ImmunoINTEL™: A flow-cytometry based platform for characterization and quantitative functional interrogation of tumour and immune populations contained within the Tumour-Microenvironment

Puneet Singh[1], Paulina Sindrewicz-Góral[1], Nupur Gupta[1], Seija Nevalainen[1], Santosh Putta[2], Norman Purvis[1]

[1]Pierian Biosciences, Liverpool, UK
[2]BioLegend Inc, San Diego, CA, USA

ABSTRACT

Background: Tumour heterogeneity makes each patient tumour unique, and it can significantly impact patient outcomes. Traditional methods of analysis of TME and tumour-infiltrating leukocyte (TIL) rely on a limited selection of histology and IHC markers, mutational analysis by PCR and NGS. However, most traditional methods fall short in adequately describing tumour cell heterogeneity, TIL functional status, and expression of immune checkpoint receptors and their ligands. More recently, single-cell transcriptomic profiling has demonstrated more complete characterization of TME using either scRNA-Seq or scDNA-Seq, but these are costly and not widely available for use.

Methods: Quantitative multi-parameter flow cytometry panels and automated sample/data processing procedures are employed to support: 1) Phenotypic and functional analysis of TILs to characterize the immune status within TME and 2) Quantification of TIL and tumour cell (co-)expression profiles of immunomodulatory receptors (IMR) and ligands (IMR-L).

Results: Antibody cocktails were specially designed to utilize 37 fluorophore signatures in a single tube that can be differentiated using a spectral flow cytometer. Phenotypic composition of all major cell-types was evaluated. The panels were complemented with drop-in IMR and IMR-L markers profiling the functional status of TILs, and to study the cytotoxic potential of tumour TILs.

Conclusions: Stringent QC processes were developed and implemented to ensure quality and precision of phenotypic and functional analyses of dissociated TILs and tumour cells, and quantitative characterization of IMR and/or IMR-L interactions.

INTRODUCTION AND OBJECTIVES

ImmunoINTEL™ platform delivers clinically important information on immune cell landscape within the tumour microenvironment (TME)

- Characterization of tumour-infiltrating leukocytes (TILs) within tumour microenvironment (TME) is key for targeted immunotherapies. However, traditional approaches fail to adequately address the heterogeneity of the TME and detect rare immune subsets present within the microenvironment.
- Our flow cytometry-based ImmunoINTEL™ platform can be used for identification and quantitative characterization of the various immune cell populations within TME. Thus, by providing a detailed phenotypic subset analysis of TILs as well as information on their functional status (anti-tumour, pro-tumour), ImmunoINTEL™ has a potential to serve as a treatment directing assay for immunotherapeutics.
- The platform can also deliver information on the immune checkpoint receptor/ligand interactions (such as PD-1/PD-1L, TIGIT/CD112, TIGIT/CD155, and OX-40/OX-40L) within the tumour microenvironment which could help to identify potential responders to immune checkpoint inhibitors
- The ImmunoINTEL™ platform is utilised as a part of our ongoing ChemoINTEL Algorithm Development Study. Our main objective is to develop a platform that will guide the selection of the most effective immunotherapy, predict response to checkpoint inhibitor-specific biologics and help to minimize toxicities of combined immunotherapies.

METHODS AND WORKFLOW

The ImmunoINTEl™ platform utilizes a semi-automated process for sample processing and computational sciences for data analysis

- ImmunoINTEL™ is a multi-parameter spectral flow cytometry assay designed to utilize 37 different fluorophore signatures in a single tube. Additional information on IMR/IMR-L interactions is obtained by complementing the backbone antibody cocktail with drop-in IMR and IMR-L markers
- Highly standardized procedures and tools: tumour digestion, tumour cell enrichment, cell staining, and flow cytometry acquisition are performed according to the SOPs developed in-house and using validated reagents and QC'd equipment
- Automated assay: utilises liquid handling workstations from Hamilton Robotics, web-based software for batch sample/plate processing and analysis. Can be further automated to use a plate washer for antibody staining procedures and a 40-tube rack or 96-deep well plate with Cytek Aurora
- Computational sciences platform: web-based proprietary and customizable Ryvett software is used for FCS batch sample/plate processing, automated gating, flow cytometry metrics extraction, analysis and visualisation. Further visualisation and data analysis is carried out using Tableau Cloud.

