Development of MAGE-A4 IHC 1F9 pharmDx for Melanoma-Associated Antigen 4 (MAGE-A4) Detection in FFPE Synovial Sarcoma Specimens for Afamitresgene Autoleucel Therapy

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Introduction

Melanoma Associated Antigen Gene-A4 (MAGE-A4) is a cancer-testis antigen localized in both the nucleus and cytoplasm. It is expressed in spermatogonia within the testis and in the villous and extravillous trophoblasts of the placenta.[1] MAGE-A4, like other cancer-testis antigens, is aberrantly and highly expressed in various tumor types and is immunogenic, making it a promising target for cancer immunotherapy.[2]

MAGE-A4 is notably overexpressed in synovial sarcoma and other malignancies. Its overexpression may facilitate cancer cell proliferation and survival by inhibiting apoptosis and growth arrest.[3]

MAGE-A4 IHC 1F9 pharmDx is a qualitative immunohistochemistry assay developed as a companion diagnostic for afamitresgene autoleucel therapy to detect MAGE-A4 expression in synovial sarcoma specimens.[4] The assay's analytical performance has been validated on the Autostainer Link 48 Platform using mouse monoclonal anti-MAGE-A4 antibody, clone OTI1F9, in formalin-fixed paraffin-embedded (FFPE) human synovial sarcoma.

Methods

Analytical validation of the MAGE-A4 IHC 1F9 pharmDx assay included sensitivity, precision, and robustness testing of MAGE-A4 expression in Synovial Sarcoma (SyS) and squamous Non-Small Cell Lung Carcinoma (sqNSCLC) specimens based on the MAGE-A4 TIPS (≥ 2+) ≥ 75% cutoff. Unless otherwise noted, testing was performed on commercially-procured human formalin-fixed, paraffin-embedded (FFPE) SyS and sqNSCLC tissues*. sqNSCLC specimens were used in the analytical performance evaluation to supplement sample size and support the use of sqNSCLC as a positive control due to the rarity of SyS.

MAGE-A4 protein expression was determined 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+ (Table 1).

SyS and sqNSCLC data were analyzed together using negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) with two-sided 95% bootstrap confidence intervals (CIs) based on MAGE-A4 binary status (positive/negative) at the cutoff. The following acceptance criterion (AC) was applied: the lower bound (LB) of the two-sided 95% CI computed on percent agreement (NPA, PPA, and OA) ≥ 85%.

*The data and biospecimens used in this project was provided by Contract Research Ltd (Charlestown, Nevis) with appropriate ethics approval and through Azenta Life Sciences.

Methods Continued

Staining and Scoring

The MAGE-A4 IHC 1F9 pharmDx assay shows nuclear and/or cytoplasmic staining of tumor cells.

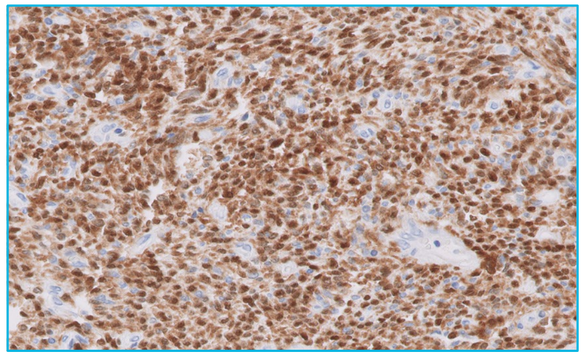

Figure 1. Synovial sarcoma specimen stained with MAGE-A4 IHC 1F9 pharmDx showing a dynamic range (1+, 2+ and 3+) of nuclear and/or cytoplasmic staining of tumor cells

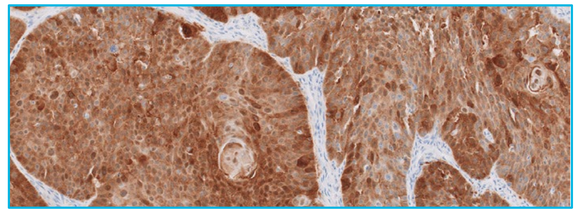

Figure 2. sqNSCLC specimen stained with MAGE-A4 IHC 1F9 pharmDx showing weak to moderate (1+ and 2+) nuclear and/or cytoplasmic staining of tumor cells

Table 1. Intensity Scale

Intensity Bucket	Staining Intensity	Example
3+	Strong Staining	
2+	Moderate Staining	
1+	Weak Staining	
0	No Staining	

Results

MAGE-A4 IHC 1F9 pharmDx demonstrated acceptable performance characteristics (sensitivity, precision, robustness) and met all predefined AC.

Results Continued

MAGE-A4 IHC 1F9 pharmDx detected a dynamic range of staining levels in SyS and sqNSCLC (Figures 3 and 4).

The prevalence of MAGE-A4-positive cases per the cutoff in the commercially procured FFPE SyS specimens tested was 30.3%.

