%

Precision Study	Study Design*	% Agreement (95% CI)
Inter-instrument	• 17 SS specimens ○ 10 MAGE-A4(+) & 7 MAGE-A4(-) • 3 Autostainer Link 48 instruments	PPA 100.0% (88.6–100.0%) NPA 100.0% (84.5–100.0%) OA 100.0% (93.0–100.0%)
Inter-operator	• 24 SS specimens ○ 13 MAGE-A4(+) & 11 MAGE-A4(-) • 3 operators • 1 Autostainer Link 48 instrument	PPA 100.0% (91.0–100.0%) NPA 93.9% (84.8–100.0%) OA 97.2% (93.1–100.0%)
Inter-day	• 24 SS specimens ○ 13 MAGE-A4(+) & 11 MAGE-A4(-) • 5 non-consecutive days • 1 Autostainer Link 48 instrument	PPA 100.0% (94.4–100.0%) NPA 96.4% (89.1–100.0%) OA 98.3% (95.0–100.0%)
Inter-lot	• 17 SS specimens ○ 10 MAGE-A4(+) & 7 MAGE-A4(-) • 3 reagent lots • 1 operator • 1 Autostainer Link 48 instrument	PPA 100.0% (88.6–100.0%) NPA 100.0% (84.5–100.0%) OA 100.0% (93.0–100.0%)
Intra-run precision (repeatability)	• 24 SS specimens ○ 13 MAGE-A4(+) & 11 MAGE-A4(-) • 5 replicates within a run • 1 Autostainer Link 48 instrument	PPA 100.0% (94.4–100.0%) NPA 94.5% (85.5–100.0%) OA 97.5% (93.3–100.0%)
Inter-observer precision	• 36 SS specimens ○ 23 MAGE-A4(+) & 13 MAGE-A4(-) • scored by 3 certified observers • 3 reads / observer • ≥14 days washout period between reads	PPA 96.1% (90.3–100.0%) NPA 94.0% (84.6–100.0%) OA 95.4% (90.7–99.1%)
Intra-observer precision	• 36 SS specimens ○ 23 MAGE-A4(+) & 13 MAGE-A4(-) • scored 3 times by 3 certified observers. • 3 reads / observer • ≥14 days washout period between reads	PPA 99.0% (97.5–100.0%) NPA 99.1% (97.2–100.0%) OA 99.1% (97.8–100.0%)

Positive (+); Negative (-)
TIPS Tumor Intensity Proportion Score.
* All positive specimens used included in study included a range of MAGE-A4 IHC expression

4. External Reproducibility

The reproducibility of MAGE-A4 IHC 1F9 pharmDx was evaluated at three external testing sites (testing laboratories). PPA, NPA, and OA were computed. The confidence intervals for PPA, NPA, and OA were calculated using a two-sided 95% percentile bootstrap method. If 100% agreement was observed, confidence intervals for PPA, NPA and OA were calculated using 95% Wilson score limits. The results for each part are provided below.

The study was assessed in a two-part study: Part A assessed inter- and intra-site reproducibility and Part B assessed inter- and intra-observer reproducibility.

Part A included a set of 19 specimens stained at three laboratories over five non-consecutive days using one reagent lot. One observer at each laboratory evaluated the Part A set of specimens for each day.

The assessment of inter-observer reproducibility (Part B) included 3 independent observers evaluating MAGE-A4 TIPS score for 26 pre-stained tissue sections. The specimens were stained and evaluated three times by each of three external pathologists, with a washout period of 14 to 29-days between reads. All slides were relabeled between rounds/reads to blind the study pathologists from specimen identity from previous evaluations. The slide sets were read by study pathologists trained and certified on the scoring algorithm. Lower-bound CI on the NPA point estimate for inter-observer precision was less than 85% due to low sample size in this rare cancer type and most discordant cases were in the near cutoff samples. Actual NPA point estimates were > 90% (Table 10).

Table 10. Reproducibility of MAGE-A4 IHC 1F9 pharmDx in synovial sarcoma (SS), tested at three external sites (MAGE-A4 TIPS (≥ 2+) ≥ 75%

Reproducibility Study	Study Design*	% Agreement (95% CI)
Inter-site	• 19 SS specimens ○ 10 MAGE-A4(+) & 9 MAGE-A4(-) • 5 non-consecutive days • 3 sites Inter-site analysis was performed between sites on a total of 285 comparisons to majority call.	PPA 94.7% (86.7–100.0%) NPA 100.0% (97.2–100.0%) OA 97.2% (93.0–100.0%)
Intra-site	• 19 SS specimens ○ 10 MAGE-A4(+) & 9 MAGE-A4(-) • 5 non-consecutive days • 3 sites Intra-site analysis was performed for sites on a total of 285 comparisons to majority call.	PPA 97.2% (93.6–99.3%) NPA 99.3% (98.0–100.0%) OA 98.2% (96.1–99.6%)