The ImmunoINTEL™ ™ technology

- Samples are shipped to Pierian Biosciences under temperature-controlled conditions for processing within 24 hours of surgical resection

Pre-Analytical Steps
- Sample collection and shipment
- Sample receipt and processing within 24 hours

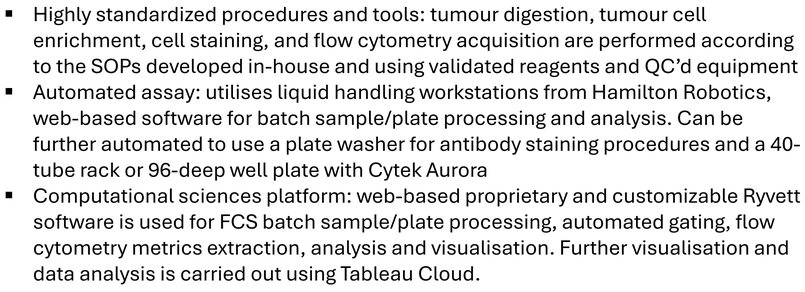
Sample Processing
- Semi-automated dissociation of tissues into single-cell suspensions using benchtop gentleMACS™ dissociator
- Automated enrichment of tumour cells using benchtop autoMACS™ ProSeparator

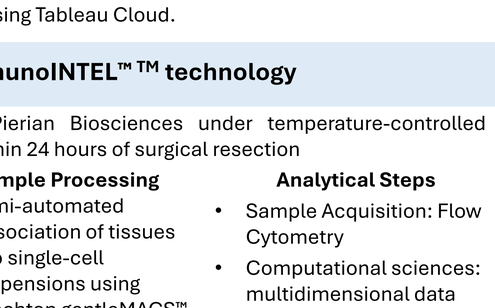
Analytical Steps
- Sample Acquisition: Flow Cytometry
- Computational sciences: multidimensional data extraction/analysis software (Ryvett software)

Cloud-based software used in ImmunoINTEL™ for automated experiment design and multivariate data analysis enables HTP processing and minimizes errors

Experimental design: templating and rapid plate cloning; export all meta-data to layout files (antibodies, modulators, sample, cytometer settings, and compensation).

Manual or automated plate-based data acquisition on Cytek Aurora





Comprehensive and integrated cloud-based data analytics (automated gating, metrics and data extraction, data mining, analysis and visualisation).

RESULTS

Figure 1. ImmunoINTEL™ metrics extraction, reporting and visualization for high complexity multi-dimensional data sets are performed in an automated system

