**Abstract**

The field of immuno-oncology (IO) covers a broad set of research disciplines and presents a highly varied set of experimental requirements. Experimental challenges include validation of ontologically diverse cell populations and functional response. Two technologies that have great potential for the clinical adoption of immune markers is the HTG EdgeSeq™ system, a targeted, high-throughput, RNA sequencing assay. The HTG EdgeSeq™ system combines HTG's proprietary quantitative nuclease protection assay (uRNA) chemistry with next-generation sequencing. Functional response is evaluated by expressing the biological readout(s) of interest. This assay shows high reproducibility and dynamic range for a broad set of immune markers. The assay also requires very little sample input (≤ 0.78 mm FFPE), making the assay adaptable to small clinical specimens. The assay requires very little sample input (≤ 0.78 mm FFPE), making it adaptable to small clinical specimens.

**Methods**

- **Sample Input**
  - Tissue samples are input into the HTG EdgeSeq™ Immuno-Oncology Assay.
  - The assay is performed on FFPE samples.$^{50}$

- **Gene Expression**
  - The assay measures gene expression levels using a set of immune markers.$^{50}$

- **Gene Expression Reproducibility**
  - Reproducibility is measured on at least three FFPE tissue samples from DLBCL patients with progression.$^{50}$

- **Assay Dynamic Range**
  - The assay covers a broad set of immune markers.$^{50}$

**Results**

- Similar immune cell compositions using classic IHC markers
- Different tumor immune response seen using new markers

**Conclusions**

- Do not require RNA extraction from samples tested.
- Are amenable to small clinical specimens - require very little sample input (< 2 mm FFPE tissue).
- Detect expression of several hundreds of genes in different sample types tested.
- Have excellent reproducibility (p < 0.05).
- Are linear over wide range of sample inputs.
- Display similar biology across multiple immune markers with different assays.
- Identify differential gene expression within tumor type.
- Identify differential gene expression between progresses and non-progressors.