Reproducibility Study	Study Design*	% Agreement (95% CI)
Inter-observer	• 26 SS specimens ○ 13 MAGE-A4(+) & 13 MAGE-A4(-) • Scored by 3 pathologists (1/site) • 3 study sites • Washout period of 14-29 days between each read Inter-observer analysis was performed between sites on a total of 234 comparisons to majority call.	PPA 95.7% (88.0–100.0%) NPA 91.5% (83.8–98.3%) OA 93.6% (88.0–97.9%)
Intra-observer	• 26 SS specimens ○ 13 MAGE-A4(+) & 13 MAGE-A4(-) • Scored by 3 pathologists (1/site) • 3 study sites • Washout period of 14-29 days between each read Intra-observer analysis was performed for sites on a total of 234 comparisons to majority call.	PPA 98.4% (95.8–100.0%) NPA 99.1% (97.2–100.0%) OA 98.7 % (97.4–100.0%)

Positive (+); Negative (-)
TIPS Tumor Intensity Proportion Score.
* All positive specimens used included in study included a range of MAGE-A4 IHC expression

5. Robustness

Robustness of staining performance of MAGE-A4 IHC 1F9 pharmDx was tested using synovial sarcoma specimens while varying the assay conditions as described below. Agreement estimates were calculated for MAGE-A4 TIPS (≥ 2+) ≥ 75%. The following parameters were evaluated in robustness testing.

a. Target Retrieval Time at three incubation times
   - 18 minutes
   - 20 minutes (standard)
   - 22 minutes
b. Target Retrieval Temperature at three incubation temperatures
   - 95 °C
   - 97 °C (standard)
   - 99 °C
c. Target Retrieval Solution pH at three pH levels
   - pH 8.8
   - pH 9.0 (standard)
   - pH 9.2
d. Target Retrieval Solution Re-use after first use and third use
e. Tissue section thickness at three thicknesses:

- 3 µm
- 4 µm (standard)
- 5 µm

f. Slide type tolerance: Superfrost Plus and FLEX IHC Microscope Slides (standard).

Robustness studies were conducted with 16 synovial sarcoma specimens. PPA, NPA, and OA for pairwise comparison against the consensus diagnostic status and the 95% CI calculated with bootstrap method were evaluated. Acceptance criteria for the study specified that lower bound of the 95% CI would meet or exceed 85.0% for each condition tested. All robustness studies, except slide type, passed acceptance criteria for the conditions tested. The failure was due to the limited number of samples in the study due to the rarity of the tissue and not the performance. The slide type study did result in PPA, NPA and OA point estimates of 100%.

Overall, the robustness studies demonstrated that the use of MAGE-A4 IHC 1F9 pharmDx on synovial sarcoma tissues produced consistent results under the conditions tested when scored at the MAGE-A4 TIPS (≥ 2+) ≥ 75% cutoff.

6. Stability Studies

a. Real Time Stability
The purpose of the real-time stability study was to establish the shelf life of MAGE-A4 IHC 1F9 pharmDx. Three unique kit lots of the device were tested on two replicates for four synovial sarcoma specimens with the following MAGE-A4 TIPS (≥ 2+) expression levels: One MAGE-A4 negative synovial sarcoma specimen (< 75%); One MAGE-A4 positive synovial sarcoma specimen (≥ 75%); and two synovial sarcoma specimens near the MAGE-A4 TIPS (≥ 2+) ≥ 75% cutoff (65% - 85%). One slide from each of these specimens was also stained with the NCR. The lots were stored at 2-8 °C before and after transport (where applicable) and after each in-use/on-board cycle or test point. Testing was performed at specified intervals and compared to testing at time point zero, which served as the reference. The total shelf-life at 2-8 °C was calculated using the shortest passing result for all three test lots. The assigned product shelf-life at 2-8 °C is the total shelf-life minus two months margin of safety. Based on the real time stability study with three lots of MAGE-A4 IHC pharmDx, the stability dating is as follows:

Total Shelf-Life (Includes Margin of Safety): 12 months at 2-8 °C
Assigned Shelf-Life: 10 months at 2-8 °C

b. Transport Simulation
Transport simulation subjects 2-8 °C product class stability test samples to worst case scenario conditions for stock shipments (shipment between manufacturing sites and distributors) and customer shipments (shipment from distribution centers to end user) under ambient conditions. A passing result confirms that product may be shipped without controlled temperatures or specialized packaging for up to two

stock shipments and one customer shipment. One lot of MAGE-A4 IHC 1F9 pharmDx underwent transport simulation at temperatures between -20 °C to 37 °C. Each reagent lot was tested on four intended use specimens for each time point.