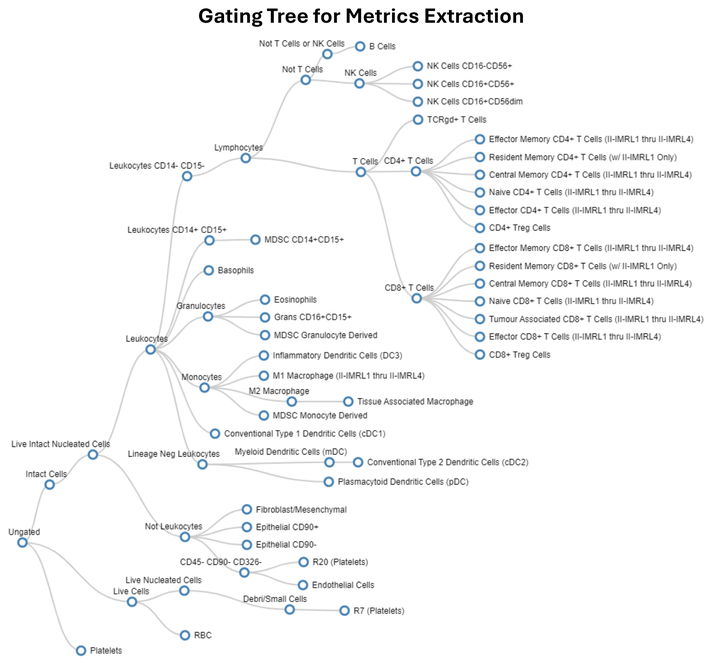
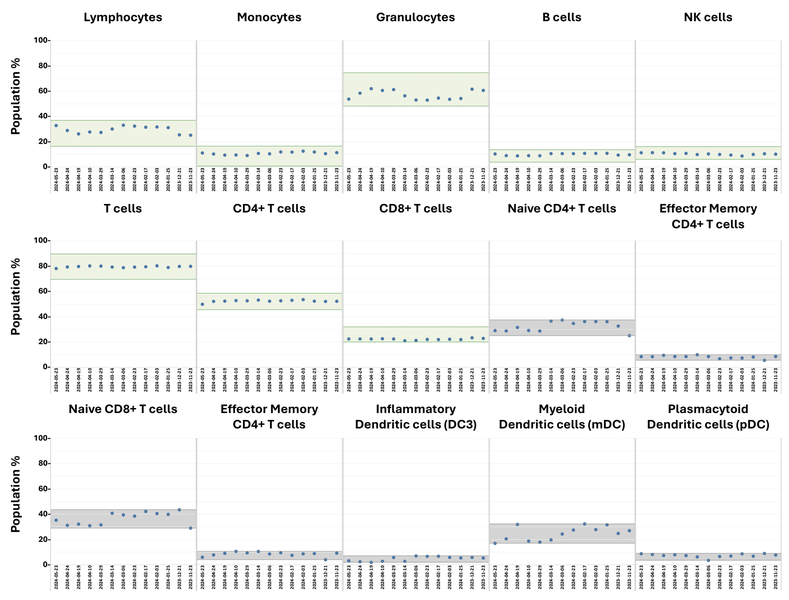
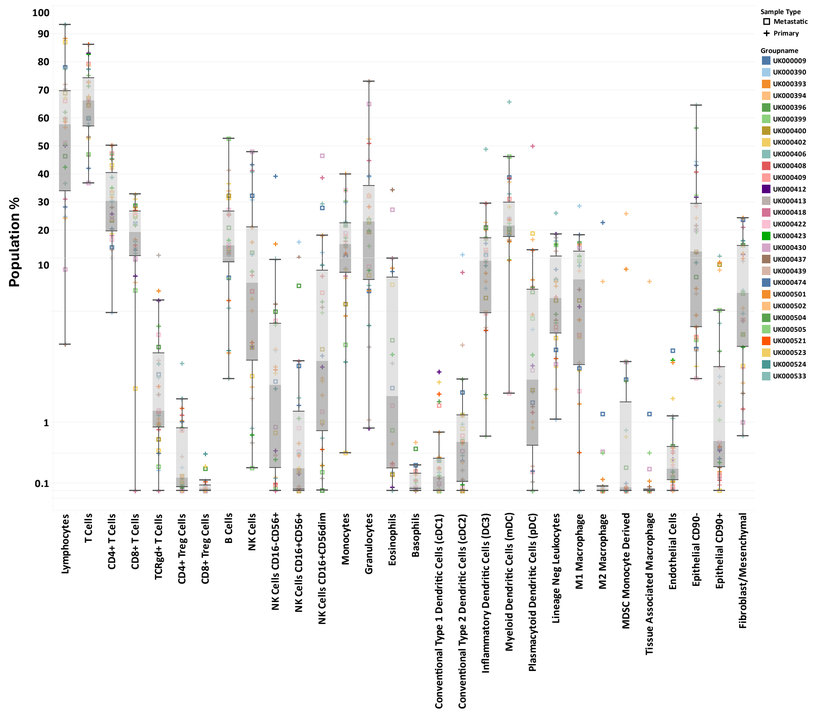


Figure 2. ImmunoINTEL™ assay performance monitoring and longitudinal tracking



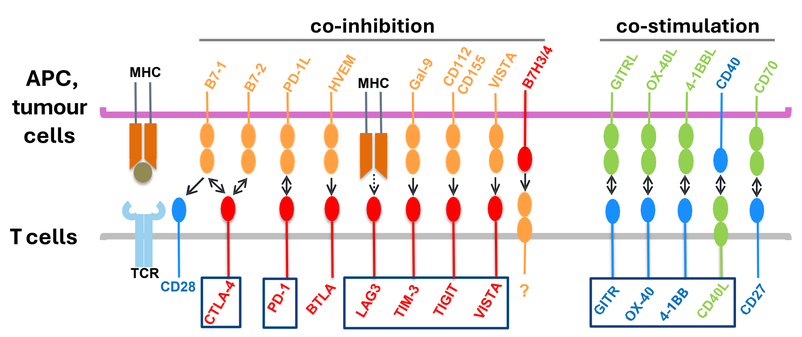
- ImmunoINTEL™ assay performance is tracked using CD-Chex Plus control (representative plots showing data over 6-month period).
- Green: expected population % range from CD-Chex plus datasheet; Grey: expected population % range defined by ImmunoINTEL™ assay.

