INTRODUCTION

NY-ESO-1 is a cancer testis antigen with documented expression in a broad range of tumors including lung, breast, ovarian, bladder, and liver cancers, melanomas, sarcoma, and myeloma. Almost all published data for NY-ESO-1 antigen expression are in tumor tissues. The nature of antigen variation and may be attributed to differences in methods or tissue quality between labs.

Cedex is developing an immunotherapy, CDA-1401, targeting NY-ESO-1 expressing cancers. CDA-1401 is a fusion protein composed of a fully human monoclonal antibody specific for a determinate NY-ESO-1 epitope linked to a flexible linker. The direct targeting of NY-ESO-1 to dendritic cells has shown robust stimulation of NY-ESO-1-specific CD4 and CD8 responses in preclinical models. In Phase 1 clinical trial, to support further development of CDA-1401, we investigated diagnostic assays for determining NY-ESO-1 expression in tumor tissues.

Immunohistochemistry (IHC) and quantitative RT-PCR (qRT-PCR) assays were developed to determine NY-ESO-1 expression in human tumors and normal adjacent tissues (NAT). A qRT-PCR assay to detect LAGE-1, a cancer testis antigen with significant DNA homology to NY-ESO-1, was also developed. An antibody to detect NY-ESO-1 by IHC was commercially available but no antibody was available that could be LAGE-1 specific.

After development and validation, assays were used to survey mRNA message and antigen expression in tumor and NAT of different tissue types.

MATERIALS & METHODS

- FFPE tumor tissues and NAT were procured (Molecular DM).
- NY-ESO-1 mouse mAb (Sigma); red chromogen (skin tissues); DAB chromogen (other tissue types).
- IHC and RT-PCR stains were performed on tissue sections stained with hematoxylin.
- Controls were normal testis, 1-2 negative cell line(s) (SK-OV-3 +/- OV-CAR-3), and DAB chromogen (other tissue types).

Intra-Run Precision: 3 replicates on a single run

NY-ESO-1 IHC ASSAY DEVELOPMENT & VALIDATION

- Selectivity/specificity of NY-ESO-1 and LAGE-1 assays.
- Red stain (3 samples)
- DAB stain (4 samples)
- Selective binding verified with cell lines positive or negative by qRT-PCR.
- Single run: Average CV = 0% stained cells, H-score = 33%
- Red stain (3 samples)
- DAB stain (4 samples)
- Average CV = 0% stained cells, H-score = 19%
- Intra-Run Precision: 6 separate runs

POSITIVE TUMOR VS. NORMAL ADJACENT TISSUES

- Both IHC and RT-PCR assays are validated for clinical trials.
- RT-PCR assay designed to detect both NY-ESO-1 and LAGE-1. NY-ESO-1 does not cross-react with LAGE-1.
- High correlation between IHC and RT-PCR, few discrepant cases. This study used a single run; however, for optimal (i.e., 95% confident to section) or even unequivocal tissue quality impacts biomarker data and may lead to false decisions in clinical trials.
- Additional testing with good quality specimens may further verify the positivity signal or in some cases, the variation in staining may be due to the clinical disease.
- Considering the pros and cons of each platform, implementing these assays, in particular, a RT-PCR assay in a preclinical setting may help the scientists (e.g., identify patients who could benefit from NY-ESO-1 vaccines, such as CDA-1401).

RESULTS

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<tr>
<th>Protein</th>
<th>NY-ESO-1</th>
<th>LAGE-1</th>
<th>Positivity</th>
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<td>NY-ESO-1</td>
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<td>15%</td>
<td>100%</td>
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<td>LAGE-1</td>
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- Samples from 10 patients with tumors and 5 patients with NAT.
- NY-ESO-1 and LAGE-1 IHC staining was quite clean (no background noise), staining of 1-2% cells positive.
- NY-RT-PCR signal Ct>35.00 considered positive.

CONCLUSIONS

- Both IHC and RT-PCR assays are validated for clinical trials.
- RT-PCR assay designed to detect both NY-ESO-1 and LAGE-1. NY-ESO-1 does not cross-react with LAGE-1.
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- NY-ESO-1 and LAGE-1 Positivity

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- Red Staining H&N (20x) vs RT-PCR

- RT-PCR and IHC assays for detection of cancer antigen NY-ESO-1 in human tissues.

- NY-ESO-1 IHC assay

- Selectivity testing

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- RT-PCR assay

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- Positive Tumor vs. Normal Adjacent Tissues

- POSITIVITY ACROSS TUMOR TYPES

- NY-ESO-1 and LAGE-1 IHC assay

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