Each of the four specimens were tested with the primary antibody in duplicate and NCR in singleton. Based on the results, MAGE-A4 IHC 1F9 pharmDx passed transport simulation.

c. In-Use /On-Board Stability Testing
One lot of MAGE-A4 IHC 1F9 pharmDx underwent in-use/on-board stability testing, which reflects the routine use of the product by the customer. The method involves cycling the test kit from storage temperature (2-8 °C) to ambient temperature and testing on-board the Autostainer Link 48 instrument. One cycle consists of storing the test kits at 2-8 °C for approximately 18 hours and then moving the test kits to room temperature for ≥ 6 hours. Based on the results, the on-board stability for MAGE-A4 IHC 1F9 pharmDx is as follows:

- 10 cycles from 2-8 °C to ambient temperature for at least 6 hours and back to 2-8 °C

d. Cut Section Stability
The stability of the MAGE-A4 antigen in synovial sarcoma cut sections was evaluated after storage at ambient temperature (25 °C) and 2-8 °C. Four synovial sarcoma tissue blocks fixed in 10% NBF (neutral buffered formalin) were selected to represent a range of MAGE-A4 expression. The blocks were cut through and mounted on slides at T0 (testing time point zero). Slide-mounted sections from each block were stained and scored at T0 and at each subsequent study timepoint. The MAGE-A4 TIPS (≥ 2+) and average intensity scores at each timepoint were compared to the scores assigned at the same time point to a T0 section from the same specimen or to the scores given at T0. Data from these comparisons were analyzed to determine cut section stability.

It is recommended that tissue sections mounted on slides are stained within 4 months of sectioning when stored in the dark at 2-8 °C (preferred) or at room temperature up to 25 °C to preserve antigenicity.

B. Animal Studies

None

C. Additional Studies

1. Pre-Analytical Variables
This study assesses the effect of pre-analytical variables on the MAGE-A4 antigen when stained with MAGE-A4 IHC 1F9 pharmDx. The study examined three testis

specimens at five different conditions for fixation time. And nine placenta specimens were evaluated at various ischemic and fixation times.
The study investigated the following:

- Ischemic time between 0 and 9 hours
- Fixation time between 6 and 72 hours

An ischemia time of < 30 minutes and fixation time of 6–72 hours in 10% neutral buffered formalin (NBF) are recommended.

2. Within-Block Heterogeneity
This study assessed intra-block heterogeneity in synovial sarcoma specimens stained with MAGE-A4 IHC 1F9 pharmDx between section across a span of 150 µm. For the purpose of this study, a block is defined as a single tumor specimen from which sequential tissue sections can be taken. Thirty synovial sarcoma specimens were included in this study. Comparison of cut sections from the approximate anterior (cut section number 2), middle (cut section number 30), and posterior (cut section number 55) of the same block were scored by a pathologist. The overall agreement was found to be 100%.

3. Within-Case Heterogeneity
This study assessed intra-case (sister block) heterogeneity between 23 unique synovial sarcoma specimens (consisting of 50 total tissue blocks) stained with MAGE-A4 IHC 1F9 pharmDx. For the purposes of this study, sister blocks are defined as blocks that were embedded separately but contain tissue from the same case (i.e., the blocks are from the same patient). The overall agreement was found to be 78%. For six of the discordant cases, each case had at least one block near the tumor diagnostic cutoff of MAGE-A4 TIPS (≥ 2+) ≥ 75%.

X. SUMMARY OF PRIMARY CLINICAL STUDY

SPEARHEAD-1 (ADP-0044-002, NCT04044768) is a phase 2 single arm open-label clinical trial of ADP-A2M4 engineered T-cells in subjects with advanced synovial sarcoma or myxoid/round cell liposarcoma. The study enrolled 52 and treated 44 HLA-A*02 positive adult patients with MAGE-A4 expressing unresectable or metastatic synovial sarcoma (Cohort 1). The MAGE-A4 expression for patient biopsy tissues was prospectively determined using an IHC clinical trial assay (CTA).

To evaluate the clinical utility of MAGE-A4 IHC 1F9 pharmDx, archived clinical study samples were retrospectively tested with MAGE-A4 IHC 1F9 pharmDx in a bridging study to the CTA.

Data from the clinical study and bridging study were the basis for the PMA approval decision. A summary of the clinical study design and outcomes are presented below.

A. Study Design

The efficacy of TECELRA was investigated in SPEARHEAD-1 (NCT04044768), a single arm, open-label clinical trial that enrolled 52 and treated 44 HLA-A*02 positive patients with MAGE-A4 expressing unresectable or metastatic synovial sarcoma (Cohort 1). Previous treatment must have included either an anthracycline or ifosfamide containing regimen. First-line metastatic treatment with TECELRA was permissible if ifosfamide +/-doxorubicin had been administered in either the pre-operative (neoadjuvant) or postoperative (adjuvant) primary tumor setting. Patients who were intolerant of both anthracycline and ifosfamide must have previously received at least one other type of systemic therapy. Patients positive for HLA-A*02:05 in either allele, or other HLA-A*02 alleles having the same protein sequence as HLA-A*02:05 in the peptide binding domains (P group), were ineligible.