Figure 3. TME cell population analysis revealed varying diversity of tumour-infiltrating immune cells and non-immune cells



- Diversity of cell population percentages observed in 28 tumour samples (log scale: 0-10; linear scale: 10-100)

Figure 4. ImmunoINTEL™ probes expression of multiple relevant immuno-modulatory receptor (IMR) /ligand (IMR-L) pairs



Probe expression of IMR ligands (orange and green) that dampen immune responses via IMRs (red and blue) could provide information on combination therapy and identify patients that are most likely to respond to immune checkpoint inhibitors.

RESULTS

Figure 5. ImmunoINTEL™ measures the expression levels of immune checkpoint/exhaustion markers and their cognate ligands on TILs and tumour epithelial cells

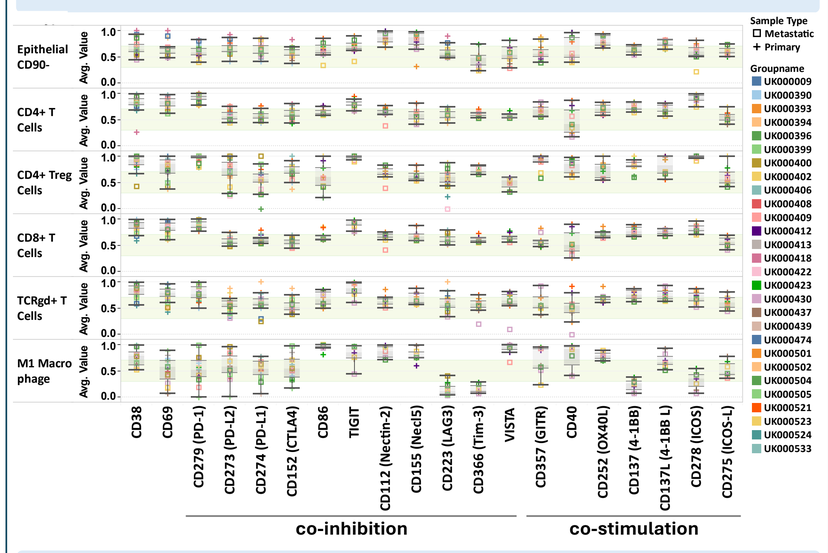
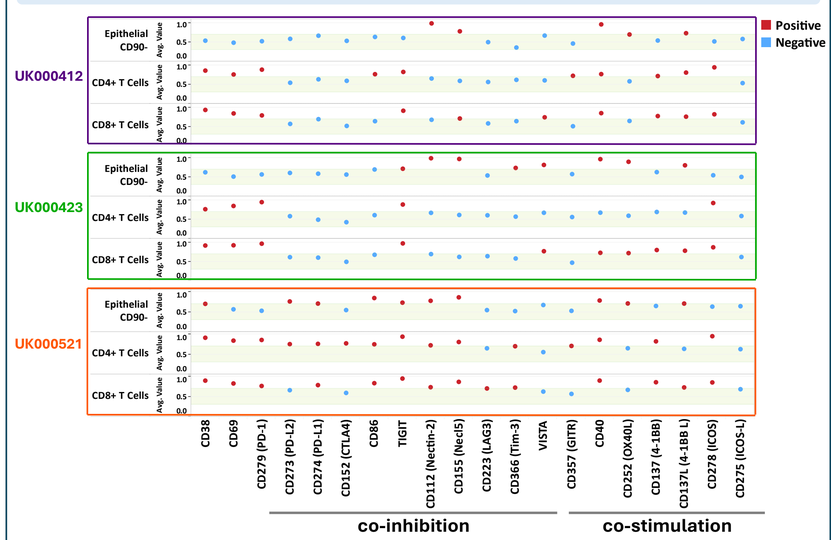
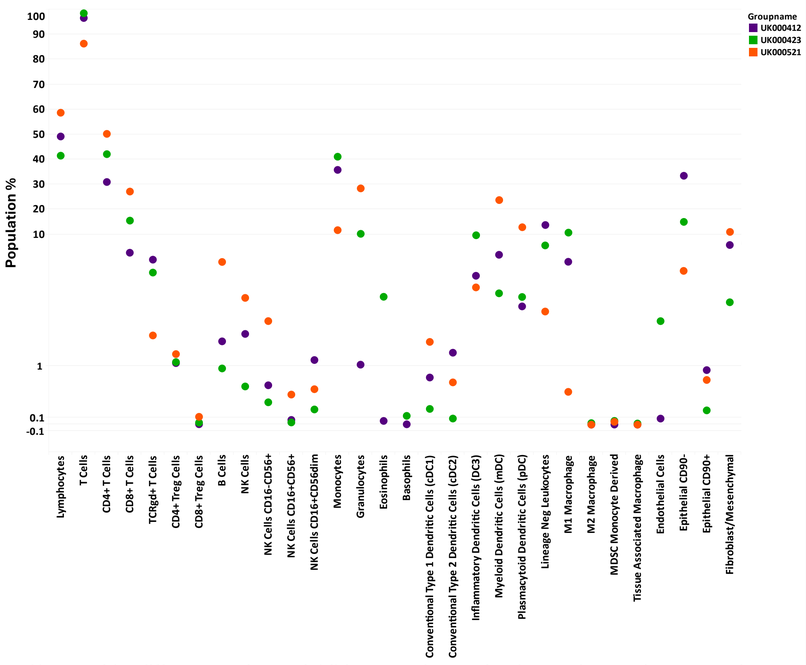