Sensitivity

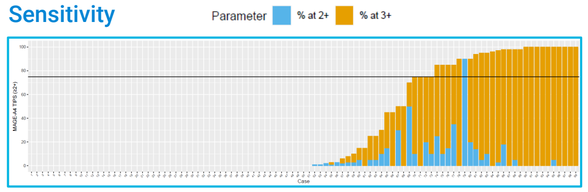

Figure 3. Sensitivity in SyS specimens

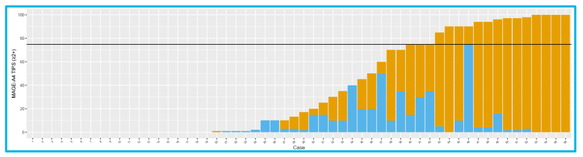

Figure 4. Sensitivity in sqNSCLC specimens

Precision

Observer Precision

To assess the inter-observer and intra-observer precision, 76 SyS and sqNSCLC specimens were tested and evaluated by certified observers, each performing three independent reads. Negative percent agreement (NPA), positive percent agreement (PPA), and overall percent agreement (OA) was calculated for inter-observer precision and intra-observer precision based on the MAGE-A4 TIPS (≥ 2+) ≥ 75% cutoff.

The MAGE-A4 TIPS (≥ 2+) scoring algorithm is practical, transferrable, and reproducible per the assessment of inter/intra-observer percent agreement point estimates being greater than 93%.

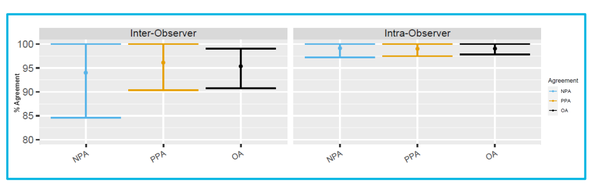

Figure 5. Inter- and Intra- observer precision for SyS specimens

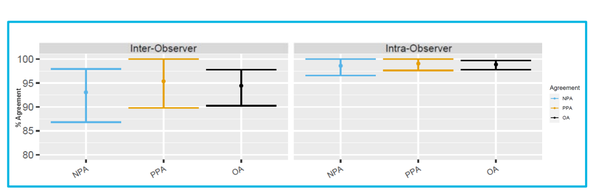

Figure 6. Inter- and Intra-observer precision for sqNSCLC specimens

Results Continued

Laboratory Precision

Laboratory precision evaluated the conditions listed in Table 2. A minimum of 27 SyS and sqNSCLC specimens were used in each sub-study. Overall agreement between test conditions within each study was calculated for each sub-study. Two-sided 95% CIs for OA were calculated using a non-parametric percentile bootstrap method. Two-sided 95% Wilson Score CIs were generated for sub-studies with 100% OA point estimates.

Table 2. Laboratory Precision Results

Sub-study	Conditions	Overall Agreement % (95% CI)
Intra-run	Five replicates within the same run	98.5 (95.9 – 100.0)
Inter-day/run	Three non-consecutive days/runs	97.4 (94.4 – 100.0)
Inter-instrument	Three instruments	98.8 (96.3 – 100.0)
Inter-operator	Three operators	97.4 (94.9 –100.0)
Inter-lot	Three unique reagent lots	100.0 (95.5 – 100.0)

Robustness

Robustness evaluated the conditions listed in Table 3. A total of 35 specimens were used in each sub-study. Overall agreement between test conditions within each study was calculated for each sub-study. Two-sided 95% CIs for OA were calculated using a non-parametric percentile bootstrap method. Two-sided 95% Wilson Score CIs were generated for sub-studies with 100% OA point estimates.

Table 3. Robustness Results

Sub-study	Conditions	Overall Agreement % (95% CI)
Target Retrieval Time	18–22 minutes	98.1 (95.2 – 100.0)
Target Retrieval Temperature	95–99 °C	99.0 (97.1 – 100.0)
Target Retrieval pH	8.8–9.2	100.0 (96.5 – 100.0)
Target Retrieval Re-use	1st and 3rd use	98.6 (95.7 – 100.0)
Tissue Thickness	3–6 µM	100.0 (97.3 – 100.0)
Slide Type	FLEX IHC and Superfrost Plus	100.0 (94.8 – 100.0)

Conclusions

These studies demonstrated high analytical assay performance and observer scoring precision of the MAGE-A4 IHC 1F9 pharmDx assay in SyS and combined with sqNSCLC specimens at MAGE-A4 TIPS (≥ 2+) ≥ 75%.

References

1. Simpson, A. J.; Caballero, O. L.; Jungbluth, A.; Chen, Y. T.; Old, L. J. Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer 2005, 5 (8), 615-625.
2. Iura, K.; Kohashi, K.; Ishii, T.; Maekawa, A.; Bekki, H.; Otsuka, H.; Yamada, Y.; Yamamoto, H.; Matsumoto, Y.; Iwamoto, Y.; et al. MAGEA4 expression in bone and soft tissue tumors: its utility as a target for immunotherapy and diagnostic marker combined with NY-ESO-1. Virchows Arch 2017, 471 (3), 383-392.
3. Bhan, S.; Chuang, A.; Negi, S. S.; Glazer, C. A.; Califano, J. A. MAGEA4 induces growth in normal oral keratinocytes by inhibiting growth arrest and apoptosis. Oncol Rep 2012, 28 (4), 1498-1502.
4. Hong, D. S.; Van Tine, B. A.; Biswas, S.; McAlpine, C.; Johnson, M. L.; Olszanski, A. J.; Clarke, J. M.; Araujo, D.; Blumenschein, G. R., Jr.; Kebriaei, P.; et al. Autologous T cell therapy for MAGE-A4(+) solid cancers in HLA-A*02(+) patients: a phase 1 trial. Nat Med 2023, 29 (1), 104-114.

For in vitro diagnostic use