After undergoing leukapheresis for the collection of autologous cells to be processed and manufactured into the therapeutic product, patients underwent lymphodepleting chemotherapy with fludarabine 30 mg/m2/day for 4 days (Day -7 to Day -4) and cyclophosphamide 600 mg/m2/day for 3 days (Days -7 to -5). This was followed by an infusion of TECELRA at a dose of 1.0×10^9 to 10×10^9 transduced cells by a single intravenous infusion on Day 1. Disease monitoring by MRI or CT scan at Baseline, Week 4, Week 8, Week 12, Week 16, Week 24, and then every 2 months until confirmed disease progression was conducted. The primary efficacy outcome measure was Overall Response Rate (ORR) based on confirmed tumor responses per RECIST v1.1 by an independent review. Duration of Response (DOR) is a secondary endpoint of the clinical efficacy.

1. Clinical Inclusion and Exclusion Criteria for synovial sarcoma patients

Key Inclusion Criteria (abbreviated list):

1) Age ≥ 16 and ≤ 75 years at the time the Pre-screening informed consent/assent is signed.
2) Diagnosis of advanced (metastatic or inoperable) synovial sarcoma or myxoid liposarcoma / myxoid round cell liposarcoma (Cohort 1 only) confirmed by cytogenetics. Inoperable refers to a tumor lesion in which clear surgical excision margins cannot be obtained without leading to significant functional compromise.
    - For Synovial Sarcoma (Cohort 1): confirmation by the presence of a translocation between SYT on the X chromosome and SSX1, SSX2 or, SSX4 on chromosome 18 [may be presented in the pathology report as t(X; 18)].
3) Must have previously received either an anthracycline or ifosfamide containing regimen. 1st-line metastatic treatment with ADP-A2M4 is permissible if ifosfamide +/- doxorubicin has been administered in either the pre-operative (neoadjuvant) or post-operative (adjuvant) primary tumor setting. (Subjects who are intolerant of both anthracycline and ifosfamide must have previously received at least one other type of systemic therapy).

4) Measurable disease according to RECIST v1.1.
5) Positive for HLA-A*02:01, HLA-A*02:02, HLA-A*02:03 or HLA-A*02:06 allele via Adaptimmune designated central laboratory testing. HLA-A*02 alleles having the same protein sequence in the peptide binding domains (P group) will also be included. Other HLA-A*02 alleles may be eligible after adjudication with the sponsor.
6) Tumor (either an archival specimen or a fresh biopsy) shows MAGE-A4 expression as defined by the clinical trial assay cutoff. All samples must have been pathologically reviewed by an Adaptimmune designated central laboratory confirming expression.
7) Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1.
8) Left ventricular ejection fraction (LVEF) ≥ 50%.
9) Fit for leukapheresis and adequate venous access can be established for the cell collection.
10) Female subjects of childbearing potential (FCBP) must have a negative urine or serum pregnancy test AND must agree to use an effective method of contraception starting at the first dose of chemotherapy and continuing for at least 12 months, or 4 months after the gene modified cells are no longer detected in the blood, whichever is longer.
    i. OR
    ii. Male subjects must be surgically sterile or agree to use a double barrier contraception method or abstain from heterosexual activity with a FCBP starting at the first dose of chemotherapy and continuing for 4 months thereafter (or longer if indicated in the country specific monograph/label for cyclophosphamide).

Key Exclusion Criteria (abbreviated list):

1) Positive for HLA-A*02:05 in either allele via Adaptimmune designated central laboratory testing. HLA-A*02 alleles having the same protein sequence as HLA-A*02:05 in the peptide binding domains (P groups) will also be excluded. Other alleles may be exclusionary after adjudication with the sponsor.
2) Toxicity from previous anti-cancer therapy must have recovered to ≤ Grade 1 prior to enrollment (except for non-clinically significant toxicities, e.g., alopecia, vitiligo). Subjects with Grade 2 toxicities that are deemed stable or irreversible (e.g., peripheral neuropathy) can be enrolled.
3) History of allergic reactions attributed to compounds of similar chemical or biologic composition to fludarabine, cyclophosphamide or other agents used in the study.
4) History of autoimmune or immune mediated disease. Subjects with hypothyroidism, diabetes, adrenal insufficiency or pituitary insufficiency that are stable on replacement therapy are eligible. Subjects with disorders such as asthma, psoriasis or atopic dermatitis that are well controlled without requiring systemic immunosuppression are also eligible.
5) Symptomatic CNS metastases including leptomenigeal disease. Subject with a prior history of symptomatic CNS metastasis including leptomenigeal disease

must have received treatment (i.e., stereotactic radiosurgery (SRS), whole brain radiation (WBRT) and/or surgery) and be neurologically stable for at least 1 month, not requiring anti-seizure medications and off of steroids for at least 14 days prior to leukapheresis and lymphodepletion. Anti-seizure prophylaxis is permitted. Subjects who have asymptomatic CNS metastases without associated edema, shift, requirement for steroids or anti-seizure medications for the treatment of seizures are eligible.