Figure 6. IMR/IMR-L expression pattern and TME cells population characterization indicate active/exhausted immune environment



- IMR/IMR-L expression profile for specimen UK000412, UK000423 and UK000521 shows multiple co-inhibitory and co-stimulatory interactions between tumour and immune cells.
- UK000521 showed signs of T cell exhaustion with high expression of co-inhibitory receptors PD-1, TIM-3, TIGIT and LAG-3 on CD8+ T cells.



- Along with differences in co-inhibitory and co-stimulatory interactions, specimens UK000412, UK000423 and UK000521 show significant differences in tumour-infiltrating immune cells in TME (log scale: 0-10; linear scale: 10-100)

CONCLUSIONS

- Pierian Biosciences has developed a semi-automated, multi-parametric platform which allows quantitative characterization of the immune cell populations and immune checkpoint receptor/ligand interactions within the TME
- The ImmunoINTEL™ platform allows for a complete evaluation of immune cell landscape within the TME and has the following advantages over other methods:
  - ✓ Requires fewer cells to characterize all the activation markers, IMR, and IMR-L across all cells within the TME
  - ✓ Panel size depends on the number of activation markers, IMR, and IMR-L measured
  - ✓ Spectral flow cytometry allows reliable utilization of 37 fluorophores simultaneously to identify a wide range of immune cell subtypes and sensitivity to detect even weakly expressed surface antigens, beyond the limited capacity of standard IHC
- The ImmunoINTEL™ platform is suitable for partnerships with pharmaceutical companies as a Pharma Support Service
  - ✓ Target Identification, drug discovery, and early-stage clinical evaluation
  - ✓ Phase 1, Phase 2 and Phase 3 Clinical Trials for patient stratification and monitoring
  - ✓ Potential applications as complimentary, companion, or treatment directing diagnostic

FOR MORE INFORMATION

- **Please see poster P-081 for more information about Pierian Biosciences ChemoINTEL platform**

US Headquarters: 479 Sam Ridley Pkwy
Suite 105 PMB 292, Smyrna, TN 37167
UK Laboratory: 131 Mount Pleasant
Liverpool Science Park, Liverpool L3 5TF
Website: www.pierianbio.com

Clinical Partners:
- The Clatterbridge Cancer Centre
- Liverpool Women's Hospital

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