6) Any other prior malignancy that is not in complete remission. Resectable squamous or basal cell carcinoma of the skin is acceptable. Prior malignancies that have been surgically resected and show no evidence of disease are acceptable.
7) Uncontrolled intercurrent illness
8) Active infection with HIV, HBV, HCV or HTLV
9) Pregnant or breastfeeding.

2. Follow-up Schedule

Disease monitoring by magnetic resonance imaging (MRI) or computerized tomography (CT) scan at Baseline, Week 4, Week 8, Week 12, Week 16, Week 24, and then every 2 months until confirmed disease progression.

Once disease progression was established, no further scans were performed for this study and subjects will switch to the long-term follow-up (LTFU) schedule of visits at Months 2, 3 and 6 followed by 6 monthly visits through Year 5 and annually thereafter for Years 6-15.

3. Clinical Endpoints

The primary efficacy outcome measure was Overall Response Rate (ORR) based on confirmed tumor responses per RECIST v1.1 by an independent review. Secondary Efficacy Endpoints: Duration of Response (DOR) is a secondary endpoint of the clinical efficacy.

B. Study Design of Bridging Study to CTA with MAGE-A4 IHC 1F9 pharmDx

A CTA for MAGE-A4 was used to prospectively screen and identify the MAGE-A4-positive patient population in the SPEARHEAD-1 clinical study.

The Clinical Re-Test/Bridging Study of MAGE-A4 IHC 1F9 pharmDx on synovial sarcoma was a comparison between the CTA and MAGE-A4 IHC 1F9 pharmDx. The objective of the study was to retrospectively test with MAGE-A4 IHC 1F9 pharmDx all available banked FFPE synovial sarcoma specimens from ADP-0044-002 screened/enrolled patients, determine the analytical agreement between MAGE-A4 IHC 1F9 pharmDx and the CTA, which is considered to be the reference assay for the bridging study, and determine the clinical efficacy with MAGE-A4 IHC 1F9 pharmDx. In alignment with the IVD intended use of MAGE-A4 IHC 1F9 pharmDx, analytical concordance and clinical utility results presented herein are limited to SPEARHEAD-1 cohort 1 patients with synovial sarcoma.

Patient samples were tested with MAGE-A4 IHC 1F9 pharmDx to assess assay concordance and support a clinical efficacy bridging study between the CTA and MAGE-A4 IHC 1F9 pharmDx. A pre-specified, prospective analysis of MAGE-A4 status was used to assess the association of MAGE-A4 IHC staining at a pre-specified cutoff with the clinical outcome, and to validate its intended use as a companion diagnostic for TECELRA.

The endpoints of the Clinical Re-Test/Bridging Study of MAGE-A4 IHC 1F9 pharmDx were to assess agreement between the assay and the CTA, in addition to the overall response rate (ORR) and duration of response (DOR) of patients identified using MAGE-A4 IHC 1F9 pharmDx [Market Ready Assay (MRA)].

C. Accountability of PMA Cohort

Table 11 provides a summary of specimen accountability for inclusion in analytical concordance and clinical utility analyses.

Table 11: Summary of PMA Cohort Accountability

Analytical Concordance	108 valid results from both CTA and MRA, Cohort 1
Efficacy Evaluation (cohort 1 only) (29 March 2023 data cut)	52 synovial sarcoma (SS) with valid CTA results 44 (SS) with valid CTA results and received a single infusion of TECELRA 39 (SS) with valid MRA results 30 (SS) MRA+/CTA+

D. Study Population Demographics and Baseline Parameters

SPEARHEAD-1 enrolled 52 synovial sarcoma patients who underwent leukapheresis, 8 of whom did not receive TECELRA due to death (n 3), loss of eligibility prior to lymphodepleting chemotherapy (n 3), withdrawal by patient (n 1), and investigator decision (n 1). There were 44 patients with synovial sarcoma who received a single infusion of TECELRA. Of the 44 patients with synovial sarcoma who received a single infusion of TECELRA in Cohort 1 of SPEARHEAD-1, 4 patients had insufficient tissue for re-testing and 1 had an invalid MAGE-A4 IHC 1F9 pharmDx result. Therefore, 39 synovial sarcoma patients had both valid CTA and MAGE-A4 IHC 1F9 pharmDx results. Of the 39 synovial sarcoma patients treated with TECELRA, 30 patients were MAGE-A4 positive (MAGE-A4 TIPS (≥ 2+) ≥ 75%) based on MAGE-A4 IHC 1F9 pharmDx, 9 patients were MAGE-A4 negative based on MAGE-A4 IHC 1F9 pharmDx.

The baseline characteristics of the 44 patients with synovial sarcoma who received a single infusion of TECELRA were: median age 40.5 years (19 to 73), 50.0% female; 88.6% White, 6.8% Asian, 4.5% Black; ECOG PS of 0 (52.3%), 1 (45.5%), and 2 (2.3%); and 79.5% had stage IV disease at the time of last staging.

Demographic and baseline characteristics by CDx subgroup (e.g., CTA(+)/MRA (+), CTA(+)/MRA (-), overall) for synovial sarcoma patients are described in Table 12. This analysis included 30 patients in the CTA(+)/MRA (+) subgroup and 9 patients in the CTA(+)/MRA (-) subgroup.

Table 12. Demographic and Baseline Characteristics by CDx Subgroup

	CTA + /MRA + (N=30)	CTA (+)/MRA -) (N=9)	Overall (N=39)
Age			
n	30	9	39
Mean (SD)	42.1 (12.59)	42.8 (16.51)	42.3 (13.36)
Median	41.5	45.0	42.0
Min, Max	19, 71	22, 73	19, 73
Sex, n (%)			
Female	17 (56.7)	3 (33.3)	20 (51.3)
Male	13 (43.3)	6 (66.7)	19 (48.7)
ECOG, n (%)			
0	14 (46.7)	4 (44.4)	18 (46.2)
1	16 (53.3)*	5 (55.6)	21 (53.8)*
2	0*	0	0*
Baseline SLD			
n	29	9	38
Mean (SD)	122.52 (89.070)	99.33 (59.494)	117.03 (82.878)
Median	97.00	97.00	97.00
Min, Max	25.0, 326.0	20.0, 175.0	20.0, 326.0
Race, n (%)			
Asian	3 (10.0)	0	3 (7.7)
Black or African American	2 (6.7)	0	2 (5.1)
White	25 (83.3)	9 (100)	34 (87.2)
Stage of cancer at last staging, n (%)			
Stage II	1 (3.3)	0	1 (2.6)
Stage III	1 (3.3)	0	1 (2.6)
Stage IV	24 (80.0)	8 (88.9)	32 (82.1)
Unknown	4 (13.3)	1 (11.1)	5 (12.8)
Histology Grade, n (%)			
G1 – Well differentiated (Low Grade)	0	0	0
G2 – Moderately well differentiated	6 (20.0)	2 (22.2)	8 (20.5)
(Intermediate Grade)	14 (46.7)	5 (55.6)	19 (48.7)
G3 – Poorly differentiated (High	3 (10.0)	0	3 (7.7)
Grade)	7 (23.3)	2 (22.2)	9 (23.1)
G4 – Undifferentiated (High Grade)			
Unknown			
P score (CTA)			
n	30	9	39
Mean (SD)	92.26 (9.545)	50.05 (11.386)	82.52 (20.529)
Median	95.61	46.14	92.38
Min, Max	65.8, 100.0	34.2, 69.0	34.2, 100.0

	CTA + /MRA + (N=30)	CTA (+)/MRA -) (N=9)	Overall (N=39)
P score (MRA)			
n	30	9	39
Mean (SD)	93.67 (8.384)	46.67 (13.323)	82.82 (22.214)
Median	99.50	48.00	91.00
Min, Max	75.0, 100.0	17.0, 62.0	17.0, 100.0

MAGE-A4 status of 'Missing' / or 'Not evaluable' are not being considered.
*Note: ECOG statistics were revised due to a data discrepancy for a patient in which ECOG status was revised to ECOG 1.

E. Safety and Effectiveness Results

1. Safety Results

In general, risks of MAGE-A4 IHC 1F9 pharmDx are associated with the possibility of inaccurate or false results that may lead to mismanagement of patient treat. Analytical validation data supports the test as a reliable method for detecting MAGE-A4 expression.

MAGE-A4 primary antibody, clone OTI1F9, cross-reacts with MAGE-A8 and MAGE-A10 proteins and presents a risk of false positive staining. However, in synovial sarcoma, the prevalence of MAGE-A8 is very low (0% of 105 specimens), and MAGE-A10 prevalence (15% of 105 specimens) is low, while MAGE-A4 prevalence is high (73% of 105 specimens) based on RNA sequencing data. All sixteen MAGE-A10 expressing synovial sarcoma specimens co-express MAGE-A4, and of the sixteen specimens, thirteen express MAGE-A4 at equal or higher levels. Two independent RNA-Seq datasets show concordant MAGE-A4, MAGE-A8, and MAGE-A10 prevalence rates. From peptide inhibition studies in IHC, anti-MAGE-A4 antibody (OTI1F9) shows differential affinity to MAGE-A proteins: MAGE-A4 > MAGE-A10 >> MAGE-A8 >> MAGE-A9/A9B. The low prevalence and expression of MAGE-A8 and A10, co-expression of MAGE-A4 and MAGE-A8/MAGE-A10 in synovial sarcoma, combined with the higher affinity of the anti-MAGE-A4 antibody to MAGE-A4 compared to MAGE-A8 and MAGE-A10 support low risk of cross-reactivity in synovial sarcoma. MAGE-A4 IHC 1F9 pharmDx diagnostic cutoff for MAGE-A4 positivity is high (≥ 75% tumor cell staining at ≥ 2+ intensity). Therefore, staining of MAGE-A8 and/or MAGE-A10 by MAGE-A4 IHC 1F9 pharmDx due to cross-reactivity is unlikely to cause a false positive result.

2. Effectiveness Results

The efficacy of TECELRA (afamitresgene autoleucel) was investigated in SPEARHEAD-1 (NCT04044768), a single arm, open-label clinical trial. An immunohistochemistry (IHC) clinical trial assay (CTA) was used to prospectively screen patient biopsy tissues for MAGE-A4 expression to identify the MAGE-A4 positive patient population in the SPEARHEAD-1 clinical study.

To evaluate the clinical utility of MAGE-A4 IHC 1F9 pharmDx, archived clinical study samples were retrospectively tested with MAGE-A4 IHC 1F9 pharmDx in a

bridging study. Of the 115 synovial sarcoma patients with valid CTA results from the population screened for potential enrollment in SPEARHEAD-1 (Cohort 1), 5 were excluded from statistical analysis due to insufficient tissue for re-testing, and 2 had invalid MAGE-A4 IHC 1F9 pharmDx results. Hence, a total of 108 also had valid MAGE-A4 IHC 1F9 pharmDx results from retrospective testing. Concordance between the CTA and MAGE-A4 IHC 1F9 pharmDx was evaluated in a bridging study in which the diagnostic outcomes from each assay were compared per specimen (Table 13). The conservative estimate for MAGE-A4 positivity rate is 53.0% (61/115) in synovial sarcoma clinical samples based on the MAGE-A4 IHC 1F9 pharmDx cutoff, assuming all 7 excluded samples would have resulted in a negative MAGE-A4 status. Point estimates for positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OA) between the CTA and MAGE-A4 IHC 1F9 pharmDx were 83.6%, 100.0%, and 88.9%, respectively. The level of agreement achieved between the CTA and MAGE-A4 IHC 1F9 pharmDx is shown in Table 14.

Table 13: Analytical Concordance for SPEARHEAD-1 (Cohort 1) Synovial Sarcoma Screening Specimens

		CTA		Totals
		Positive	Negative	
MAGE-A4 IHC 1F9 pharmDx	Positive	61	0	61
	Negative	12	35	47
Totals		73	35	108

Table 14. Analytical concordance for SPEARHEAD-1 (Cohort 1) synovial sarcoma screening specimens (n = 108)

Performance Criteria	Point Estimate of Agreement 95% CI, Wilson Score)
PPA	83.6% (73.4, 90.3)
NPA	100.0% (90.1, 100)
OA	88.9% (81.6, 93.5)

SPEARHEAD-1 enrolled 52 synovial sarcoma patients who underwent leukapheresis, 8 of whom did not receive TECELRA due to death (n 3), loss of eligibility prior to lymphodepleting chemotherapy (n 3), withdrawal by patient (n 1), and investigator decision (n 1). There were 44 patients with synovial sarcoma who received a single infusion of TECELRA. Of the 44 patients with synovial sarcoma who received a single infusion of TECELRA in Cohort 1 of SPEARHEAD-1, 4 patients had insufficient tissue for re-testing and 1 had an invalid MAGE-A4 IHC 1F9 pharmDx result. Therefore, 39 synovial sarcoma patients had both valid CTA and MAGE-A4 IHC 1F9 pharmDx results. Of the 39 synovial sarcoma patients treated with TECELRA, 30 patients were MAGE-A4 positive (MAGE-A4 TIPS (≥ 2+) ≥ 75%) based on MAGE-A4 IHC 1F9

pharmDx, 9 patients were MAGE-A4 negative based on MAGE-A4 IHC 1F9 pharmDx.

For the 44 patients screened as MAGE-A4 positive with the CTA and who were treated with TECELRA, the ORR was 43.2% (95% CI: 28.4, 59.0). The median time to response was 4.9 weeks (95% CI: 4.4 weeks, 8 weeks) by Kaplan Meier estimation. For the 30 patients with MAGE-A4 positive expression (MAGE-A4 TIPS (≥ 2+) ≥ 75%) based on MAGE-A4 IHC 1F9 pharmDx, and who were treated with TECELRA, the MAGE-A4 IHC 1F9 pharmDx+/CTA+ ORR was calculated as 43.3% (95% CI: 25.5, 62.6). Assuming an ORR of 0% in the MAGE-A4 IHC 1F9 pharmDx+/CTA- population, the ORR in the MAGE-A4 IHC 1F9 pharmDx positive population is 43.3% (95% CI: 25.6, 61.1). For the 44 patients screened as MAGE-A4 positive with the CTA and 30 patients with MAGE-A4 positive expression (MAGE-A4 TIPS (≥ 2+) ≥ 75%) based on MAGE-A4 IHC 1F9 pharmDx, the median DOR for either group was 6.0 months [95% CI: (4.6, NR) for CTA+, (4.4, NR) for MAGE-A4 IHC 1F9 pharmDx+/CTA+] (Table 15).

Table 15: Efficacy results for MAGE-A4 positive patients with synovial sarcoma

Endpoint	MAGE-A4 IHC 1F9 pharmDx +/CTA+ (N=30)	TECELRA Treated population (N=44)
Overall Response Rate	43.3%	43.2%
(95% CI [1])	(25.5, 62.6)	(28.4, 59.0)
Complete Response Rate, n%	2 (6.7%)	2 (4.5%)
Partial Response Rate, n%	11 (36.7%)	17 (38.6%)
Median Duration of Response in Months	6.0	6.0
(95% CI [2])	(4.4, NR)	(4.6, NR)
Min, Max	2.1, 36.1+	1.9, 36.1+
Patients with DoR ≥ 6 months, %[2]	51.9%	45.6%
Patients with DoR ≥ 12 months, %[2]	43.3%	39.0%

CI Confidence Interval; NR Not Reached.
Response is defined as achieving a complete response (CR) or partial response (PR) according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.1
Duration of response only applies to patients with confirmed CR or confirmed PR
[1] Two-sided 95% confidence interval based on exact Clopper-Pearson (exact Binomial) method
[2] Two-sided 95% confidence interval and % of patients with response duration ≥ 6 and ≥ 12 months based on Kaplan-Meier method

3. **Pediatric Extrapolation**
   The data from SPEARHEAD-1 extrapolates a reasonable assurance of effectiveness and safety regarding the use of MAGE-A4 IHC 1F9 pharmDx in the older adolescent population (i.e. 18 years of age to less than 22 years of age) and the adult population. The safety and effectiveness of TECELRA has been established in patients 18 years and older for synovial sarcoma whose tumor

expresses the MAGE-A4 antigen (please see the TECELRA product label for specific clinical circumstances guiding MAGE-A4 testing).

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study and bridging study included multiple laboratory investigators. None of the laboratory investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XIII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

Not applicable

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The ORR met the protocol-defined criterion for demonstrating efficacy: the lower limit of the 95% exact Clopper-Pearson CI was greater than the prespecified null hypothesis rate of 18% in the synovial sarcoma Cohort 1 group. The ORR in the MAGE-A4 positive Cohort 1 synovial sarcoma group is 43.3%, as identified as positive by MAGE-A4 IHC 1F9 pharmDx. Compared to the clinical trial assay population (CTA positive) in which the ORR is 43.2%, the clinical utility in the population selected by the MAGE-A4 IHC 1F9 pharmDx is comparable.

B. Safety Conclusions

Safety of the device for patient management is related to safety and efficacy of the therapeutic. TECELRA infusion with prior administration of fludarabine and cyclophosphamide lymphodepleting chemotherapy was well tolerated and had a favorable safety profile in subjects with synovial sarcoma. No on-study deaths were related to the TECELRA infusion. For adverse events of special interest (AESIs), all cytokine release syndrome (CRS), Immune Effector Cell- Associated Neurotoxicity Syndrome (ICANS), and prolonged cytopenia events were reversible and successfully clinically managed per the study protocol. In general, risks of MAGE-A4 IHC 1F9 pharmDx are associated with the possibility of inaccurate or false results that may arise due to the failure of the device to perform as expected, or failure to correctly interpret test results.

C. Benefit-Risk Determination

The probable benefits of this device are based on the data collected in a bridging study to ADP-0044-002 SPEARHEAD-1 clinical study which demonstrated improved overall response rate and duration of response to treatment with TECELRA over available second-line therapies in patients with advanced synovial sarcoma who were MAGE-A4 positive. The risks associated with MAGE-A4 IHC 1F9 pharmDx are the possibility of inaccurate or false results, which may lead to patients having no benefit from the treatment. The safety and efficacy of TECELRA in MAGE-A4 positive patients was determined to have clinical benefit when compared to the risks. The MAGE-A4 IHC 1F9 pharmDx analytical validation conducted supports the test as a reliable method for detecting MAGE-A4 expression in synovial sarcoma.

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

In conclusion, given the available information above, the data support that, for adult synovial sarcoma patients who are being considered for treatment with TECELRA, the probable benefits of MAGE-A4 IHC 1F9 pharmDx use outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of MAGE-A4 IHC 1F9 pharmDx when used in accordance with the indications for use and product labeling. The provided studies support use of MAGE-A4 IHC 1F9 pharmDx as an aid in identifying patients with synovial sarcoma for whom TECELRA, a MAGE-A4 directed genetically modified autologous T-cell immunotherapy is being considered.

XV. CDRH DECISION

CDRH issued an approval order on August 1, 2024.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.